Genomic investigation of the pathomechanism of allergic asthma

Summery of Ph.D. thesis

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Introduction

In our studies, we examined the genomic background of the pathomechanism of the complex multifactorial disorder, *asthma bronchiale,* whose increasing prevalence (vagy increasing prevalence of which) causes serious public-health problem worldwide, especially in developed countries. Asthma is a complex chronic lung disease characterized by airway inflammation, hyperresponsiveness, remodeling, obstruction, enhanced mucus production, and the peribronchial and perivascular infiltration of inflammatory cells. Allergic (extrinsic) asthma is related to allergen specific IgE production, which can cause exaggerated activation of the immune system, leading to the development of airway inflammation. The disease is known to be caused by the failure of the coordinated function of genes regulating the immune system, but the environmental factors (airway pollution, allergens, and physical exertion) are also essential for its clinical manifestation.

Intensive research is in progress at present to identify the genetic background of the pathomechanisms of allergic diseases. The main difficulty that hampers the efforts to identify the specific genes involved in asthma is the multigenic nature of the disease. It means that more than one gene in each individual might interact to produce the disease phenotype (polygenic inheritance), different disease alleles might exist in different individuals (genetic heterogeneity), and interaction with the environment might lead to incomplete penetrance. Due to the sequencing of the human genom, the expanding molecular genetic and informatic knowledge, a new science was arisen some years ago, which created the genom scaled molecular genetics, the genomics. One of the most important trends of genomics is the structural genomics, main task of which is to identify disease related gene variants (mutations and polymorphisms) and haplotypes. Functional genomics, the other important trend of genomics, allows us to monitor the expression of genes, which can help to identify disease related gene expression changes and patterns. The introduction of high-throughput microarray technologies offers a

new opportunity to gain insight into the global gene-expression profiles in asthma, leading to the identification of new asthma-associated genes and pathways. The developing bioinformatics offers powerful tools to compare our data derived from microarray measurements to the observations made by other researchers. Using advanced statistical methods we are able to relate gene expression changes to cellular processes and to integrate our results into multiple levels of information available in public databases.

In our studies, we applied both functional and structural genomical approaches to examine the genomic background of the pathomechanism of allergic asthma.

Goals of our study

Goals of our study were the following:

1. In our animal experiments, we used an ovalbumin-induced murine model of asthma. We applied microarray gene and microRNA expression analysis of the lung at different time points after allergen challenges to monitor the gene expression changes related to the different progression state during the development of allergic airway inflammation. In these experiments, we focused on the followings:

- Applying different bioinformatical techniques, we aimed to identify new genes, gene groups and pathways involved in the pathogenesis of experimental asthma.
- Validation of the selected genes on multiple levels.
- Identifying genes, gene groups that have not yet been investigated in asthma earlier.
- Identification of those microRNAs, which show different expression profile in the lung between the control and allergic mice, and validate these selected microRNAs with real time PCR.

- If we are able to identify new gene (genes) using the animal model of asthma, then investigate the role of those in human asthmatics.

The gene expression analysis revealed the strong down-regulation of paraoxonase 1 (*PON1*) in the lung of mice undergoing experimental asthma. Paraoxonase 1 is an extensively studied antioxidant enzyme, which plays an important role in other multifactorial diseases including atherosclerosis and diabetes, but it has not yet been studied in connection with asthma earlier. According to our results, it is reasonable to hypothesize that development of acute asthma coincides with reduced expression of *PON1* in humans, too. To examine this, we addressed the following questions:

- The two most investigated *PON1* polymorphisms are the Q192R, which was found to influence the activity of the enzyme, and the -108 T/C promoter polymorphism, which is associated with altered expression of the gene. Is there any difference in the distributions of these two polymorphisms between asthmatic and control subjects?
- Is there any association between the serum PON1 activity and the severity of asthmatic symptoms in patient with serious asthma?

2. In our gene-environment interaction studies, we investigated the role of the TNF-α -308A promoter polymorphism in *Chlamydophila pneumoniae* infection, and its possible modifying effect on the susceptibility to asthma in children.

Materials and methods

Experimental animals

6-8-weeks-old female mice with Balb/c genetic background were used in our experiments.

