Role of galectin-1, -3, -9 and vascular endothelial growth factor in the pathomechanism of allergic asthma

Ph.D. Thesis

Erna Sziksz

Semmelweis University
Doctoral School of Clinical Medical Sciences

Consultants:
Prof. Dr. András Szabó, D.Sc.
Dr. Ádám Vannay, Ph.D.

Examiners:
Dr. Edit Hídvégi, Ph.D.
Dr. Ádám Balogh, Ph.D.

Examination comittee:
Dr. Csaba Szalay, D.Sc.
Dr. Szilvia Bősze, Ph.D.
Dr. Beáta Dérfalvi, Ph.D.

Budapest
2010

INTRODUCTION

Asthma bronchiale is a complex chronic lung disease characterized by a reversible obstructive ventillatory disturbance, airway inflammation, mucosal enlargement and bronchial hyperreactivity, which molecular mechanisms are not clear. Recently sugar-binding molecules, such as galectins (Gal), came into the focus, which role were described during the adaptive and innate immune responses as well, but their function in allergic asthma is not fully understood. Galectins are ß-galactoside binding lectins, which can take part in cell-cell, cell-matrix and protein interactions. Research of recent years revealed, that Gal-1 has anti-apoptotic, immune suppressive and anti-inflammatory features. Based on these we hypothesize, that it may have important role in the inhibition of asthmatic inflammation. It is also known, that this lectin is able to polarize the immune balance toward the Th2 immune response, because it increases the IL-5 production, which may facilitate asthma progression. However the precise role of Gal-1 in asthma is little-known. Gal-3 is known to initiate cell polarization, inhibit apoptosis of epithelial cells, T cells, fibroblasts, monocytes, neutrophil granulocytes, mast cells and basophiles, and may promote the chemotaxis of cells, which are important during inflammation, such as neutrophil and eosinophil granulocytes, monocytes, macrophages. In the lung Gal-3 may interact with some IgE isoforms, which leads to activation of mast cells and basophils and to release of histamine. Based on this, Gal-3 may be involved in the pathogenesis of asthma, but the exact mechanisms are not fully understood. Gal-9 may have several functions: eosinophilic chemoattractant, urate-transporter, tumor antigen, regulator of tymocyte-epithelial cell interactions and mediator of apoptosis. Gal-9 induces the apoptosis of Th1 cells, but not Th2 cells. Researchers suppose that Gal-9 regulates eosinophil traffic during allergic reactions, but its role in asthma is not known. The permanent inflammation in asthma may lead to the structural changes of airways, which is known as airway remodeling. The vascular endothelial growth factor (VEGF) may have crucial role in this progress, because it is one of the important angiogenic factors, which may inhibit the proliferation of vascular smooth muscle cells and also have pro-inflammatory features. One of the central mediators of asthma is histamine, for which synthesis one enzyme, the histidine decarboxylase (HDC) is responsible. But there are only few data about the interaction between histamine and VEGF.

AIMS

Our aim was the better understanding of the pathomechanism of asthma, especially the potential role of some members of a specific lectin molecular family called Galectins, such as Gal-1, -3 and -9. Further we investigated the involvement of VEGF as an important angiogenic factor in the pathogenesis of asthma. In several cases we also examined the effect of lack of histamine on these molecules, whether there are any connection of the examined molecules and histamine.

During my PhD work I investigated the following topics:

1. Our questions about Gal-1 and Gal-3:

- Does the mRNA expression of Gal-1 and Gal-3 change in asthmatic mice compared to controls?
- Are there any changes in the Gal-1 and Gal-3 protein levels in the lung and bronchoalveolar lavage?
- Which immune cells are Gal-1 and Gal-3 positive and how does their number and amount change during asthma?
- Does the presence or absence of histamine influence the Gal-1 and Gal-3 production of the cells? If yes, how?
- Does the exogenously administrated histamine any effects on the Gal-1 and Gal-3 productivity of the alveolar macrophages?

