Role of genetic factors in the pathomechanism of allergic rhinitis.
Association of TNF-alpha promoter (-238,-308), TLR-4 (Arg299Gly, Thr399Ile) and PPAR-α, PPAR-γ (Pro12Ala) polymorphisms with clinical symptoms and cytokine levels (sTNF-alpha, sFas) in patients with allergic rhinitis.

Doctoral Thesis

Dr. Magdolna Krasznai

Semmelweis University
Doctoral School of Clinical Sciences

Supervisor:
Dr. Cseh Károly, professor, Dsc.

Official reviwers:
Dr. Tamási Lilla PhD
Dr. Major Tamás PhD

President of final exam comittee:
Dr. Gergely Péter professor DSc.

Members of final exam comitte:
Dr. Gálffy Gabriella PhD
Dr. Tamás László PhD
Dr. Katona Gábor PhD

Budapest
2011

The prevalence of allergic rhinitis (AR) is increasing worldwide. According to the geographic location, 17-29% of the population in Europe is suffering from the disease.

The objective of the study was to determine the frequency of TNF-alpha −238, −308 G/A promoter, TLR-4 299 D/G and 399 T/I, PPAR-gamma Pro12Ala, C161T exon6 and PPAR-alpha 2468 G/C intron7 polymorphisms in healthy population and in patients with seasonal allergic rhinitis, and to examine the influences of the allelic variants on serum TNF-alpha, TNF receptor-1, Fas, Fas-ligand, IgE levels and on clinical symptoms.

66 patients (35 female, 31 male; mean age ± SE: 35.91 ±1.40 years) with minimum four years of history of ragweed pollen allergic rhinitis were enrolled into the study after having their written informed consent approved by the local ethical committee. Clinical symptoms and laboratory parameters were registered during and after ragweed pollen season. Patients with other types of pollen allergy or with concomittant bronchial asthma were not enrolled. Ten clinical manifestations (rhinorrhea, nasal congestion, nasal itching, sneezing, conjunctival itching, lacrimation, redness of eyes, itching of throat, cough, dyspnea) of patients were evaluated and scored numerically (0: no clinical symptom, 1: mild symptom, 2: moderate symptom, 3: severe symptom). Average total score values were calculated by summarizing the score numbers of each clinical symptom for each patient divided with the number of patients. Peak nasal inspiratory

flow (PNIF) values of patients were measured during and after the pollen season.Venous blood samples were taken in fasted state before starting and after finishing nasal steroid therapy and samples were stored at -80 C until they were used for detection of the cytokines, serum sTNF-alpha, sTNF-R1, sFas, sFasL and IgE concentrations.

The distribution of genetic polymorphisms of the TNF-alpha promoter, TLR-4, PPAR-gamma and alpha in allergic patients was compared to 161 non-allergic, healthy referent subjects free from allergic family history with similar age range (87 female, 74 male; age±SE= 36.40±7.11 years).

TNF-alpha -238 (rs361525), -308 (rs1800629), TLR-4 299 (rs 4986790, var 012739) and 399 (rs 4986791, var 012740), PPAR-γ2 Pro12Ala (rs: 1801282), exon6 C161T (rs: 3856806) PPAR-α intron7 2468 G/C (rs: 4253778 polymorphisms were studied by PCR-RFLP technique.

Serum soluble (s) TNF-alpha (TNFSF2) and serum sTNF-R1 (TNFRSF1A) concentrations were measured by TNF-alpha EASIA and sTNF-R1 EASIA (Biosource Europe S.A. Nivelles, Belgium cat. No. KAC 1752 and KAC 1762, 2x96 determinations) according to the manufacturer's instructions.

Serum sFas (TNFRSF6) concentrations were determined by a human s APO-1/ELISA kit (Bender MedSystems GmbH, Vienna, Austria cat No. BMS 245 TENCE). Serum sFasL (TNFSF6) concentrations

were measured by an ELISA kit manufactured by Abnova GmbH (Heidelberg, Germany cat. No. KAO 0159).

Serum IgE values were detected by an ELISA kit (TM Microwell IgE EIA, Syncron Bio Inc, Karlsbad, California, USA).

PNIF values were measured by Youlten- flow meter (l/min, Clement Clarke International LTD, Sales Office, Edinburg Way, Harlow Essex CM 20 2 ED, England).

Statistical analysis (Mann-Whitney test, Spearman linear correlation, Fisher's exact test and multiple linear regression) was performed by using PRISM 4 (Graph Pad Software Inc. La Jolla, California, USA) and SPSS-15.0 for Windows (SPSS Inc., Chicago, California) programs. Graphical illustration of data was performed by using the PRISM 4 program.

Serum sTNF-alpha, sFas, sFasL and IgE concentrations of patients with ragweed allergic rhinitis were significantly higher (Mann-Whitney t-test, $p<0.01$) during the pollen season as compared to those of the after season values. PNIF values were significantly lower during the pollen season. sTNF-R1 concentrations did not differ significantly during and after the pollen season.

Serum TNF-alpha levels were in a significant (Spearman, $p<0.05$) negative linear correlation with PNIF values (in season(s): $r=-0.33$, after season (as): $r=-0.30$) and significant positive linear correlation with total clinical score values (s: $r=0.38$ as: $r=0.31$) and IgE levels

(s: r=0.34, as: r=0.26) in patients with seasonal allergic rhinitis both during and after pollen season.

Serum sTNF-R1 concentrations were found to be in a significant negative linear correlation with PNIF values (s: r=-0.29, as: r=-0.24) and in a significant positive correlation with IgE levels (s: r=0.32, as: not significant (ns)), sFas concentrations (s: r=0.30, as: ns) and total clinical score values (s: r=0.30, as: ns) in our patients during and after pollen season.

