

**Summary of PhD thesis**

**Polymorphism of HLA class II alleles and tumor necrosis factor alpha promoter alleles in Hungarian patients with systemic lupus erythematosus and with primary Sjögren's syndrome**

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2005.

**List of publications related to the Ph.D. thesis:**

**I. Kovács A, Endreffy E, Petri I, Varga É, Kovács L, Pokorny Gy.: Primer Sjögren syndromás betegek HLA II. osztályú allélpolimorfizmusa  
Transzfúzió, 1999. (32) 33-38.**

**II. AJG Swaak, HG van den Brink, RJT Smeenk, K Manger, JR Kalden, S Tosi, A Marchesoni, Z Domljan, B Rozman, D Logar, G Pokorny, L Kovács, A Kovács, PG Vlachoyiannopoulos, HM Moutsopoulos, H Chwalinska-Sadowska, B Dratwianka, E Kiss, N Cikes, A Branimir, M Schneider, R Fischer, S Bombardieri, M Mosca, W Graninger, JS Smolen: Systemic lupus erythematosus: clinical features in patients with a disease duration over 10 years, first evaluation  
Rheumatology, 1999. (38) 953-958. IF: 4.102**

**III. A Kovács, E Szekeres, E Endreffy, L Berek, E Makula, L Kovács, J Eller, G Pokorny, I Petri: Relationship between the occurrence of anti-SSA, anti-SSB autoantibodies and HLA class II alleles from the aspect of in vitro inhibitory effect of glucocorticosteroid on the of antibody-dependent cellular cytotoxicity in patients with primary Sjögren's syndrome  
Acta Microbiologica et Immunologica Hungarica, 2000. (47) 421-431.**

**IV. E Endreffy, A Kovács, L Kovács, G Pokorny: HLA class II allele polymorphism in Hungarian patients with systemic lupus erythematosus  
Annals of the Rheumatic Diseases, 2003. (62) 1017-1018. IF: 3.916**

**V. A Kovács, E Endreffy, I Petri, L Kovács, G Pokorny.: HLA class II allele polymorphism in Hungarian patients with primary Sjögren's syndrome  
Scand J of Rheumatology, 2006. (35) 75-76. IF: 1.685**

## **1. Introduction, background and aims**

The pathogenesis of the autoimmune diseases is a diverse, ramifying complex, in which environmental, genetic and ethnic factors may have an important role. Concerning the relationship between the development of the so-called prototype of these disorders, systemic lupus erythematosus (SLE) and genetic background of the disease, the studies on different ethnic populations revealed different associations. Genetic predisposition is associated with different clinical subsets of disease and autoantibody production by the same genes in different populations, while many of the susceptibility haplotypes differ for most ethnic populations. Beside the human leukocyte antigen (HLA) system, other susceptibility candidate genes may include tumor necrosis factor alpha, mannose binding protein, interleukin (IL)-10, angiotensin converting enzyme and deficiencies of components of the complement system (**Correa, 2003**). The main purpose of our studies was to collect new data about the different genetic factors which contribute to SLE and primary Sjögren's syndrome (SS) in Hungarian patients, searching for the associations of the pheno- and genotypes.

### **1.1. HLA DRB1, DQA1, DQB1 allele polymorphism in Hungarian patients with SLE**

Systemic lupus erythematosus (SLE) is a complex autoimmune disease which is strongly influenced by genetic factors, e.g. HLA class II alleles. The disease is characterized by diverse clinical manifestations and the presence of specific autoantibodies. The pathogenesis of SLE is multifactorial but there is considerable evidence that the development of this autoimmune disorder has a strong genetic basis. Association studies in humans suggest the existence of genetic effects by the alleles encoded in the HLA, deficiencies in the complement genes and the low-affinity variants of Fcγ-receptors. In mouse models of SLE at least 13 loci have been identified, including the MHC, linked to lupus-related phenotypes such as nephritis and production of autoantibodies. Recently, linkage studies have been performed in human SLE. (**Lindquist et al. 1999.**) Complete deficiency of the early components C1q, C2 and C4 of classical pathway of complement are associated with a most striking risk to developing SLE (**Tokunaga et al. 1985.**). The apoptosis genes FAS and FASL are candidate contributory genes in human SLE, as mutations in these genes result in autoimmunity in several murine models of this disease. **Wu et al. (1996)** studied DNA from

75 patients with SLE using SSCP analysis for potential mutations of the extracellular domain of FASL. **Barreto et al. (2004)** examined the association between a 49A-G SNP in exon 1 of the CTLA4 gene and SLE. The authors found that individuals with the GG genotype were at significantly higher risk of developing SLE; carriers of the A allele had a significantly lower risk of developing the disease, and the AA genotype acted as a protective genotype for SLE. **Su et al. (2004)** identified 10 SNPs in the first FCGR2B promoter in 66 SLE patients and 66 controls. They determined that the proximal promoter contains 2 functionally distinct haplotypes, and showed that the less frequent haplotype was associated with increased gene expression. A case-control study of 243 SLE patients and 366 matched controls demonstrated that the less frequent haplotype was significantly associated with the SLE phenotype and was not in linkage disequilibrium with FCGR2A and FCGR3A polymorphisms. They concluded that an expression variant of FCGR2B is a risk factor for SLE.

The first report of an association between SLE and a HLA genotype (B8) was reported in 1971 (**Grumet et al.**). Later studies have shown that the association is stronger with HLA class II DR2 and DR3 (DRB1\*0301) alleles, the latter actually being in linkage disequilibrium with B8 (**Schur et al. 1982**). Several studies suggest that the contribution of HLA class II genes in SLE is predominantly at the level of production of specific autoantibodies rather than with SLE itself. **Doherty's et al. (1992)** data suggest that the susceptibility lies at/or telomeric to the DR locus, and that DRw15 and C4 deletions may act synergistically in conferring disease susceptibility. In lupus patients in Chinese HLA-DR2 and its subtype DRw15 were seen more frequently. No association was found with DR3. None of the DQB1 nucleotide or amino acid sequence variants were associated with any of the clinical subsets of SLE. In Japanese and Chinese populations (**Ogahara et al., 1996, Hong et al., 1994**) the DR2 genotype is associated with a relative high incidence of nephritis (**Colombo, 1996**) Studies on different ethnic populations showed that there are substantial differences in HLA class II allele frequencies between different races. While studies have revealed associations of SLE with HLA-DR3 and/or DR2 in Caucasians, those on Koreans, Malaysians and Chinese (**Doherty, 1992, Hong, 1994, Lu, 1997**) in black South Africans (**Rudwaleit et al., 1995**) only the frequency of DR2 was found to be increased.

The clinical features were studied in patients with SLE with disease duration at least 10 yrs in a European multicentre study. Our working group participated in it, a lot of our SLE patients were enrolled in this study. Outcome parameters were given according to the SLEDAI, the European Consensus Lupus Activity Measure (ECLAM), and further the Systemic Lupus

International Collaborative Clinics/ American College of Rheumatology Damage Index (SLICC/ ACR), a global damage index (DI) and required treatment. Conclusions were that after 10 yr, a high proportion of patients in our cohort continued to show evidence of active disease, defined by the SLEDAI as well as ECLAM. The DI was related to the involvement of the central nervous system, renal involvement and the presence of hypertension. Seventy-two per cent of the patients were on maintained GS therapy, because of the presence of some disease activity. The overall accumulated end-organ damage in our patient group was comparable with previous studies (**Swaak, G Pokorny, A Kovács et. al. 1999. and Swaak, G Pokorny, A Kovács et. al. 2001**).