2. Questions about the role of Gal-9 in asthma:

- Are there any changes in the Gal-9 mRNA expression and protein amount in the lung of airway challenged mice?
- What kind of BAL cells are Gal-9 positive?
- How does the number of Gal-9 positive immune cells and their Gal-9 production change in asthma?
- Where is Gal-9 localized in the lung of allergized and control animals?
- Does Dexamethasone - a steroid, which is known to diminish inflammation and used to treat asthma – any effects on Gal-9 mRNA expression and protein level?

3. Our questions about the potential role of VEGF in asthma:

- Are there any changes in the mRNA expression and/or VEGF protein level in the lung of mice after ovalbumin (OVA) sensitization and airway challenge compared to controls?
- How does the amount of VEGF positive immune cells and their intracellular VEGF amount change in the BAL of asthmatic animals?
- Is there any direct association between histamine and VEGF production in asthma? Does the lack of endogenous histamine influence the amount of VEGF in the lung? If yes, which cells are important in this?

METHODS

Experimental animals and allergization procedure

6-8 weeks old female, specific pathogen free wild type (WT) BALB/C and histidine decarboxylase "gene knock out" ($HDC^{-/-}$) mice were applied, which genotype – except of HDC gene – were similar to WT. Animals (n=7/group) were sensitized on day 1. and 14. of the experiment intraperitoneally administrated OVA injection (20 μg/animal diluted in PBS and 2,25 mg $Al(OH)_3$). Controls received only PBS and $Al(OH)_3$ as placebo. On days 28, 29 and 30 1% OVA solution was inhaled for 20 minutes (allergen provocation). Controls received PBS aerosol. Measurement of airway and sample collection was performed on day 31 or on day 32 (in experiments with $HDC^{-/-}$ mice) after further provocation (20 minutes, 5% OVA) 5 hours before the end of the experiment.

Steroid treatment

The steroid treated group received dexamethasone- sodium phosphate injection on day 29 and 30 (5 mg/kg doses/day in saline). Controls received only saline.

Airway responsiveness

We measured airway responsiveness – or AHR – so the changes of lung resistance after administration of metacholin (MCh) at invasive manner on mechanically ventilated animals. First dose of MCh was 43 μg/kg, and we always used triple doses according to protocols described earlier.

Bronchoalveolar lavage (BAL) and cytokines of the supernatant

After measuring airway reactivity the lungs of the animals were washed using 3x600μl sterile PBS. After centrifugation we determined the total cell number using Bürker-chamber. With Cytomix pearls we measured the amount of IL-1α, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, IFNγ, TNFα, and granulocyte-monocyte colony stimulating factor (GM-CSF) according to the protocol of the manufacturer.

Histology

After taking BAL the lungs of the animals were collected. One part of them were immediately fixed in formaldehyde. After dehydration they were embedded in paraffin and cutted to 5 μm sections. Hematoxiline-eosin and PAS (periodic-acid- Schiff) stainings were fulfilled. We

examined the perivascular and peribronchial granulocyte infiltration and goblet cell metaplasia and mucus hypersecretion with light microscopy.

Gal-1, -3, -9, VEGF protein level of BAL lymphocytes using FACS

After blocking the non-specific Fc receptor binding sites we stained the immune cells of BAL with Gal-1, Gal-3, Gal-9 and VEGF specific antibodies. Cells were fixed in 4% paraformaldehide, and then permeabilized by saponine. Samples were incubated with specific isothiociante (FITC) conjugated polyclonal IgG primer and secondary antibodies. As negative controls only secondary antibodies were used. We measured fluorescence intensity by FACS method. Alveolar macrophages, lymphocytes, eosinophil and neutrophil granulocytes were determined using anti-F4/80, -ECP, -CD3, -B220 antibody, respectively.