A significant negative linear correlation (Spearman, $p<0.05$) was observed between serum sFas concentrations (s: r=-0.27, as: r=-0.26) and PNIF values and a significant positive linear correlation with total clinical score values (s: r=0.33, as: ns) and serum IgE levels (s: r=0.28, as: r=0.27) during pollen season.

Serum sFasL levels negatively correlated with PNIF values (s: r=-0.24, as: ns) and positively with IgE levels (s: r=0.34, as: ns) and total clinical score values (s: r=0.30, as: r=0.24) during and after pollen season (not illustrated in graphical form).

Serum IgE levels were in a significant negative linear correlation (Spearmann, $p< 0.05$) with PNIF values (s: r=-0.30, as: r=-0.29) and in a positive correlation with total clinical score values (s: r=0.36, as: r=0.34) in our patients during and after ragweed pollen season (not illustrated).

In a multivariate regression analysis using SPSS 15.0 program we found serum sTNF-alpha (standardized coefficient beta= 0.372, t=

3.599, p=0.01) and IgE (standardized coefficient beta=0.289, t=2.680, p=0.009) concentrations to be significant predictors for the patients' PNIF values. Serum sTNF-alpha (standardized coefficient beta= 0.241, p=0.046) and sFasL (standardized coefficient beta=0.279, p=0.026) concentrations proved to be significant predictors for the patient IgE levels during pollen season.

The allelic frequency of the TNF-alpha promoter -238A allele was 5.3% (A: 7/132, 0.053, G: 0.947) in our patients with allergic rhinitis. The carrier ratio was 9.09% among patients (6/66 patients, 1 homozygous for the -238A allele). The ratio of carriers of the -238A allele in the referent subjects' group (n=161) was 2.48% (4/161). The carrier ratio of the -238A allele in the allergic rhinitis group was significantly higher (Fisher's exact test, p=0.01, OR: 4.45, CI 95%:1.28-15.48) compared to the referent subjects' group (4/322, all of them heterozygous, A: 0.012, G: 0.988).

In patients carrying the -238A allele, significantly higher serum TNF-alpha concentrations, IgE levels, average total clinical score- and lower PNIF values were detected as compared to patients with the -238G allelic variant. The -238 G/A polymorphism of the TNF-alpha promoter had the most significant influence on the patients' clinical symptoms and cytokine concentrations both during and after the pollen season as compared to the other allelic variants (-308 G/A and the TLR-4 299D/G, -399 T/I).

The allelic frequency of the -308A allele of the TNF-alpha promoter was found to be 9.09% (A: 12/132, 0.091, G: 0.909) in our allergic patients (11/66, 1 homozygous for the -308A allele), and 18.01% in the referent subjects (A: 58/322, 0.180, G: 0.820; 54/161 patients, 4 of them homozygous for the -308A allele). The difference in the allelic distribution was statistically significant, measured by using the Fisher's exact test (p=0.02, OR: 0.45, CI95%:0.23-0.88).

Significantly higher sTNF-alpha, sTNF-R1, sFasL, IgE concentrations, average total clinical score values and lower PNIF values were found in patients (n=54) carrying the -308G alleles during and after pollen season as compared to those (n=12) with the -308A allelic variant.

The allelic frequency of the TLR-4 Asp299Gly and Thr399Ile polymorphisms in our patient group with allergic rhinitis was 6.06 % (G: 8/132, 0.061, D: 0.939; in Hardy-Weinberg equilibrium). The mutational frequency was not different in the referent population: 5.59 % (G: 18/322, 0.056, D: 0.944, p=0.82, RR=1.062, OR=1.09, 95%, CI 0.46-2.57, Fisher's exact test). No homozygous mutation was found either in the patient's group, or in the referent population. The presence of the Asp299Gly and Thr399Ile polymorphisms were linked together and occurred in the same patients together.

Patients carrying the mutant 299G/399I alleles had significantly lower serum sTNF-alpha, sFas, sFasL, IgE levels, average total clinical score values and higher PNIF values as compared to the

carriers of the wild type alleles (299D/399T) during and after pollen season. All patients carrying the mutant -238A allele of the TNF-alpha promoter had the -308G allelic variant and the TLR-4 299/399 D/T alleles, too. After the correction of data for -238A carrier status, in season cytokine levels, IgE concentrations and average total clinical score values remained significantly higher and PNIF values lower ($p<0.05$, Mann Whitney test) in those of carrying -308G (n=48) and 299D/399T alleles (n=52), as compared to patients with the -308A and 299G/399I alleles. Lowest in season total clinical score values, cytokine and IgE levels and the highest PNIF values were found in those patients who carried the combination of the -238G, -308A and TLR-4 299G/399I alleles.

Allelic distribution of PPAR-gamma and alpha studied in patients with allergic rhinitis and referent subjects was not statistically different.

Patients carrying the mutant PPAR-gamma and alpha alleles (Ala12, exon6 T and intron7 C) had lower clinical score and higher PNIF values both in allergic season and after season. Moreover in these patients sTNF-alpha, sFasL and IgE levels were significantly lower.

In conclusion, members of the TNF system may play a role in the pathogenesis of allergic rhinitis. sTNF-alpha and sFasL concentrations correlate with the clinical symptom score and PNIF values of patients in allergic season.

Polymorphisms of the TNF-alpha promoter, TLR4, PPAR-gamma and alpha influence the clinical symptoms and cytokine levels of patients with allergic rhinitis. Carriers of the mutant alleles of TLR4, PPAR-gamma and alpha have lower clinical score values, sTNF-alpha, sFasL and IgE levels and higher PNIF values.

Polymorphisms of the TNF-alpha promoter, TLR4, PPAR-gamma and alpha may contribute to the heterogeneity of the clinical symptoms in ragweed allergic rhinitis.