The purpose of our present study was to analyse the MHC/HLA class II DRB1, DQA1 and DQB1 allele polymorphisms in Hungarian patients with SLE, and to evaluate the relationship between the genetic and clinical features of the disease. We paid a special attention to the connection between HLA polymorphism and organ manifestations, mainly renal manifestation. Most of our patients were recruited from the above mentioned international study.

## **1.2. HLA DRB1, DQA1 and DQB1 alleles in Hungarian patients with primary Sjögren's syndrome**

Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by lymphocytic inflammatory infiltrations of lacrimal and salivary glands resulting in dry eye and mouth. SS can exist as a primary condition (primary SS) or in association with other autoimmune diseases such as RA or SLE (secondary SS) (**Moutsopoulos et al., 1979**). A wide variety of extraglandular manifestations may occur in primary Sjögren's syndrome, including skin (vasculitis, hyperglobulinaemic purpura), lung (lymphocytic interstitial pneumonitis), renal (interstitial nephritis), central nervous system (vasculitis and demyelinating syndromes), heart (pericarditis) and hematopoietic abnormalities. SS patients produce a variety of autoantibodies including rheumatoid factor (RF) and antinuclear antibodies (ANA). In particular, SS patients produce autoantibodies against ribonuclear proteins SS-A [Ro] and SS-B [La], that are involved in the transport and post-transcriptional modification of mRNA. Investigating the immunoserological markers in SS, rheumatoid factor was found in 43/65 cases, while hypergammaglobulinaemia was detected in 34/65, ANA positivity in 28/65 and anti-SSA/SSB antibody positivity appeared in 31/65 cases. HLA B8 antigen occurred in

50%, DR3 in 20/36, DR5 in 11/36 and DR2 positivity in 10/36 SS patients (**Pokorny, 1991**). Genetic factors including HLA-DR and HLA-DQ predispose to SS syndrome. In secondary SS associated with rheumatoid arthritis (RA), the genetic predisposition is HLA-DR4 and antibodies against SS-B are rarely present.

The precipitating cause of SS remains unknown but exogenous agents such as Epstein-Barr virus have been proposed as a co-factor perpetuating the immune response against the salivary gland epithelial cells. In contrast to normal salivary glands, the SS gland contains increased proportion of glandular epithelial cells expressed in high levels of HLA-DR antigens. B-cells within the salivary gland produce autoantibodies including rheumatoid factor. B-cells undergo small clonal expansions that can be detected on Southern blot of immunoglobulin gene rearrangement and SS patients have a markedly increased risk of developing non-Hodgkin's lymphoma.

SS suggests a cell mediated destruction rather than an immune complex deposition mechanism. To characterize the cell-cell interactions in SS, immunohistologic methods have been used to identify lymphocyte subsets in salivary gland biopsies (**Adamson et al., 1983**). The majority of lymphocytes are mature T cells (CD3+) of the T helper (CD4+) subset. These T cells express the antibody cell surface receptor, although a small proportion of T-cells can be detected. Salivary gland epithelial cells in biopsies from SS patients react with anti-HLA DR antibodies, in contrast to no salivary gland biopsies that lack anti-HLA DR reactivity (**Lindahl et al., 1985**). The induction of HLA-DR on the epithelial cells may play an important role in pathogenesis, since CD4+ T cells (the major lymphocyte subset in the SS salivary gland) only interact with peptide antigen presented by HLA-DR molecules. As an important factor of pathogenesis, **Kovács L et al. (2000)** proved an impaired microvascular response to cholinergic stimuli in patients with primary Sjögren's syndrome. Expression of HLA-DR antigen and intracellular adhesion molecule-1 (ICAM-1) in human conjunctival epithelium is upregulated in patients with dry eyes associated with SS. **Pisella et al. (2000)** reported that a significant increase of HLA-DR and ICAM-1 expression by epithelial cells was consistently found in patients with keratoconjunctivitis sicca compared with normal eyes.

Even though the clinical and serological features of the primary Sjögren's syndrome are well known, the main triggering cause of the disorder still remains unknown (**Rader et al., 1989; Rischmüller et al., 1996; Tan, 1989**). Initial studies demonstrated the different genetic predispositions between primary SS patients and secondary SS patients associated with RA

(HLA-DR4), referring to the influence of additional HLA encoded genes such as HLA-DQ (**Ben-Chetrit et al., 1988**). In particular, Caucasian patients with the extended haplotype HLA-DR3, DR52a, DQA4 and DQB2 show an increased frequency of anti-SSA/anti-SSB autoantibodies. According to the recently adopted nomenclature for HLA, this extended haplotype is named HLA DRB1 \*0301, -DRB3\*0101, -DQA1\*0501, -DQB1\*0201. Increased risk for SS may be associated with HLA-DR5 in Negroids, with HLA-DR4 in Japanese and with HLA-DR8 in Chinese. Recent advances have clarified the self-MHC molecule - T-cell interactions in antigen presentation, thus certain polymorphisms of HLA susceptibility alleles might be a precipitating factor in autoimmune processes. It has been postulated that specific conformations of HLA-D associated molecules and of peptides derived from autoantigens may cause CD4<sup>+</sup> T cells to generate an autoimmune response against the salivary gland epithelial cells. **Rischmüller et al. (1996)** found that certain HLA class II phenotype control the autoantibody response in primary SS. The alleles of susceptibility to SS in the Hungarian population have not been clarified yet.

The aim of this study was to determine the HLA II class (DRB1, DQA1 and DQB1) allele polymorphism and to analyse the pheno- and genotype correlations in Hungarian patients with primary Sjögren's syndrome (SS).

### **1.3. Tumor Necrosis Factor alpha allele polymorphism in systemic lupus erythematosus and in primary Sjögren's syndrome**

As a result of the examinations of the past few decades, it is well known that tumor necrosis factor (TNF) alpha is a central mediator of the inflammatory response, which is regulated at the transcriptional and posttranscriptional levels (**Sariban et al., 1988**). The TNF alpha gene is localized at the class III region of the major histocompatibility complex (MHC). A very strong association has been confirmed between the uncommon TNF allele and HLA A1, B8 and DR3 alleles (**Wilson et al., 1993**). This association between TNF alpha and HLA alleles increases the possibility that certain TNF alpha alleles may contribute to autoimmune diseases in association of the above mentioned HLA haplotype. **Borodin et al. (2002)** published that elevated TNF alpha concentrations were found in 49% of the SLE patients. Concentration of TNF alpha, SLE activity and development of antiphospholipid syndrome were correlated. The findings indicate that TNF alpha is involved in the pathogenesis of SLE. Quantitation of TNF alpha may serve as useful tool for monitoring SLE activity. Our goal

was to investigate the association between the TNF alpha genotypes and HLA class II DRB1-DQA1-DQB1 allele frequencies in patients with SLE and with SS and to study the correlations of the pheno- and genotypes.