Real time reverz transcriptional polymerase chain reactions (RT-PCR)

One part of the lungs we have frozen immediately, and we isolated RNA using RNeasy™ Mini Kit according to the instructions of the manufacturer. Quality and quantity of the RNA was determined by photometry. 1 μg RNA was reverse transcripted to cDNA. With SYBR Green based real time PCR we measured the mRNA expression changes of Gal-1, -3, -9 and VEGF. Gliceraldehid-3-phosphate dehidrogenase was used as housekeeping gene.

Western blot of Gal-1, -3, -9, VEGF and actin

Lung samples were homogenized in lysing solution, and total protein content was determined with spectrophotometry using Bradford reagent. After denaturation 12% sodium-dodecil-sulphate (SDS) gel electrophoresis was performed. The separated proteins were transferred into nitrocellulose membranes, which were blocked in 5% nonfat milk solution. Membranes were incubated with specific primer and then secondary horse radish conjugated secondary antibody. ß-actin was used as reference. Chemiluminescence was detected with ECLplus reagent using camera. Immunoreactive bands were determined using densitometry.

.

Immunfluorescent staining of Gal-9

The frozen lung samples were embedded into criomatrix, then 5 μm sections were stained with Gal-9 specific primer and Alexa Fluor® 488 conjugated secondary antibodies. DNA were stained with Hoechst 33342. Sections were covered with Vectashield mounting medium, and then examined with laser scanning confocal microscope.

Immunhistology of Gal-1

The lungs were embedded into paraffin and 5 μm sections were prepared and deparaffined and then treated with microwaves in Target Retrieval solution. Endogenous peroxidase activity was blocked in 0**,**03% hydrogen-peroxide and then sections were incubated in 3% non fat milk diluted in PBS. We incubated them using Gal-1 specific primer and biotinilated secondary antibody. We used peroxidase/DAB kit. Sections were stained with hematoxilin-eosin also and mounted with Vecta medium.

J774.2 mouse alveolar macrophage cell culture and histamine treatment

A J774.2 alveolar macrophage cell line derived from BALB/c mouse were maintained in 4,5 mg/ml glucose and 10% FBS containing DMEM. Cells were divided into groups: only medium (control, non-stimulated), or 0**,**5 μg/well lipopolisaccharide (LPS) treated, or 0**,**5 μg/well LPS and 10^{-5}M α-fluoromethil histidine (LPS+FMH) treated, or 0,5 μg/well LPS and 10^{-5}M histamine (LPS+H) treated cells. After 12, 24 and 48 hours of treatment cells were prepared for FACS analysis and stained using Gal-1 and Gal-3 specific antibodies.

Statistical analysis

Normality was tested with Shapiro-Wilk test, and one way or two way variance analysis (ANOVA) and Newman-Keuls post hoc test were used to determine differences between groups. Data were considered to be significantly different if p was less than 0.05. Values for all measurements were expressed as mean + SD.

RESULTS AND DISCUSSION

Asthmatic symptoms and animal model

OVA sensitization and airway challenge induced AHR, marked peribronchial and perivascular inflammatory cell infiltration, goblet cell metaplasia and increased mucus production in the lung. Changes in the number and type of BAL cells were also representative to allergic asthma. The inflammatory response in the asthmatic lung is characterized by infiltration of the airways by lymphocytes, especially with activated Th2 cells. Cytokines and chemokines derived by Th2 lymphocytes promote eosinophilic infiltration and play pivotal role in the pathogenesis of allergic asthma. Based on these we successfully performed the model of allergic asthma.

Gal-1 in asthma

In the lung of WT animals the Gal-1 mRNA expression and protein level were increased after OVA sensitization and airway challenge compared to controls. Using immunhistochemistry we found, that the major sources of Gal-1 in the lung are the alveolar macrophages and lymphocytes. Based on the literature we suppose that Gal-1 could diminish the symptoms of asthma through elimination of inflammatory cells (except of eosinophils) and inhibits inflammation. On the other hand it may exacerbate asthma because it may polarize the immune system towards Th2 immune response. Further studies are needed to clarify the exact role of Gal-1 during asthma. Therefore our aim was to examine, whether the presence or absence of histamine may influence Gal-1 expression. In the lung of $HDC^{-/-}$ the Gal-1 mRNA expression was also increased after OVA sensitization and airway challenge in the $HDC^{-/-}{}_{ova}$ group compared to control animals. But the mRNA expression of Gal-1 was lower in the lung of $HDC^{-/-}$ animals compared to WT mice. The changes in the Gal-1 protein level were similar to the mRNA expression changes, but no significant differences were detectable. Furthermore the Gal-1 producing cell types in $HDC^{-/-}$ were also similar to that of WT animals.