Sensitization and airway challenge

Mice were sensitized by intraperitoneal injection of 20 μg ovalbumin (OVA) emulsified in 2.25 mg aluminium hydroxide (Alum) in a total volume of 100 μL phosphate-buffered saline (PBS) on days 0 and 14. Non-sensitized animals received Alum and PBS only. On days 28, 29 and 30, mice were placed in a plastic chamber and challenged with 1% OVA aerosol (in PBS) or PBS only for 20min by an ultrasonic nebulizer. Bronchoalveolar lavage (BAL), lung tissue, RNA and protein sample collection was performed on day 28 (group one) and 30 (group 2), 4 h after the first and third allergen challenge, and on day 31, 24h after the third (last) allergen challenge (group 3 and controls), after the lung resistance measurements.

Airway responsiveness

Airway responsiveness was measured as a change in lung resistance (R_L) in response to aerosolized methacholine (MCh).

Bronchoalveolar lavage

BAL was performed in each mouse via a tracheostomy tube using 600 μL PBS three consecutive times on day 28 and on day 30 4 hours after the allergen challenge (the first and the third), and on day 31, after the lung resistance measurements. BALF was centrifuged at 700g for 10 min at 4 ^{o}C, and supernatants were aliquoted, immediately frozen and maintained at -80 ^{o}C for further protein analysis. Cell pellets were resuspended in 1 ml PBS, and 50 μl of cell suspension was added to 50 μL trypan blue stain (0.4%) and the total cell count was determined under a light microscope using. The remaining cell suspension was diluted with PBS and centrifuged in a cyto-chamber. Slides were stained with a hematological dye, and differential cell counts were determined under a light microscope by counting at least 300 cells. The cell types were identified according to the standard morphology.

Histology

After BAL collection, the chest wall of the animals was opened and removed from the thoracic cavity. The left lung was immersed in a fixative for 24 h, and then dehydrated, embedded in paraffin block and 5 μm sections were stained with haematoxylin and eosin for light microscopy examination.

RNA isolation, quality determination

RNA from mouse lung was prepared by RNeasy columns, the quality and quantity of total RNA was determined with an Agilent 2100 Bioanalyzer.

Microarray experiments

For microarray experiments Agilent Whole Mouse Genome Oligo Microarray 4x44K chips were used. Samples from OVA sensitized and challenged mice from group 1, 2 and 3 were identically labeled with Cy5 dye, while lung

tissue RNA samples from group 4 (control, placebo sensitized and challenged) were pooled and labeled with Cy3 dye, and served as a common reference. Cy3 and Cy5-labeled samples were mixed and hybridized to Whole Mouse Genome Oligo 4x44k microarrays, then the slides were washed with an ozone safe method and scanned by Agilent Microarray Scanner. All steps were carried out according to the manufacturer. Data were then normalized by the Feature Extraction software version 7.5 with default parameter settings for two-color oligonucleotide microarrays.

Statistical and bioinformatics analysis of the microarray data

Statistical and bioinformatics analysis of the microarray data was performed by the GeneSpring 9.02 program. In GeneSpring the normalization and data transformation steps recommended by Agilent Technologies for two-color data were applied. Experiment interpretation was built by GeneSpring, and the expressed genes showing a >2.0-fold differential expression were further analyzed by statistical tests. The statistical comparisons were performed between the groups and the respective groups against the common reference control by one-way ANOVA and T-test, respectively. A multiple testing correction method by Benjamini-Hochberg was applied with $p<0.05$ cutoff in our statistical tests.

Gene Ontology analysis

Based on the results of the microarray data analysis, the differentially expressed genes were studied for Gene Ontology (GO) terms, which provide information on the cellular component, biological process, and molecular function of the protein. The Genespring program calculates P values for each GO term using a standard hyper-geometric distribution to compare the frequencies of individual GO terms within the selected list of genes to the frequencies of those terms on the entire microarray (P values = 0.01 were considered to be significant). The p-value for individual GO terms, also known as the enrichment score, signifies the relative importance or significance of the GO term among the entities in the selected gene list compared to the entities in the whole dataset. False discovery rate (FDR) was controlled at 5% for all analyses. This strict correction ensures the enrichment is statistically significant.

Gene set enrichment analysis (GSEA)

The GSEA was carried out by GSEA v2.0 software. For the GSEA, a collection of canonical pathway gene set containing 639 was used. In addition, six sets of genes were generated consisting of genes considered to be associated with multiple models of lung disease, including bacterial infection, bleomycin induced lung disease and Th2 inflammation models. These gene sets were confirmed previously by other authors using other microarray platforms. Gene sets were assessed as to whether they individually scored high when compared with other possible choices of gene sets. This provided an unbiased means of assessing

pathways and test gene lists with respect to enrichment or degree of representation of highly regulated genes. Positive normalized enrichment score (NES) indicates correlation with group 3, while negative value indicates correlation with group 1. The nominal p value estimates the statistical significance of the enrichment score for a single gene set.