#### **1.4. Relationship between HLA class II alleles and in vitro steroid inhibition of antibody - dependent cellular cytotoxicity in patients with primary SS**

Though at present there is no evidence-based algorithm for the treatment of primary Sjögren's syndrome, it is generally accepted that corticosteroids must be used in cases with severe systemic manifestations. As the side-effects of the corticosteroids are well-known, it would be useful to know in advance how the patients will respond to this type of treatment. An important feature is the expression of HLA-DR antigens on the glandular epithelial cells showing their activation in SS. B-cells are also activated in exocrine glands. In cases with severe, sometimes life-threatening organ manifestations (kidney, lung, vascular, etc.), corticosteroids are generally used. Taking into account the well-known short and long-term side-effects of GS therapy, it would be essential to know in advance, whether a patient will or will not respond to steroids. For this reason, we measured antibody-dependent cellular cytotoxicity (ADCC) reaction and in-vitro steroid inhibition of this reaction in patients with SS by methods described by **Petri et al. (1985.)**

We wanted to evaluate whether these in-vitro examinations may help to determine the in-vivo steroid sensitivity of SS patients if corticosteroid therapy is required. Another question was if HLA class II genotype, autoantibody positivity and clinical manifestations had any influence on steroid sensitivity or not.

## **2. Patients and methods**

### **2.1. HLA DRB1, DQA1, DQB1 allele polymorphism in Hungarian SLE patients**

In the period between 1998 and 2000, fifty patients (48 female, 2 male) with SLE were investigated for HLA class II polymorphism. All patients met at least 4 of the ARA revised criteria for the classification of SLE (**Tan et al., 1982**). Their mean age was 38 years (range: 18-76 years) at the onset of the disease and 41 years (range: 21-76 years) at the time of the examinations. As controls, 50 healthy blood donors (47 female, 3 male) of the similar age

(mean age 35 years, range: 18-52 years) were also genotyped. Subjects in both groups were unrelated.

Genomic DNA was extracted by a standard phenol/chloroform proteinase K method (**Davies, 1986**). Genotyping of HLA-DRB1 alleles was carried out with the Dynal RELI SSO HLA-DRB, a direct DNA probe test, which, after the nucleic acid amplification, uses a nucleic acid hybridization method for the differentiation of 70 HLA-DRB 1 alleles, and 9 supertypes (Dynal, Oslo, Norway). We used the terminology DR1-10 as no subtypes in the DR region were taken into account. DR2 subtyping was carried out by method of **Ota et al (1991)**. DQA1 determination was performed with PCR-restriction fragment length polymorphism (RFLP) method of **Ota et al. (1991)** to differentiate 8 alleles. The DQB1 typing was carried out using INNO-LIPA DQB PCR-reverse hybridization kit (Innogenetics, Gent, Belgium), for discrimination of 30 alleles.

Immunoserological examinations were carried out on all patients: antinuclear antibodies (ANA; indirect immunofluorescence on rat liver substrate), lupus erythematosus (LE) cell investigation (rotatory method utilizing heparin and glass beads), anti-double-stranded DNA (anti-dsDNA), anti-SSA, anti-SSB, anti-Sm and anti-RNP antibodies (enzyme-linked immunosorbent assay; ImmunoDOT, Epignost, Leonding Linz, Austria), IgG anticardiolipin antibodies (Cardiolipina, Biochem Immunotest, Italy) and concentrations of complement C3 (rocket immuno-electrophoresis) and serum immunoglobulins (Mancini technique) in all patients.

The differences of allele frequencies between SLE patients and controls were analysed by chi-square and Fisher's exact tests, where appropriate. We also evaluated the differences in allele frequencies in SLE patient subgroups with and without organ manifestations, compared to each other and controls. Odds ratio (OR) values were calculated, too.

As a spreading of our examinations, 33 SLE patients with lupus nephritis and 34 SLE patients without LN were studied for DRB1, DQA1 and DQB1 allele polymorphism by genotyping, PCR- RFLP or slot-blot hybridization methods. The data were compared to 50 healthy controls. The morphological classification of lupus nephritis was given according to the WHO classification system (**Weening et. al. 2004**).

## **2.2. HLA DRB1, DQA1 and DQB1 allele distribution in Hungarian patients with primary Sjögren's syndrome**

HLA II class (DRB1, DQA1 and DQB1) allele polymorphisms were investigated in 48 Hungarian patients (47 females and one male) with primary SS (age: 33-83 y, onset between 21-64 y, duration: 1-29 y). They all fulfilled the European-American classification criteria for SS syndrome. Identification of the HLA class II alleles, immunoserological examinations and statistical analysis were performed as it was described in Chapter 2.1.

Associations were examined between clinical manifestations and immunoserological data: ANA, anti-Ro/SSA, anti-La/SSB autoantibody positivity and MHC II allele polymorphism in the patient population. The amino acids were studied at position 34 of the outermost domain of DQA1 chains and at position 26 of the outermost domain of DQB1 chains.

### **2.3. TNF alpha polymorphism in systemic lupus erythematosus and in primary Sjögren's syndrome**

Fifty patients with systemic lupus erythematosus (SLE) were studied for HLA class II (DRB1, DQA1, DQB1) polymorphism and 49 of them for TNF alpha-308 and 53 SLE patients for the TNF alpha-238 polymorphism were investigated. As another study, 50 Hungarian patients with SS for the TNF alpha-308 (n=50) and 52 for the TNF alpha-238 (n=52) promoter polymorphisms and 50 SS patients for MHC class II polymorphisms were investigated. The genotype and allele frequencies of the TNF-alpha gene in Hungarian healthy blood donors as controls (n=248) were determined by **Szalai et al. (2002)**. Subjects in both groups were unrelated.

The TNF-alpha-238 G/A and - 308 G/A polymorphisms were determined by DNA amplification by PCR using the primers suggested by **Day et al. (1998)**. The PCR products were digested at 37°C with MspI to detect the -238 G/A polymorphism and NcoI to detect the -308 G/A polymorphism. The products were separated on a 4 % agarose gel and stained with ethidium bromide. Immunoserological examinations were carried out on all patients, as described previously. Allele frequencies for -308 and -238 of TNF alpha were calculated by allele counting and given with an estimate of standard errors (SE). Data were analysed using MedCalc and Arlequin software (**Schneider et al., 1997**). Allele frequencies, mutant alleles and different genotypes, as heterozygote (AG) and homozygote genotypes (AA) were counted. The differences of allele frequencies between SLE patients and controls were analysed where appropriate by chi-square ( $\chi^2$ ) and Fisher's exact t-test. Differences in allele

frequencies in SLE patient subgroups with and without certain organ manifestations were also evaluated. Odds ratio (OR) values were calculated as well.

#### **2.4. Relationship between HLA class II alleles and in vitro steroid inhibition of antibody - dependent cellular cytotoxicity in patients with primary Sjögren's syndrome**

ADCC activity and steroid inhibition of the reaction were determined in 29 patients with Sjögren's syndrome and in 28 healthy blood donors as controls. All SS patients met the European Diagnostic Criteria (1993). The mean age of patients was 55 years (range: 32-74 years) and the mean duration of the symptoms was 10.3 years (range: 1-20 years). In the ADCC reaction fresh, human "0" Rh (D) positive red blood cells were used as target cells. Human anti-D serum was adsorbed onto the cells labelled with  $^{51}\text{Cr}/\text{Na}_2\ ^{51}\text{CrO}_4$ ; 7-8 Gbq/mg Cr, Amersham. The effector lymphocytes were isolated on Ficoll Uromiro gradient after treatment of the whole blood with colloidal iron powder (GAF, USA). The effector/target cell ratio was adjusted to 10:1. Methylprednisolone was added to the culture medium for a final concentration of 10  $\mu\text{g}/\text{ml}$ . The spontaneous activity was given by the count rates for cultures without anti-D antibody. The total activity was calculated as the radioactivity of labelled red blood cells lysed in distilled water. The cells were incubated at 37 °C in a 5%  $\text{CO}_2$  thermostat for 18 hours.