One cause for that the Gal-1 protein amount in the lung of $HDC^{-/-}$ mice was only slightly elevated, could be, that in these genetically modified animals the airway hyper-responsiveness is also less than in WT animals. Our research group earlier described, that these animals have less mast cells and mast cell precursors, the content of the granules is less and the degranulation is also impaired. After OVA sensitization and challenge the airway inflammation and hyper reactivity were not so serious in $HDC^{-/-}$ animals compared to WT animals, and the expression of Th1 cytokines and chemokines was also elevated.

Our experiments show that the alveolar macrophages and lymphocytes in the BAL of $HDC^{-/-}$ animals are able to produce Gal-1 and to migrate from the tissue to the alveolar surface. In WT animals the number of Gal-1 positive alveolar macrophages and lymphocytes was unchanged after OVA provocation and challenge, in $HDC^{-/-}$ mice their number was elevated compared to $HDC^{-/-}{}_{control}$ and $WT_{ova.}$ This suggests that the lack of histamine induce the migration of Gal-1 positive immune cells to the alveolar space. Furthermore the Gal-1 content of alveolar macrophages of OVA sensitized $HDC^{-/-}$ mice was higher than in control macrophages. These results suggest that the Gal-1 content in the alveolar macrophages of $HDC^{-/-}$ animals is highly elevated during asthma because of the lack of histamine. But alveolar macrophages are not the lonely sources of Gal-1 in the lung, which could explain, why the Gal-1 level is a little bit lower in the lung of $HDC^{-/-}$ animals compared to WT. To examine the role of alveolar macrophages we used J774.2 alveolar macrophage cell line in vitro. The LPS stimulated cells produced more Gal-1 compared to non-stimulated cells. This result suggests, that the cell stimulation has important role in promoting of Gal-1 production.
After LPS stimulation HDC blocker (FMH) was used, which significantly elevated the Gal-1 amount of J774.2 cells compared to only LPS stimulated (LPS) and LPS and histamine treated (LPS+H) groups. Not significantly, but histamine decreased the Gal-1 level of LPS stimulated J774.2 cells. Based on our results and literature we hypothesize, that Gal-1 and histamine may be regulators of each other.

Gal-3 and asthma

After OVA sensitization and airway challenge in the lung of WT animals the Gal-1 mRNA expression and protein level were increased compared to controls. We found, that the major sources of Gal-3 in the lung are the monocytes, macrophages, but lymphocytes of the BAL and newly immigrated eosinophils and neutrophils produce this lectin. In the BAL of histamine deficient $HDC^{-/-}$ mice the number of Gal-3 positive alveolar macrophages and their intracellular Gal-3 content were less than in WT animals after OVA treatment. These results suggest that either histamine may have direct effect on Gal-3 regulation in alveolar macrophages, or the different immune mechanisms in $HDC^{-/-}$ animals may lead to the reduced Gal-3 level.
To examine this we studied the effect of histamine on Gal-3 production using J774.2 cells. Neither histamine nor the HDC blocker FMH have an effect on Gal-3 production of the cells, which suggests, that histamine has not direct effect on Gal-3 synthesis, but it regulates Gal-3 production indirectly through mediators.