MicroRNA expression microarray measurement

For microRNA expression microarray measurement, Agilent Mouse miRNA Microarray 8x15k chips were used, which measures the expression of 567 different mouse microRNAs. In our experiments, we compared the microRNA expression profile of mice in group 3 to the placebo treated controls. Sample preparation, labeling and hybridization steps were carried out according to the manufacturer. Statistical and bioinformatics analysis of the microRNA microarray data was performed by the GeneSpring 9.02 program, similar to the gene expression microarray data analysis.

Reverse transcription and mRNA expression analysis by TaqMan real-time PCR

One μg total RNA was reverse transcribed to cDNA, and then the real-time PCR reactions were carried out for selected genes (*mChia, mPon1 and mClca3*) in an ABIPrism 7000 instrument according to the manufacturer. HGPRT-normalized signal levels were calculated using the comparative Ct method and expressed in percents of the respective marker level measured in placebo controls.

MicroRNA expression analysis by TaqMan real-time PCR

MicroRNA expression analysis for selected genes (miR-21, miR-135b and miR-155) was carried out using TaqMan MicroRNA Assay in two-step RT-PCR reaction according to the manufacturer in an ABIPrism 7000 instrument. For internal controls, specific snoRNAs were used, and the snoRNA-normalized signal levels were calculated using the comparative Ct method and expressed in percents of the respective marker level measured in placebo controls.

Immunohistochemical analysis

Protein level validation of paraoxonase-1 expression in the lung of mice was performed by immunohistochemistry using Biogenex i6000 automated staining system according to the manufacturer's protocol. Signal intensities were determined densitometrically with the NIH Image by measuring three randomly chosen areas per slide at 10x magnification.

Western blot analysis

The specificity of the antibody against the PON1 protein was confirmed by Western-blotting. Immunoreactive bands were visualized with the ECL Plus Western blotting Detection System. Specific band size was determined with the Full Range Rainbow Molecular Weight Marker.

Subjects for human studies

The -108 T/C and Q192R polymorphisms in the *PON1* gene were genotyped in 302 children with physician-diagnosed asthma (170 boys, 132 girls; age 3-18, mean 10.1 years, SD: 3.9) and in 188 healthy control (99 boys, 89 girls; age 3-18; mean 11.1 years, SD: 4.2) subjects. The TNF-α -308A polymorphism was genotyped in 144 asthmatic (80 boys, 64 girls; age 3-18; mean 10.4 years, SD: 4.3) and 174 healthy control (95 boys, 79 girls; age 3-18, mean 11.5 years, SD: 4.3) children. The asthmatic children attended the Allergic Outpatient Consultation of the Budai Children's Hospital. All the asthmatic children had specialist physician-diagnosed asthma with the following characteristics: (1) recurrent breathlessness and expiratory dyspnea requiring treatment; (2) physician diagnosed wheeze; (3) reversibility of the wheezing and dyspnea by bronchodilator treatment measured as forced expiratory volume 1s (FEV_1) by a spirometer. . Informed parental consent was obtained for each patient and control and the study was approved by the Institutional Review Board of Budai Children's Hospital. The investigation conforms to the principles outlined in the Declaration of Helsinki. All children were from Budapest and at least 95% of them belonged to the Hungarian Caucasian population.

Paraoxonase and arylesterase activities were monitored in serum of eight adult asthmatic patients (3 males and 5 females; age: 54.5 ±14.3) at three time points: (1) day 0: the patient was taken to the hospital with serious asthma exacerbation; the blood was taken before the start of *iv.* corticosteroid (80 mg methylprednisolon/day) treatment; (2) day 4: switch from *iv.* to oral methylprednisolon (16 mg); (3) day 10: the exacerbation symptoms were relieved and the patient was released from the hospital. Informed consent was obtained for each patient and the study was approved by the Institutional Review Board of Semmelweis University.

Genomic DNA isolation

Total genomic DNA was extracted from white blood cells using the QIAamp DNA Blood Midi Kit of Qiagen.

Genotyping the *PON1* -108C/T polymorphism (rs705379) by TaqMan 5' nuclease allele specific PCR

The *PON1* -108C/T polymorphism (rs705379) was determined by TaqMan Pre-Designed SNP Genotyping Assay (assay ID: C_11708905_10) on ABI Prism 7000 instrument according to the manufacturer.