The cytotoxicity and steroid inhibition were calculated by the following formulas:

$$\text{cytotoxicity \%} = \frac{\text{test supernatant cpm} - \text{spontaneous cpm}}{\text{incorporated total activity cpm}} \times 100$$

$$\text{steroid inhibition \%} = \frac{\text{ADCC with steroid \%}}{\text{ADCC without steroid \%}} \times 100$$

Grade of sensitivity to GS was given as a percentage of the inhibition of the ADCC reaction due to the steroid. Glucocorticosteroid sensitivity was defined when inhibition of basic ADCC was >30 %.

Furthermore, HLA class II polymorphism was evaluated in 28 of 29 SS patients by molecular genetic methods as it was described before. We searched for correlations between GS sensitivity and HLA polymorphism and autoantibody positivity. Immunological examinations and statistical analysis were performed by the same methods as it was described previously.

### 3. Results

#### 3.1. HLA DRB1, DQA1, DQB1 allele polymorphism in Hungarian SLE patients

In our 50 patients with SLE, the most common clinical manifestation was the articular involvement, which occurred in nearly all patients (92 %). In order of frequency, it was followed by anemia (72 %), leukopenia (54 %), serositis (54 %) and different types of skin involvement (46 %).

According to our results, DR2 (1501 subtype), DR3 and DR7 alleles occurred significantly more frequently in lupus patients than in controls. The presence of these alleles means an increased risk for the development of disease (Odds Ratio: 4.4, 2.25 and 3.2, respectively). In contrast, DR4 allele was significantly less common in SLE patients (Odds Ratio: 0.16). DR5 allele was analyzed further for subtypes. Interestingly, 4 of the 16 DR5 alleles of 15 patients (1 homozygote) proved to be 1126 subtype, while there was none of this subtype in the 25 DR5 alleles of 21 controls (4 homozygotes) ( $p < 0.04$ ). As concerns the DQA1 alleles, 0102 and 05011 were significantly more common in SLE patients. Both alleles represent more than 2-fold increased risk for the disease (2.23 and 2.53, respectively). Among the DQB1 alleles, 0201 and 0602 were detected significantly more frequently in SLE patients than in controls (Odds Ratio: 2.87 and 6.05, respectively). In SLE patients, the DR2 (1501 subtype)-DQA1\*0102-DQB1\*0602 haplotype, the DR3-DQA1\*05011-DQB1\*0201 haplotype and the DR7 allele occurred significantly more frequently (12vs3,  $p = 0.045$ , 18vs9,  $p = 0.02$  and 15vs5,  $p = 0.045$ , respectively), than in the controls. In contrast, the DR4-DQA1\*0301-DQB1\*0302 and the DR5-DQA1\*05012-DQB1\*0301 haplotypes were detected significantly more frequently in controls than in patients with SLE (14vs2,  $p < 0.01$  and 22vs11,  $p < 0.04$ , respectively).

Evaluating the connections between the genetic and clinical characteristics of the disease we found that DR2 positivity was less frequent in patients with lupus nephritis (LN), than in

patients without lupus nephritis (3/16, 18% vs 16/34, 47%,  $p=0.05$ ). In contrast, DR3- and DR7-associated haplotypes were more common in LN than in patients without LN (8/16, 50% vs 11/34, 32%, and 7/16, 43% vs 8/34, 24%,  $p<0.03$  and  $p<0.01$ , respectively). In addition, it was interesting that in patients with central nervous system (CNS) affection the DR3-positivity occurred significantly less frequently than in patients without CNS manifestation (0/6, 0% vs 9/44, 20%,  $p=0.046$ ). In patients with pleuritis and/or pericarditis, only the DR7 positivity was significantly more frequent than in patients without serositis (12/27, 44% vs 3/23, 13%,  $p=0.016$ ). Similarly, the DR7-associated haplotype was detected significantly more frequently in patients with one or more severe (renal, cardiorespiratory, CNS) manifestations as compared to patients without these major features of the disease (16/36, 44% vs 0/14, 0%,  $p=0.02$ ). DQB1 molecules with leucine (L) in position 26 was significantly more frequent in SLE patients than in healthy Hungarian blood donors (64/100 vs 42/100,  $p<0.01$ ). DQA1 molecules with glutamine (Q) in position 34 did not differ from the controls (80/100 vs 63/100, respectively). All patients with anti-SSA antibody positivity had glutamine at position 34 of the DQA1 chain, and/or leucine at position 26 of the DQB1 chain.

#### **HLA class II allele examinations in SLE patients with and without lupus nephritis (LN)**

As an extension of this SLE study, 33 patients with LN and 34 SLE patients without renal manifestation were examined HLA class II allele polymorphism and compared to each other and to the controls. 16 out of 33 LN patients belonged to the type IV and 17 to other histological types of LN groups. Kidney biopsy was performed in 29 LN cases. The most frequent alleles were: DRB1\*0301 (51.5 % vs. 20%,  $p<0.01$  Bonferroni's  $pc$  ns., OR=3.4), the DRB1\*1501/2 (39,4% vs. 22%,  $p<0,05$  \* $pc$  ns.), DQA1\*05011 (51.5% vs. 18%,  $p<0.01$  \* $pc$  ns., OR=3.8), DQB1\*0201 (51.5% vs. 16%,  $p<0.01$  \* $pc$  ns., OR=4.3) and DQB1\*0601/2 alleles (18 % vs. 4%,  $p<0.05$  \* $pc$  ns., OR=4.3), as susceptibility alleles to LN. The frequency of the DRB1\*08, \*09 and \*10 DQA1\*0401, \*0301 and DQB1\*0402 and \*0303 alleles decreased significantly ( $p<0,05$ ) in the 33 LN patients comparing to the SLE patients without renal affection.

The frequency of DRB1, DQA1 and DQB1 alleles did not differ significantly in the WHO type IV. and non-IV. type patients, except that of DQB1\*0601 allele (0 % vs. 17.6 %  $p<0.05$ ) decreased significantly in the WHO type IV. cases.

### 3.2. HLA DRB1, DQA1 and DQB1 alleles in Hungarian patients with primary SS

In Hungarian SS patients the haplotypes of susceptibility have been found to be:

HLA DRB1\*03 (03011) - DQA1\*05011 - DQB1\*0201 (26/48, 54% vs 9/50, 18%,  $p < 0.05$ ), and HLA DRB1\*15/16 (DR2)02(1601) - DQA1\*0102 - DQB1\*0602 (14/48, 29% vs 3/50, 6%,  $p < 0.05$ ), while these alleles were detected significantly more frequently in SS patients.

In Hungarian SS patients the haplotype of defense seems to be:

HLA DRB1\*11/12(DR5) - DQA1\*05012 - DQB1\*0301 (11/48, 23% vs 22/50, 44%,  $p < 0.05$ ), occurring significantly less frequently as compared to the controls.