We hypothesize, that not only histamine is able to influence the Gal-3 production of alveolar macrophages after OVA sensitization and challenge. There might be other factors too, because the immune responses of these animals mediated by basophil and eosinophil granulocytes and mast cells are damaged. Others described, that Gal-3 can promote mast cell and basophil activation by crossbinding of IgE receptors, which leads to degranulation and to release of different mediators. In contrary Gal-3 inhibits IL-5 transcription in eosinophils and allergen induced T cells, which regulates growth, differentiation, activation and survival of eosinophils. In this context Gal-3 has anti-asthmatic effect.

In summary we suppose, that Gal-3 produced by alveolar macrophages, eosinophil and neutrophil granulocytes and lymphocytes may be an important mediator of asthmatic reactions. It seems, that histamine haven't got direct regulatory role in Gal-3 production, but it influences indirectly the number of Gal-3 positive macrophages and their intracellular Gal-3 amount. We hypothesize, that in histamine deficient $HDC^{-/-}$ animals Gal-3 may modulate the pathogenesis of asthma, but further studies are needed to clarify these regulatory processes.

Gal-9 in asthma

In our model the percentage of the Gal-9 immunopositive lymphocytes and their intracellular Gal-9 amount and the level of Th2 cytokines, such as interleukin IL-4, IL-5 and IL-6 were increased in the BAL of the allergized (G_{OVA}) mice compared to G_{PBS} and steroid treated ($G_{OVA+DEX}$) animals. Based on our results and data in the literature we suggest that Gal-9 may have role in the development of Th2 dominancy and elevated Th2 cytokine production.

In the present study we also characterized the synthesis of Gal-9 in the lung of allergized mice. Interestingly, while the mRNA expression of Gal-9 decreased, the protein level of Gal-9 increased in the lung of G_{OVA} compared to G_{PBS} mice. These data suggest that the OVA sensitization and airway challenge may alter both the transcription and the translation of Gal-9 as well. Elevation in the protein level of Gal-9 in the presence of decreased mRNA expression suggests the importance of the post-transcriptional regulation in the synthesis of Gal-9. Another possibility, that the decreased mRNA expression results in a decreased protein level of Gal-9 in a later, than the investigated time point in the G_{OVA} mice. However, based on the literature we suggest that the OVA sensitization and airway challenge may lead to an increased protein level of Gal-9.

DEX treatment did not alter the mRNA expression of Gal-9 of the OVA sensitized and airway challenged $G_{OVA+DEX}$ compared to G_{OVA} mice. However, the protein level of Gal-9 was decreased in $G_{OVA+DEX}$ compared to G_{OVA} mice. Our findings suggest that while DEX

treatment alters the translation, it does not affect the transcription of Gal-9. However, there is an apparent discrepancy in the literature, Gal-9 may participate in the pathomechanism of allergic inflammation via different ways.

In line with our results Yamamoto et al. found increased Gal-9 level in the lung of sensitized guinea pigs and suggested that Gal-9 may induces chemotaxis of eosinophil granulocytes and apoptosis of T cells. On the contrary Katoh et al. showed that different doses of intravenously administrated recombinant Gal-9 inhibits allergic inflammation by modulating CD44-dependent leukocyte recognition of the extracellular matrix. Based on the literature and our results we suggest that the effect of Gal-9 may be dose-dependent. While physiological amount of Gal-9 may induce chemotaxis of eosinophil granulocytes and contribute to the pathomechanism of allergic asthma, the higher concentration of the exogenously administrated Gal-9 reduces the recruitment of these cells and diminishes inflammation.

We assume that the elevated Gal-9 level in the lungs influences the progress and severity of asthma in different manners. Gal-9 may induce the death of the Th1 cells, which may lead to the development of Th2 dominancy and the secretion of Th2 cytokines during the allergic processes. Th2 cytokines including IL-4, IL-5 and IL-6 play an important role in the pathophysiology of allergic asthma by promoting eosinophil inflammation. Based on our results we suppose that DEX may exert its anti-inflammatory effect also by diminishing Gal-9 protein amount. We suggest that Gal-9 may have important role in the pathogenesis of asthma by promoting Th2 dominancy and recruiting eosinophil granulocytes.