Genotyping the *PON1* Q192R polymorphism (rs662) by single base primer extension method on Beckman SNPstream Genotyping System

The Q192R polymorphism (rs662) of the *PON1* gene was measured by single base primer extension method on Beckman SNPstream Genotyping System with primers: PCRU: ATG TTT TAA TTG CAG TTT GAA TGA; PCRL: TAG ACA ACA TAC GAC CAC GCT; SNPU: GGC TAT GAT TCG CAA TGC TTC ACT ATT TTC TTG ACC CCT ACT TAC.

Genotyping the *TNF-α* -308A promoter polymorphism by PCR-RFLP

Genotyping analysis of the *TNF-α* -308A promoter polymorphism was performed by PCR-RFLP.

Measurement of paraoxonase activity

Paraoxonase activity was determined using paraoxon (O,O-diethyl-O-p-nitrophenylphosphate) as substrate, and measuring the increase of the absorbance at 412 nm due to formation of 4-nitrophenol. Activity was measured by adding 50 μL serum to 1 mL Tris/HCl buffer (100 mmol/L, pH=8.0) containing 2 mmol/L $CaCl_2$ and 5.5 mmol/L paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm, 25°C, using a Hewlett-Packard 8453 UV-visible spectrophotometer. Enzyme activity was calculated from the molar extinction coefficient 17100 $M^{-1}cm^{-1}$. One unit of paraoxonase activity is defined as 1 nmol of 4-nitrophenol formed per minute under the assay conditions mentioned above.

Measurement of arylesterase activity

Arylesterase activity was measured spectrophotometrically. The assay contained 1 mM phenylacetate in 20 mM Tris-HCl pH=8.0. The reaction was started by the addition of the serum, and then the increase in absorbance at 270 nm was read. Blanks were included to correct for the spontaneous hydrolysis of phenylacetate. Enzyme activity was calculated using the molar extinction coefficient 1310 $M^{-1}cm^{-1}$. Arylesterase activity is expressed in U/mL; 1 U is defined as 1μmol phenylacetate hydrolyzed per minute.

Measurements of *Chlamydophila pneumoniae*-specific IgA and IgG antibodies

Chlamydophila pneumoniae-specific IgA and IgG were determined from serum used Sero CP-IgA, IgG protein according to the manufacturer. Serum was designated as positive if the cut-off index (COI) was >1.1.

Measurements of total and specific serum IgE levels

Total serum IgE levels and specific IgE levels to more than 100 allergens were determined by the Pharmacia CAP System. IgE levels were defined as high in

subjects whose total serum IgE exceeded 100 kU/l, and specific IgE was considered positive in those having detectable allergen-specific IgE (>0.35 kU/l).

Statistical analysis

Data were analyzed using MedCalc, Microsoft Excel or Statistica 7 programs. Depending on data type, unpaired t-test, one-way ANOVA and Tukey HSD post hoc test were applied to analyze the statistical significance of our results.

In our genotyping studies, allele frequencies were calculated by allele counting. Data were analysed using MedCalc 5.0, SPSS 11.0 and Arlequin 1.1 programs. Hardy-Weinberg equilibrium was tested by using a $\chi 2$ goodness-of-fit test. $\chi 2$ test was used to test for differences in allele distribution between the groups. Confidence intervals were calculated at the 95 percent level.

Results and discussion

In our functional genomic studies, we investigated the development of allergic airway inflammation in a mouse model system. We have demonstrated that the first airway inhalation of allergen led to a marked neutrophil and macrophage recruitment in the BAL of OVA-sensitized mice. This early neutrophil inflammation decreased during the progression and almost disappeared by the end of the protocol, when the asthma related Th2-type eosinophilic airway inflammation and hyper-reactivity developed.

We carried out gene expression microarray profile analysis in the lung of mice undergoing allergen-induced experimental asthma at different time points during the experimental protocol. The study has shown extensive changes in gene expression in the lungs in response to allergen at all time points. Since the classical microarray data analysis generated a very high number of significantly differentially regulated genes, we applied GO and Gene Set Enrichment Analyses to be able to gain a comprehensive insight into the biological processes and functions of the asthmatic responses. GO analysis clearly revealed highly significant overrepresentation of gene set families related to immune response, inflammatory response, cytokine and chemokine activity in all groups compared to the controls. We identified the overrepresentation of ontology sets