Nine SS cases exhibited purpura as a vascular manifestation and the DRB1\*03 (10/11 cases;  $p < 0.02$ ) - DQA1\*05011 (10/11 cases;  $p < 0.02$ ) - DQB1\*0201 (9/11 cases;  $p < 0.05$ ) haplotype was significantly more frequently detected than in patients without purpura.

The most frequent sensitive haplotype in SS patients with renal tubular acidosis (RTA) was the HLA DRB1\*03(03011)/DQA1\*05011/DQB1\*0201, while defensive alleles were HLA DRB1\*07/DRB1\*08,09,04/ DQA1\*0201/DQB1\*0402,0203 and 0303. In SS patients with overt renal tubular acidosis, the DRB1\*03(03011)-associated haplotype was significantly more frequent as compared to the SS cases without kidney affection (9/11, 82% vs 18/37, 49%,  $p < 0.02$ ). This association was stronger expressed in 4 of the 11 SS patients with RTA in whom renal biopsies were performed and tubulointerstitial nephritis was revealed (4/4, 100% vs 23/44, 52%,  $p < 0.01$ ). In the SS patients with overt RTA, the DRB1\*15/16(DR2)02 positivity occurred significantly less frequently than in the SS cases without renal manifestation (0/22, 0% vs 11/74, 15%,  $p < 0.05$ ). Thirty-three of our 48 SS patients were seropositive for anti-SSA autoantibody, and 17 of them were also anti-SSB positive. Altogether 20 patients were seropositive for anti-SSB. In the seronegative cases the DQB1\*0201 and/or DQB1\*0301 alleles increased significantly.

In concern of the immunoserological characteristics of the SS patients, we found that the DRB1\*03011-linked haplotype was significantly more frequent in anti-SSB positive than in anti-SSB negative cases (10/11, 91% vs 19/37, 51%,  $p < 0.02$ ). The HLA DRB1\*03(03011) ( $p < 0.05$ ) - DQA1\*05011 ( $p < 0.01$ ) - DRB1\*0201 ( $p < 0.005$ ) and HLA DRB1\*15/16(DR2)(1601) ( $p < 0.05$ ) - DQA1\*0102 ( $p < 0.005$ ) - DQB1\*0602 ( $p < 0.05$ ) haplotypes occurred significantly more frequently in anti-SSA (Ro) seropositive patients, as compared to the seronegative group. In the anti-SSA positive SS patients the frequency of

HLA DRB1\*0101 allele decreased significantly as compared to those of anti-SSA negative patients, while in the anti-SSB positive SS cases the frequency of DRB1\*03(03011) allele increased significantly, and the frequency of DRB1\*15/16(DR2)(1601) subtype and DRB1\*01 alleles decreased significantly as compared to the anti-SSB seronegative group.

Four SS patients developed non-Hodgkin lymphoma and one more patient Hodgkin-lymphoma during the course of the disease. Three out of 4 SS patients with non-Hodgkin lymphoma (NHL) had HLA DRB1\*03(0101) – DQA1\*05011 – DQB1\*0201 haplotype, two of them in homozygous form. We observed that all of the patients with lymphoma carried the DRB1\*03 allele (6/10 vs 24/86 alleles,  $p < 0.05$ , Yates's correction: ns). In patients with SS, the glutamine proved to be the most frequent amino acid at position 34 of the DQA1 chain (80 %). At the position 26 of the DQB1 chain the leucine was the most frequent amino acid in anti-SSA positive patients (54.2 %), the frequency of glutamine did not differ significantly from those of the anti-SSA negative SS patients.

### 3.3.1. TNF alpha polymorphism in systemic lupus erythematosus

The allele frequency ( $p = m/2n$ ,  $m =$  mutations) of TNF-alpha-308A increased significantly in the SLE patients' group comparing to the data of controls. The frequency of TNF-alpha-308A alleles proved to be 0.286, the number of mutant alleles was 28. Normal (wild) TNF-alpha genotype (GG) occurred in 29 patients (59.2 %), heterozygote mutant genotype (AG) was found in 14 (28.6%), while homozygote mutant (AA) in 6 (12.2%) SLE patients. The frequency of TNF-238 allele was determined in 53 SLE patients, number of mutant (A) alleles was 3, so this allele frequency proved to be 0.028. Distribution of genotypes for TNF-238 was normal in 50 (96.23 %), heterozygote mutant (AG) in 3 patients (5.7%), while homozygote mutant (AA) variant did not occur. The occurrence of the TNF -238 mutant (A) allele did not differ from the control population (2.8 +/- 3.2% vs 4.9 +/- 1.7%, respectively).

The DRB1\*0301 allele linked haplotype – DRB1\*0301-DQA1\*05011 - DQB1\*0201 occurred in 93% - 83.3% - 72% in the heterozygous AG, homozygous AA and the wild (GG) genotypes for TNF-alpha-308, respectively. The DRB1\*0301 allele didn't occur in the AG heterozygous group for TNF-alpha-238 allele (0% vs 44%) against of the GG (wild) SLE group.

The distribution of the DRB1\*0301 haplotype in the different genotypes of TNF-alpha-308 A/G allele was the next: 5/6 in the AA homozygotes (80%), in the AG heterozygotes 13 of 14

(93%), while in the GG homozygous group that was 93% vs 76%. The DRB1\*03 linked haplotype occurred only in the GG genotype-bearing patients as compared to the heterozygous AG genotype for TNF-alpha-238 allele (44% vs 0%, respectively).

The DRB1\*15/16(DR2) allele occurred in 33.4% in the heterozygous -238AG group, whereas in 26% in the homozygous GG group, the most frequent allele was the DRB1\*07 in the 238AG genotype comparing to GG genotype (66.7% vs 34%, respectively).

The DQA1\*0201 allele was the most frequent in the AG heterozygous group (66.6% vs 28%) against of the GG genotypes, while the frequency of the HLA DQA1\*05011 allele was significantly decreased in the AG group (0% vs 84%,  $p < 0.02$ ) comparing to the GG (wild) group.

### **3.3.2. TNF alpha genotypes in primary Sjögren's syndrome**

The allele frequency for TNF-alpha-308A was significantly increased (0.310, SE  $\pm$  0.046 vs. 0.139 SE  $\pm$  0.031,  $p < 0.001$ ) in the 50 SS patients versus controls. The number of mutant (A) alleles was 31 in the TNF-alpha-308 locus. The frequency of normal (wild-type) genotypes (GG) was 44% (n=22); the TNF-alpha heterozygous genotype (AG) was found in 25 (50%), while mutant allele (AA) homozygosity was found in 3 (6.0%) SS patients ( $p < 0.001$ ).

The frequency of the TNF-alpha-238A allele was determined in 52 SS patients. The number of mutant alleles was 3, exhibiting an allele frequency of 0.029 (SE  $\pm$  0.016 vs. 0.046 SE  $\pm$  0.018, ns.). There were 49 (94.2%) wild-type genotypes (GG) whereas 3 heterozygous mutants (AG) were identified (5.8%). No homozygote for the mutant alleles (AA) was found.

The frequencies of HLA class II alleles were determined in the subgroups with different of TNF-alpha genotypes. The DRB1\*0301-DQA1\*05011-DQB1\*0201 haplotype was detected in 20 of 25 SS patients with the TNF-alpha -308 AG genotype and in 10 of 22 wild-type homozygous (GG) patients (80% vs. 45%,  $p < 0.05$ ). All of the 3 homozygous TNF-alpha -308AA SS patients had the DRB1\*03 haplotype.