VEGF and asthma

We have found increased protein level of VEGF in the lung tissue and in the lymphocytes and alveolar macrophages of the BAL after OVA sensitization and airway challenge of $HDC^{-/-}$ and WT mice compared with their controls. Moreover, VEGF immunopositive eosinophil granulocytes appeared in the BAL. In accordance with our findings Asai et al have found increased total VEGF amount in the sputum of asthmatic patients.

In contrast of increased protein level the mRNA expression of VEGF was unchanged after OVA sensitization and airway challenge in the lung of WT and $HDC^{-/-}$ mice compared with their controls. These data in accordance with previous results found in rat kidneys propose that the post-transcriptional mechanisms are important elements in the regulation of VEGF synthesis in the allergized lung as well. Previously, it has been shown that binding of RNA-binding protein HuR to the AU rich region of the 3' untranslated region of VEGF mRNA leads to the stabilization of the transcript. Moreover two internal ribosomal entry site on the 5'

untranslated region of VEGF ensure the efficient translation of VEGF mRNA even when the other translational mechanisms are inhibited.

Previous studies demonstrated significant increase in the number, size and surface of the newly synthesized blood vessels in the inflamed asthmatic lung. There is a growing body of evidence that angiogenesis and chronic inflammation depend from each other. VEGF – a potent angiogenic factor – has also been implicated as a driver of enhanced allergic sensitization and upregulated T-helper type 2 (Th2) inflammatory responses. The persistent inflammation can lead to structural changes of the airways known as remodeling. VEGF as a driver of airway angiogenesis, goblet cell metaplasia, and matrix metalloproteinase (MMP)-9 activation, may be an important mediator of progression of structural lung remodeling in asthma.

We have found similar VEGF protein levels in the lung and in the BAL of allergized $HDC^{-/-}$ and WT mice. To our present knowledge the literature about the role of histamine in the regulation of VEGF synthesis is contradictory. Numata Y et al. using $HDC^{-/-}$ animals demonstrated that histamine is crucial in the wound-healing process. In HDC–/– animals they found reduced basic fibroblast growth factor, but similarly to our present findings unaltered VEGF synthesis at the edge of skin wounds. On the contrary, we and others have demonstrated previously that both endogenous and exogenous histamine increased the level of VEGF via the H2 receptors. The observed discrepancy in the literature may be due to the different models used in the various experiments (mice or rats, wound healing, granulation tissue or kidney ischemia). However, based on our present data the endogenous histamine is not necessary for increased synthesis of VEGF in our mouse model of allergic asthma. However, further experiments are needed to investigate the possible complex connection between histamine and altered synthesis of VEGF.

Based on our present results, however, we conclude that there might be other essential mechanisms, which are independent of histamine mediated inflammatory pathways, and which also may have major effects on the progression of the disease (by promoting remodeling). Thus, our observations may provide some theoretical explanation of the failure of antihistamine treatment in bronchial asthma, despite of well known involvement of histamine in allergic processes, and in the pathomechanism of asthma.

In summary, increased level of VEGF was demonstrated in the lung tissue and BAL cells of allergized HDC–/– and WT mice. These data support the involvement of VEGF in the pathogenesis of allergic asthma. The level of VEGF did not differ between HDC–/– and WT animals suggesting that the lack of histamine in HDC–/– mice has no effect on the allergen

induced VEGF synthesis. Our data suggest that a part of the processes, which ends in allergic asthma may proceed without histamine. We suggest that VEGF, as a potent inducer of lung remodeling, could be an important target of further research to clarify the precise role of this molecule in allergic diseases and asthma.

THESES

The most important statements of my Ph.D. thesis are:

1. We found elevated Gal-1 production in asthma, and primarily alveolar macrophages, lymphocytes and in part bronchial epithelial cells are responsible for this.

2. The lack of histamine enhances the Gal-1 production of macrophages and lymphocytes, while histamine decreases it, so we suppose that histamine and Gal-1 may be regulators of each other.