related to acute inflammatory response, serine-type endopeptidase inhibitor activity only in group 1 (representing the allergen-evoked early response), while sets corresponding to positive regulation of phagocytosis, humoral immune response, cell cycle and mitosis were strongly enriched in groups 2 and 3 (representing a developed OVA-induced experimental asthma response). Taking advantage of an unbiased approach offered by GSEA to rank gene expression to pathways, as opposed to individual genes, we showed that pathways involved in cytokine and IL-R1 signaling had the strongest correlation with group 1. In contrast, pathway sets related to cell cycle, T cell receptor signaling, T helper and T cytotoxic response correlated only with group 3. Besides the canonical pathway gene sets, the tested gene sets derived from other gene expression microarray studies using different lung disease models of mice, were found to be coherent with our microarray data. According to the observed marked neutrophil infiltration at the early time point in group 1, we found highly significant enrichment of the gene set representing the gene expression changes related to bacterial infection models characterized by neutrophilic inflammation and macrophage infiltration in the lung. This phenomenon has been completely replaced by the asthma related Th2-type airway inflammation, indicated by the significant overrepresentation of gene sets related to Th2 type inflammation models in group 3. These results validated our observation about the kinetics of the leukocyte influx into the airways after allergen challenge. Furthermore, GSEA also provided a rapid cross platform comparison of the microarray data.

In our microRNA microarray measurements, we observed the up-regulation of several microRNAs (miR-135b, miR-139-3p, miR-155, miR-21, miR-21*, miR-449a, miR-449c, miR-582-3p, miR-689, miR-711) in the lung of mice in group 3 compared to the placebo controls. Expression level of a miR-155, miR-135b and miR-21, the three most important of them, was successfully validated with real-time PCR.

We could confirm not only many of the previous observations of differential gene expressions made by others, but we also identified paraoxonase-1 as one of the genes that was strongly down-regulated in experimental asthma. Reduced *PON1* expression in the lungs of groups 2 and 3 mice was also confirmed at the protein level by immunohistochemical analysis. PON1 is an extensively studied antioxidant enzyme, which is able to metabolize pro-inflammatory lipids formed during the oxidation of low-density lipoprotein and is therefore potentially anti-atherogenic. There is increasing evidence that asthma is linked to oxidative stress. Cell derived reactive oxygen species (ROS) are enzymatically produced by inflammatory (macrophages, eosinophils, neutrophils) and epithelial cells within the lung as part of an inflammatory-immune response to pathogens or allergens. Generation of ROS close to cell membranes can lead to lipid peroxidation, which can destabilize membrane function, inactivate membrane-bound receptors and enzymes and increase tissue permeability, and these events cause tissue dysfunction and destruction.

According to these results it is reasonable to hypothesize that development of acute asthma coincides with reduced expression of *PON1* in humans, too. In clinical samples we demonstrated that serum PON1 activity increased parallel with improving asthma symptoms. The observation suggests, that there could be an inverse relationship between serum PON1 activity and symptoms of asthma in humans. The mechanism of the reduction of serum PON1 activity in these patients is not clearly understood. This reduction could be related to increased lipid peroxidation caused by ROS produced by inflammatory and epithelial cells, since oxidized lipids are reported to inhibit *PON1* activity.

It must be noted however, that the results of the animal experiments, in which the amount of the *PON1* was reduced on the mRNA level, suggested another hypothesis. According to these results, the allergen-induced processes reduced the PON1 activity not by inhibiting the enzyme, but by lowering the expression of the gene. Currently it is hard to give a coherent explanation of these

observations. Additional studies are necessary to elucidate the exact mechanisms how the activity and level of the *PON1* are regulated. The two most investigated *PON1* polymorphisms are the Q192R, which was found to influence the activity of the enzyme, and the -108 T/C promoter polymorphism associated with altered expression of the gene. Both SNPs have been found associated with several diseases like atherosclerosis, diabetes complications, and polycystic ovary syndrome. As oxidative stress plays an important role also in asthma and polymorphisms influencing the antioxidant capacity of *PON1* might change the susceptibility of the disease, we studied the distribution of these SNPs in asthmatic and control patients, but found no difference. This means that these polymorphisms do not play an important role in the susceptibility to the disease.

Our observations suggest the hypothesis that reduced PON1 activity may be involved in the pathogenesis of asthma. Treatments directly raising its level could offer new therapeutic tools in the management of asthma.