The distribution of the HLA class II alleles differed significantly between the two TNF-alpha-308 genotypes. The DRB1\*0301-DQA1\*05011-DQB1\*0201 haplotype was demonstrated in 80% of the TNF-alpha-308 AG heterozygous SS group as compared to the 45% of the patients with TNF-alpha-308 GG (wild-type) genotype ( $p < 0.05$ ). The other frequent alleles in these two TNF-alpha genotype groups were the DRB1\*11/12 allele (32% vs. 10% in AG vs. GG subgroups, respectively, ns) and the DQA1\*05012 allele (36% vs.

20% in AG vs. GG, respectively, ns). The most frequent HLA class II haplotype, the DRB1\*0301-DQA1\*05011-DQB1\*0201 was increased in the TNF-alpha-238 AG heterozygous group only in comparison with homozygotes for the G allele (67% vs. 46%, respectively; ns.). Exclusively, the DQA1\*0301 allele was significantly increased (100% vs. 17%,  $p < 0.05$ ) in the TNF-alpha -238 AG heterozygous genotype group.

### **3.4. Relationship between HLA class II alleles and in-vitro steroid inhibition of antibody - dependent cellular cytotoxicity in patients with primary Sjögren's syndrome**

The results of ADCC reaction exhibited a tendency for an elevated ADCC reaction in SS patients ( $48.3 \pm 15.7\%$ ) comparing to those of controls ( $41.4 \pm 14.1\%$ ). In contrast, the in vitro steroid inhibition of the ADCC reaction was significantly lower ( $p < 0.01$ ) in SS patients ( $42.4 \pm 15.8\%$ ) than in controls ( $53.1 \pm 13.1\%$ ). Twenty-three of 29 patients (79.3%) proved to be steroid sensitive. This rate of sensitivity did not differ from those of the controls. We analyzed the results of steroid inhibition in SS patients on the basis of antibody profile and HLA status. The steroid sensitivity decreased significantly in patients with anti-SSA and/or anti-SSB antibody positivity as compared to the controls. The difference proved also to be significant between the results of anti-SSB negative SS patients and controls.

In HLA-DR2 and/or -DR3 positive patients the steroid sensitivity was significantly lower than in controls. Though the steroid sensitivity was lower in SS patients with DR2 or DR3 positivity than in DR2 or DR3 negative patients, the differences were not statistically significant. In contrast, in cases with DR2/3 heterozygosity the difference proved to be statistically significant not only in comparison to controls, but also to SS patients carrying neither DR2 nor DR3. Moreover we also evaluated the influence of combinations of both HLA haplotype (DR2, DR3), anti-SSA and SSB antibody positivity on in vitro steroid resistance. We observed a tendency that SS patients with at least one of these alleles plus one of these autoantibodies exhibited lower sensitivity to steroid than SS patients who possessed none or only one of the above mentioned immunological and genetic markers. However, the difference proved to be significant only between DR2 plus anti-SSA positive and DR2 plus anti-SSA negative subgroups ( $p < 0.05$ ).

## **4. Discussion**

#### 4.1. HLA alleles in Hungarian patients with SLE

Evidence for genetic influence in SLE is based on observations as a three-to ten-fold increase in the disease in monozygotic twins compared with dizygotic twins and that 10 to 20 percent of SLE patients have a first-or second-degree relative with lupus. **Reinharz et al. (1991)** observed the absence of DR2/DR3 and DR2/DR7 heterozygosity in their patients with SLE. In the present study, we searched for associations between the genetic and clinical manifestations in Hungarian lupus patients. We detected two typical Caucasian susceptible haplotypes to SLE. DR3-DQA1\*05011DQB1\*0201 and DRB1\*1501-DQA1\*01021-DQB1\*0602 haplotypes ( $p=0,022$  and  $0,045$ , respectively) could be detected significantly more frequently in Hungarian patients, and the DRB1\*07 allele was also associated with disease ( $p=0,045$ ). All of these alleles confers an increased risk for the development of SLE. Opposingly, the DR4- and DR5-positivity was significantly less frequent in patients than in controls, suggesting a possible resisting role against SLE. However, 1126 subtype of DR5 allele was detected significantly more commonly in SLE patients.

In an earlier Hungarian study (**Stenszky et al., 1986**) published a strong correlation between DR3 but not DR2 positivity and severe course of the disease. In agreement with their results, we could confirm the association between DR3 positivity and SLE in Hungarian patients, especially in more severe cases. In addition, we found a similar or even stronger correlation between the severe course of the disease and DR7 allele, while patients with milder manifestations presented an association with DR2 allele. **Gladman et al. (1999)** investigated the relationship between HLA antigens and the disease manifestations in 117 SLE patients. They found a lower prevalence of DR6 in patients with renal involvement and of DR1 and DR7 in patients with vasculitis. We confirm the possibility of another association between HLA class II haplotypes and clinical features of SLE. In our experience, the DR7 positive cases were often associated with a severe, while patients with DR2 positivity with a milder clinical course. We observed a decreased frequency of DR7 allele in our SLE patients with leucopenia comparing to SLE cases without leucopenia ( $p<0.01$ ), whereas DR5 allele frequency decreased in patients with vasculitis comparing to patients without vasculitis ( $p<0.04$ ).

**Reveille et al. (1991)** suggested an association between anti-SSA and/or anti-SSB autoantibody response and presence of glutamine in position 34 of the DQA1 chain, and

leucine at position 26 of the DQB1 chain. All their patients with anti-SSA had glutamine and/or leucine residues in these positions. Patients with anti-SSA plus anti-SSB were more likely to have all four of their DQA1/DQB1 chains containing these amino acid residues than either anti-SSA negative SLE patients or controls. Similarly to previous authors, we found also glutamine and/or leucine residues in our patients at this position, but DQA1 molecules with glutamine in position 34 did not occur more frequently in patients than in controls (71/100, 71% vs 63/100, 63%, ns). DQB1 molecules with leucine in position 26 were significantly more frequent in SLE patients compared to the controls (64/100, vs 42/100,  $p<0.01$ ). We found only a non-significant increase of these amino acids in both anti-SSA and anti-SSB positive patients compared to both anti-SSA and anti-SSB negative patients (80 vs 63%, respectively). In our earlier SLE study with less LN patients' group, the DRB1\*02 positivity was less frequent in patients with LN ( $n=16$ ), than without LN ( $n=34$ ), (3/16, 18% vs. 16/34, 47 %,  $p=0.05$ ) (**Endreffy et al., 2003**). With more LN patients in this study, the frequency of DRB1\*15/16 allele reached the significantly elevated level (16/66, 24.2% vs. 11/100, 11%,  $p<0.05$ ). The presence of DRB1\*04 allele significantly decreased in LN as compared to the controls ( $p<0.01$ ).

The DRB1\*0301 allele proved to be a sensitive marker for anti-SSA and anti-SSB antibody production. The most severe LN WHO type IV. and the WHO non-IV. type LN groups did not differ in the distribution of HLA class II alleles, except in the significant decrease of DQB1\*0601 allele in WHO type IV cases. The above mentioned negative associations may represent a new aspect of the LN pathomechanism, suggesting DRB1\*11/12 as a protective allele for lupus nephritis.