3. We have shown that Gal-3 mRNA expression and protein production is elevated in the lung of asthmatic mice. In these progress alveolar macrophages, lymphocytes and the newly immigrated eosinophil and neutrophil granulocytes have important role. The lack of histamine *in vivo* did not cause any changes in the amount of Gal-3, which we confirmed in vitro also. This suggests that histamine do not influence the Gal-3 production directly.

4. Based on our measurement we confirmed, that the mRNA expression of Gal-9 is decreased after allergization and the protein level is increased, so we hypothesize, that posttranscriptional regulation may have important role in the synthesis of Gal-9. Although all immune cell types of the BAL were positive to Gal-9, for the elevated Gal-9 level primarily lymphocytes, eosinophil and neutrophil granulocytes and epithelial cells are responsible.

5. We firstly have shown that the steroid DEX treatment diminishes the amount of Gal-9 in the lung, and the tissue localization of Gal-9 in the DEX treated animals is similar to controls. As Gal-9 is chemotactic to eosinophil granulocytes, we suppose, that DEX may diminish inflammation also through reducing the amount of Gal-9.

6. We described that the protein amount of VEGF is elevated in the lung after asthmatic procedure and the VEGF production of alveolar macrophages, lymphocytes and eosinophil granulocytes is enhanced.

7. We firstly demonstrated that there are no differences in the VEGF mRNA expression and protein production between the genetically histamine deficient and wild type allergized animals. Based on this we suggest, that histamine does not influence directly the allergen induced VEGF synthesis. We hypothesize, that there might be a histamine independent pathway which may also lead to asthma, and which would partially explain, why anti-histamine products applied to treat asthma are in same cases ineffective.

ACKNOWLEDGEMENTS

First of all I would like to thank Professor Dr. Tivadar Tulassay, who established the intellectual creative workshop and who often encouraged me and gave me new impetus to fulfill challenges.

I would also like to thank my consultants Professor Dr. András Szabó and Dr. Ádám Vannay for always standing by me, thanks for the advice and invaluable assistance in scientific approach of research and also for creating the necessary material conditions for work.

I would like to express my gratitude to Dr. Gergely Kozma and Dr. Zsolt Komlósi, who played important role in this work.

I would like to thank Dr. Barna Vásárhelyi for helping me with his experiences and for shaping my scientific thinking.

I'm grateful to Dr. Éva Pállinger for her selfless aid and professional advice.

Furthermore I thank to Prof. Dr. András Arató, Prof. Dr. György Reusz, Dr. Attila Szabó and Dr. Gábor Veres, who also contributed to present our results at professional conferences.

I would like to thank Dr. Beáta Szebeni for standing by me in everything, for her friendship and professional help.

I'm grateful to all of the other researchers of our lab, Dr. Andrea Fekete, Dr. Krisztina Rusai, Dr. Kálmán Tory and Leonóra Himer, that they always answered to my professional questions.

I would like to thank all my fellow PhD students, who contributed to the good and youthful atmosphere of our lab.

I'm grateful to Maria Bernáth for her excellent technical assistance and humanity. I would like to express my gratitude to all colleague of the 1st Department of Pediatrics that I have an opportunity to do my work among friendly circumstances.

Finally I would like to express my unfailing gratitude to my parents, every family members and to my groom for they love, solicitude, and encouragement.

PUBLICATIONS

Publications in the topic of the thesis:

1) **Erna Sziksz**, Gergely Tibor Kozma, Éva Pállinger, Zsolt István Komlósi, Csaba Ádori, Lajos Kovács, Beáta Szebeni, Krisztina Rusai, György Losonczy, András Szabó, Ádám Vannay. Galectin-9 in allergic airway inflammation and hyper-responsiveness in mice. – Int Arch Allergy Immunol. 2010; 151(4): 308-17. (IF: 2,131)