In our genotype-environment interaction studies, we investigated the role of the *TNFα* -308A promoter polymorphism in *Chlamydophila pneumoniae* infection, and its possible modifying effect on the susceptibility to asthma in children. There were no significant differences in the proportion of patients positive for any *Chlamydophila pneumoniae*-specific antibodies between asthmatic and control children. No significant differences were observed in the genotype and allele frequencies of the *TNFα* promoter polymorphism between the two groups. To evaluate the modifying effect of the polymorphism on the susceptibility to asthma in *Chlamydophila pneumoniae* infection, children were stratified according to their genotypes, and the asthmatic and control children were compared on the basis of their infection status. Among asthmatic children carrying the *TNFα* -308A allele, there were significantly more patients positive for *Chlamydophila pneumoniae*-specific IgG, than among control children carrying the same allele (20.1% vs. 9.2% of asthmatic vs. control children, respectively; P=0.002; Odds ratio

(95% confidence interval) (OR(95%CI))=4.08(1.75-9.52). The results remained significant after adjusting for age and sex (P=0.005; OR(95% CI)=3.52 (1.52-7.53). To further evaluate the role of these polymorphisms in the susceptibility to asthma among children infected with *Chlamydophila pneumoniae*, the prevalence of the different genotypes in subjects positive for *Chlamydophila pneumoniae*-specific antibodies was compared in asthmatics and controls. The distribution of the *TNFα* -308 genotypes differed significantly between asthmatic and control children who were positive for *Chlamydophila pneumoniae*-specific IgG. The proportion of children with the rare *TNFα* allele was higher in asthmatics than in healthy controls comparing it with children with the G/G genotype (45.3% and 54.7% of asthmatics vs. 19.0% and 81.0% of controls had G/A+A/A vs. G/G genotypes, respectively; adjusted OR (95% CI) = 3.31(1.62-6.88); P=0.005).

Conclusions

1. In our functional genomics studies, we demonstrated that even the first airway inhalation of allergen caused extensive changes in gene expression in the lung of mice undergoing the experimental protocol. We applied GO and Gene Set Enrichment Analyses in order to gain a comprehensive insight into the biological processes and functions of the asthmatic response. Using these methods, we were able to relate gene expression changes to cellular processes and to integrate our results into multiple levels of information available in public databases. Besides the canonical pathway gene sets, the tested gene sets derived from other gene expression microarray studies using different lung disease models of mice, were found to be coherent with our microarray data. Our study differs significantly from previous studies, as we have monitored the development of allergic airway inflammation in a mouse model of asthma by applying GSEA to identify differentially expressed gene pathways and gene sets from other lung disease models as opposed to individual genes. Importantly, to our knowledge we were the first who applied a

comprehensive GSEA to analyze gene expression profile in the kinetics of asthma.
2. We identified the high upregulation of miR-155, a microRNA that plays an important role in the development of immune response, in the lungs of allergic mice.

3. Among the top downregulated transcripts, we identified an antioxidant enzyme, paraoxonase-1 (*PON1*), which has not yet been studied in connection with asthma earlier.

4. The two most investigated *PON1* polymorphisms (Q192R and -108 T/C), which affect the expression and the activity of the coded enzyme, did not influence the susceptibility to the disease.

5. In human asthmatic patients we found that serum PON1 activity was reduced at asthma exacerbations, but increased in parallel with improving asthma symptoms.

Our observations suggest that an altered PON1 activity might be involved in the pathogenesis of asthma, and PON1 might be a potential new therapeutic target as well as a diagnostic tool for following up the effect of therapy.

6. In our gene-environment interaction studies, we showed that *Chlamydophila pneumoniae-specific* IgG positivity was associated with asthma, when children carrying the *TNFα* -308A allele were considered. Furthermore, children infected with *Chlamydophila pneumoniae* in the past (IgG positivity) carrying the *TNFα* -308A allele have considerably higher risk of developing asthma than children with similar infection status carrying normal genotypes.

Publications

Publications related to the subject of the thesis

1. Tolgyesi, G., M. Keszei, I. Ungvari, A. Nagy, A. Falus, and C. Szalai. Involvement of TNFalpha -308A promóter polymorphism in the development of asthma in children infected with Chlamydophila pneumoniae. Pediatr Res. 2006 Nov ; 543-8. IF= 2,619
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Other publications

1. Wiener, Z., B. Kohalmi, P. Pocza, J. Jeager, Tolgyesi G., S. Toth, E. Gorbe, Z. Papp, and A. Falus, TIM-3 is expressed in melanoma cells and is upregulated in TGF-beta stimulated mast cells. J Invest Dermatol, 2007. 127(4): p. 906-14. IF= 4,829

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