According to our data there was no significant difference in HLA class II subtypes between the seropositive and seronegative groups for anti-SSA and/or anti-SSB antibodies. Only the DRB1\*11/12 (DR5) 1101 allele frequency decreased significantly in the anti-SSA/anti-SSB seropositive patients (7.1 % vs 25 %,  $p<0.05$ ) as compared to the seronegative LN group.

We have determined the sensitive alleles (DRB1\*0301 – DQA1\*05011 – DQB1\*0201, DRB1\*15/16 - DQB1\*0601) and defensive alleles (DRB1\*11/12 (DR5), DRB1\*04 – DQA1\*05012 – DQB1\*0301, \*0303) for LN in Hungarian patients. The most severe (WHO type IV.) patients and the WHO non-IV. type LN group did not differ in the distribution of HLA II class alleles, except the significantly decreased DQB1\*0601 allele in WHO type IV.

#### **4.2. HLA alleles in Hungarian patients with primary Sjögren's syndrome**

In this study, we could confirm two haplotypes of susceptibility in Hungarian patients with primary Sjögren's syndrome. HLA DRB1\*03 (03011) - DQA1\*05011 - DQB1\*0201(26/48, 54% vs 9/50, 18%,  $p<0.05$ ), and HLA DRB1\*15/16 (DR2)(1601) - DQA1\*0102 - DQB1\*0602 (14/48, 29% vs 3/50, 6%,  $p<0.05$ ) haplotypes were detected significantly more frequently in SS patients. In Hungarian SS patients the haplotype of defense seems to be: HLA DRB1\*11/12(DR5) - DQA1\*05012 - DQB1\*0301 (11/48, 23% vs 22/50, 44%,  $p<0.05$ ).

In our SS patients the most frequent amino acid at position 34 of the DQA1 chain was the glutamine (80%). In anti-SSA positive patients, at position 26 of the DQB1 chain the leucine was the most frequent amino acid (54.2%), but the frequency of glutamine(G) did not differ significantly from the anti-SSA negative patients. **Jean et al. (1998)** confirmed that SS patients with anti-SSA or anti-SSA+SSB have higher rates of extraglandular disease than those of without anti-SSA or anti-SSB.

Summarizing the results of the present study, the distribution of HLA class II alleles was determined by molecular biological methods in Hungarian SS patients. The pheno- and genotypes were also analyzed and compared with different clinical manifestations. To our best knowledge, it is the very first description of the haplotypes of susceptibility and resistance in Hungarian patients with SS.

The DRB1\*03 allele and its associates haplotype exhibited a positive correlation with anti-SSB autoantibody production, the presence of renal tubular acidosis and the development of malignant lymphoma. The DRB1\*15/16(DR2) alleles were detected more frequently in the anti-SSB-negative patients than in the anti-SSB-positive patients, and in the anti-SSA plus anti-SSB negative patients than in the anti-SSA plus anti-SSB positive patients. Our results suggest a model of HLA-restricted presentation of Ro/La peptide determinants in primary Sjögren's syndrome.

#### **4.3.1. TNF alpha polymorphism in SLE**

**Zuniga et al. (2001)** analyzed the polymorphism of TNF-alpha promoter in Mexican Mestizo SLE patients. They found a significant increase only in the TNF G/A -238 genotype and in the TNFA-238 allele frequencies in the SLE group when compared to controls. In multivariate analysis, possession of HLA-DR3, TNF-308A, and C1\_2\_5\*192 remained

independently associated with susceptibility to SLE. The association of possession of TNF-308A with susceptibility to SLE cannot be attributed to linkage to HLA-DR3 alone, nor to other polymorphic markers in the vicinity of the TNF gene (**van den Linden et al., 2001**). TNF-alpha is the co-stimulatory factor for B cell proliferation and immunoglobulin production (**Kehrl et al., 1987**) and there is considerable evidence of B cell hyperactivity in SLE. Cultured B cells from lupus patients spontaneously produce immunoglobulins including autoantibodies (**Woods et al., 1989**) and the B8-DR3 haplotype is associated with a high autoantibody response as compared to non-B8-DR3 haplotypes. Non-HLA genes within the MHC complex may be directly involved in the predisposition to autoimmunity.

In this study, we found significantly elevated frequency of TNF alpha-308 heterozygous (AG) and homozygous (AA) alleles in SLE patients. The frequency of TNFalpha-238 A/G alleles and different genotypes were similar to the controls. Examining the distribution of the HLA DRB1, DQA1, DQB1 alleles, the sensitive allele was the DRB1\*03(03). The HLA DRB1\*04 and the DRB1\*15/16(DR2) allele were present in AG and GG subgroup as defensive alleles. We confirm that the TNF-alpha-308A allele may play a role partly in the development of SLE.

#### **4.3.2. TNF alpha genotypes in primary SS**

The allele frequency for TNF-alpha-308A was significantly increased in our SS patients in comparison with healthy blood donors. In contrast, the TNF-alpha-238 allele frequency among SS patients did not differ significantly from that in the control group.

Our results indicate that the TNF-alpha-308A allele may contribute to susceptibility for primary SS, while it seems that the TNF-alpha-238A allele is not associated with SS. According to our results the main HLA class II susceptibility haplotype (DRB1\*0301 - DQA1\*05011 - DQB1\*0201) occurs frequently together with the TNF-alpha-308AG genotype in SS patients, indicating a possible co-acting effect with respect to susceptibility to SS.

#### **4.4. ADCC reaction and in vitro glucocorticoid sensitivity in primary SS**

Concerning the explanation of steroid effect, one concept is that the in vitro and in vivo actions of GSs may be connected to the number of steroid receptors on the surface of

lymphocytes, or to the blocking of those receptors. Since mainly the IgG Fc-receptor bearing lymphocytes take part in the ADCC reaction, it is conceivable that the interindividual differences in GS sensitivity may be correlated with a shift in the proportion of different T-lymphocyte subpopulations. Considerable evidence was presented that GSs inhibit T-cell proliferation by blocking the production of T-cell Growth Factor. **Katz and Fauci (1998)** found that GSs suppressed NK-cell activity in humans. As a decreased GS sensitivity was observed in patients with anti-SSA and/or anti-SSB antibody positivity, it is expected that with a certain genetic background these autoantibodies can influence the GS sensitivity, however, the exact in vivo mechanism is unknown.

We conclude that sensitivity to GSs in patients with SS is influenced by both genetic haplotype and anti-SSA/anti-SSB seropositivity. Co-existence of these two factors may cause a decreased sensitivity to GSs. In vitro ADCC steroid inhibition test of the peripheral lymphocytes, coupled with the analysis of HLA class II allele polymorphism and autoantibody profile may have a predictive value in making a decision to introduce GS therapy in other systemic autoimmune diseases as well.

## **5. Conclusions and new findings**

### **5.1. HLA class II allele polymorphism in Hungarian patients with SLE**

To summarize the present work, we have proven associations between the HLA class II polymorphism, TNF $\alpha$  promoter allele polymorphism and the phenotype in patients with systemic lupus erythematosus and with primary Sjögren's syndrome. To our best knowledge, it is the very first description of the susceptible and defensive haplotypes in SLE by molecular genetic (but not serological) methods in our Hungarian population. According to our results, the haplotypes of susceptibility to SLE in Hungarian patients are the DRB1\*03-DQA1\*05011-DQB1\*0201 and the DRB1\*1501-DQA1\*0102-DQB1\*0602 haplotypes. Oppositely, the DRB1\*04 and DRB1\*11/12 alleles were less common in the patients with SLE than in the controls. Our patients with DRB1\*1501 (DR2) positivity exhibited a milder clinical course and a negative correlation with lupus nephritis whereas patients with DRB1\*07 positivity presented more severe clinical symptoms.