2) **Erna Sziksz,** Gergely Tibor Kozma, Zsolt István Komlósi, Éva Pállinger, Magdolna Kardos, Beáta Szebeni, György Losonczy, András Falus, András Szabó, Tivadar Tulassay, Ádám Vannay. Increased synthesis of VEGF in allergic airway inflammation in histidine decarboxylase knockout ($HDC^{-/-}$) mice. – Exp Lung Res, 2010, accepted, in press (IF:1,618)

3) **Sziksz Erna**, Kozma Gergely Tibor, Pállinger Éva, Komlósi Zsolt István, Ádori Csaba, Kovács Lajos, Szebeni Beáta, Rusai Krisztina, Losonczy György, Vannay Ádám, Tulassay Tivadar, Szabó András. Galektin-9 szerepének vizsgálata az allergiás légúti gyulladás és hiperreaktivitás kísérletes egérmodelljében. Gyermekgyógyászat 2009; 60: 1. (IF: 0,0)

Publications independent of the thesis:

4) Ádám Vannay, Andrea Fekete**,** Róbert Langer, Tibor Tóth, **Erna Sziksz**, Barna Vásárhelyi, Attila J. Szabó, György Losonczy, Csaba Ádori, Anikó Gál, Tivadar Tulassay, András Szabó. Dehydroepiandrosterone pretreatment alters the ischaemia/reperfusion-induced VEGF, IL-1 and IL-6 gene expression in acute renal failure. Kidney Blood Press Res. 2009; 32(3): 175-84. (IF:1,268)

5) Beáta Szebeni, Ádám Vannay, **Erna Sziksz**, Ágnes Prókai, Áron Cseh, Gábor Veres, Antal Dezsőfi, Hajnalka Győrffy, Ilma Rita Korponay Szabó, András Arató. Increased expression of serum-and glucocorticoid-regulated kinase-1 in the duodenal mucosa of

children with coeliac disease. J Pediatr Gastroenterol Nutr. 2010 Feb; 50(2): 147-53. (IF: 2,132)

6) Mónika Ádori, Endre Kiss, Zsuzsanna Barad, Klaudia Barabás, Edda Kiszely, Andrea Schneider, Dorottya Kövesdi, **Erna Sziksz**, István M. Ábrahám, János Matkó, Gabriella Sármay. Estrogen augments the T-cell-dependent but not the T-independent immune response. – Cell Mol Life Sci. 2010 May; 67(10): 1661-74 (IF: 5,511)

7) Ádám Vannay, **Erna Sziksz**, Ágnes Prókai, Gábor Veres, Kriszta Molnár, Dorottya Nagy Szakál, Anna Ónódy, Ilma Rita Korponay-Szabó, András Szabó, Tivadar Tulassay, András Arató, Beáta Szebeni. Increased expression of hypoxia inducible factor 1alpha in coeliac disease. Pediatr Res. 2010 May 5, accepted, in press, equally contributed first authority (IF: 2,604)

8) Szebeni Beáta, **Sziksz Erna**, Prókai Ágnes, Gál Krisztina, Vannay Ádám, Cseh Áron, Veres Gábor, Dezsőfi Antal, Korponay Szabó Ilma, Bodánszky Hedvig, Arató András. Fokozott szérum és glükokortikoid regulált kináz-1 expresszió gyermekkori cöliákiában. Gyermekgyógyászat 2009; 60: 1. Equally contributed first authority (IF: 0,0)

9) **Sziksz Erna**, Gál Krisztina, Vannay Ádám, Reusz György, Tulassay Tivadar, Szabó András. A hipoxia indukálta faktorok szerepe a hipoxiás vesekárosodások patológiai folyamataiban. Hypertonia és Nephrologia 2008; 12 (6): 197-201, review (IF: 0,0)

10) Himer Leonóra, Balog Attila, Szebeni Beáta, Nagy Szakál Dorottya, **Sziksz Erna**, Reusz György, Tulassay Tivadar, Vannay Ádám. A Th17 sejtek szerepe a reumatoid artritiszben. – Orv Hetil. 2010 Jun 20; 151(25):1003-10. (IF: 0,0)