Examining the differences of the HLA class II allele polymorphism between SLE patients with and without lupus nephritis, we observed that the DRB1\*03011, DRB1\*07,

DQA1\*05011, 0102, DQB1\*0201, 0602, 0202 seem to be susceptible alleles for LN, while the HLA DRB1\* 11/12(DR5)/DQA1\*05012 were found to be as protective alleles.

## **5.2. HLA class II allele polymorphism in Hungarian patients with primary SS**

The HLA DRB1, DQA1 and DQB1 allele polymorphism was detected with molecular biological methods in Hungarian patients with SS very first time. The haplotypes of susceptibility and of defence for primary Sjögren's syndrome were also clarified. The HLA DRB1\*03011 - DQA1\*05011- DQB1\*0201 and HLA DRB1\*15/16(DR2)1601 - DQA1\*0102 - DQB1\*0602 haplotypes proved to be susceptible in Hungarian patients with SS and the HLA DRB1\*11/12(DR5) -DQA1\*05012- DQB1\*0301 seemed to be haplotype of defence. The DRB1\*03-associated haplotype was detected more frequently in SS cases with vascular manifestations, with malignant lymphoma and in anti-SSB seropositive patients. The DRB1\*03 allele proved to be an allele of susceptibility to the development of kidney involvement. DRB1\*03-positive patients exhibited a more severe clinical course as compared with patients with DRB1\*15/16(DR2) positivity. We hope that our findings will add to the knowledge of development of systemic lupus erythematosus and primary Sjögren's syndrome new pathogenetic aspects.

### **5.3.1. TNF alpha polymorphism in SLE**

The allele frequency of TNF-alpha-308A significantly increased in the SLE patients' group in comparison to the data of controls. The frequency of TNFalpha-238A allele did not differ between the patients and the controls. We detected significantly increased of homo- and heterozygous genotypes for -308A allele frequency of TNF alpha gene in Hungarian SLE patients, therefore our data suggest a possible role of this allele in the pathogenesis of SLE.

### **5.3.2. TNF alpha polymorphism in primary SS**

Our results indicate that the TNF-alpha-308A allele may contribute to susceptibility for primary SS, while it seems that the TNF-alpha-238A allele is not associated with the development of SS. According to our results the main HLA class II susceptibility haplotype (DRB1\*0301 - DQA1\*05011 - DQB1\*0201) occurred frequently together with the TNF-

alpha-308AG genotype in SS patients, indicating a possible co-acting effect with respect to susceptibility to SS.

#### **5.4. ADCC reaction and in vitro glucocorticoid sensitivity in primary SS**

Relationship between HLA class II alleles and in-vitro steroid inhibition of antibody-dependent cellular cytotoxicity in patients with primary Sjögren's syndrome were analysed. The in vitro steroid inhibition of the ADCC reaction was significantly lower in SS patients than in controls, as a sign of the decreased glucocorticosteroid sensitivity. In SS cases with HLA DR2/ DR3 heterozygosity the glucocorticosteroid sensitivity was significantly lower not only in comparison to controls, but also to SS patients carrying neither DR2 nor DR3 alleles.

## **Practical results**

- 1. The analysis of HLA class II allele polymorphism can be useful as a marker of disease susceptibility or defence in Hungarian patients with primary Sjögren's syndrome and with systemic lupus erythematosus. These results may have predictive informative values for the prognosis and severity and the data can be used in family examinations.**
- 2. The allele frequencies and genotypes of TNF-alpha promoter gene (TNF-alpha-308 and TNF-alpha-238) were analysed in patients with SLE and SS. Their associations with HLA class II alleles were also described. Our results confirmed the role of the TNF-alpha-308A allele in the pathogenesis of systemic lupus erythematosus and Sjögren's syndrome. The data support the necessity of anti-TNF-alpha treatment in severe forms of SLE and SS.**
- 3. An examination of glucocorticoid sensitivity with a special in vitro glucocorticoid inhibition test of ADCC reaction may be suggested before the introduction of glucocorticoid treatment in the cases of severe manifestations of primary SS patients.**

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**Sum of impact factors for base references (I-V): 9.703**

**Total impact factor for the publications (without the abstracts): 47.364**

#### **Obtained scientific and professional prizes**

1998/99. Annual immunological prize by Teva-Biogal

#### **Laudation charter**

at Professor Cserhádi's Laudation meeting, 1998.- prize winner

#### **Prizes from Academical Committee, Szeged:**

1. M. Gyöngyösi, **A. Kovács**, L. Kovács: **Cardiac manifestations of primary Sjögren syndrome**  
1994. II. prize
2. E. Endreffy, **A. Kovács**: **Molecular biological analysis of autoimmune disorders: HLA antigens, pheno- and genotypic correlations in rheumatoid arthritis**  
1994. III. prize
3. **A. Kovács**: **Molecular biological features of systemic lupus erythematosus**  
1995. II. prize
4. **A. Kovács**: **Lipid peroxidation and antioxidant enzyme investigations in autoimmune disorders**  
1996. II. prize
5. **A. Kovács**: **Molecular biological analysis of autoimmune diseases: MHC II. class allele polymorphism in primary Sjögren's syndrome**  
1997. I. prize with silver medal

**6. A. Kovács: Molecular genetical analysis in lupus nephritis**

1999. II. Prize

## **Acknowledgements**

At first I wish to express my special thanks to Professor Ildikó Petri, who received me as a tutor at the Regional Blood Transfusion Center several years ago and encouraged me to perform my scientific studies. Her guidance and pragmatic advices were always of great value.

I thank Professor György Gál, the former Head of Regional Blood Transfusion Center the possibility of working in his institute. He constantly supported me whereby my scientific aims could be realized.

I am really grateful to Professor Gyula Pokorny, who gave me the opportunity to join his autoimmune team at the First Department of Internal Medicine in 1993. I thank him very much to initiate and lead my scientific work as the Head of Department of Rheumatology as well. He played an indispensable part in my scientific activities from the planning to the evaluation of the results. Beside his professional help, I deeply appreciate his support in the preparation of our manuscripts.

I wish to express my deepest thanks to Dr. Emőke Endreffy who supplied all the necessary help to perform the molecular genetic studies in our patients. From the beginning she shared with me her great experience and extremely valuable theoretical advices.

My sincerest thanks to Professor Vince Varró, the first Head of the First Department of Medicine of my scientific career for the admission to his staff after my graduation, and for the initiation of my professional and scientific activities.

I am grateful to Professor János Lonovics, who as the Head the First Department of Medicine promoted my scientific and clinical advance.

My special thanks are due to Dr. Csaba Szalai for the precise laboratory investigations and valuable corrections of manuscripts.

I thank my colleague Dr. László Kovács, who has always given me his scientific and enthusiastic support.

I would not have been able to carry my scientific work without the continuous love and support of my mother, Professor Aranka László, my son Kristóf and without the force-giving memory of my father.