

Ph.D. thesis

IDENTIFICATION OF GENETIC FACTORS ASSOCIATED WITH
SJÖGREN'S SYNDROME

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INTRODUCTION

During the autoimmune process the immune system loses the self-tolerance ability, which may be the result of abnormal selection or regulation of self-reactive lymphocytes and by abnormalities in the way that self-antigens are presented in the immune system.

Sjögren's syndrome (Ss) is a systemic autoimmune disorder that mainly affects exocrine glands and usually presents as persistent dryness of the mouth and the eyes due to functional impairment of the salivary and lachrymal glands. Therefore the most important manifestations of the disease are keratoconjunctivitis sicca and xerostomia caused by diminished lachrymal and salivary gland secretory activity. Antibodies against ribonucleoproteins (antiRo/SSA and antiLa/SSB) are frequently present in the sera of Ss patients. Ss is characterized by B cell hyperactivity and hypergammaglobulinemia. In the absence of an associated systemic autoimmune disease, patients with this condition are classified as having primary Ss (pSs). Several cytokines, including IL-10, IFN γ and some autoantibodies (Ro/SSA, La/SSB) have been proposed to have a role in the pathogenesis of the disease.

Antibodies produced against Ro/SSA and La/SSB autoantigens are not only of diagnostic value but they may even play a role in the pathogenesis of several autoimmune diseases (Sjögren's syndrome/Ss, subacute cutaneous lupus erythematosus/SCLE, neonatal lupus erythematosus/NLE and systemic lupus erythematosus/SLE). Among other factors, ultraviolet (UV) radiation and the hormonal milieu are well-known cofactors in the pathogenesis of these autoimmune diseases as well. AntiRo/SSA and antiLa/SSB autoantibodies are two of the most common antibodies found in the serum of patients with Sjögren's syndrome and SLE. Three genes have been isolated which encode three Ro/SSA protein antigens 46, 52 and 60 kDa. The 46 kDa antigen is a calcium-binding protein that

resides predominantly in the endoplasmic reticulum, while the 52 kDa and the 60 kDa Ro proteins are located in the cytoplasm or the nucleus. Ultraviolet B (UVB) radiation, estrogen treatment and some viral infections result in translocation of the Ro/SSA antigens to the cell surface, where they are available to bind circulating Ro/SSA autoantibodies. The La/SSB antigen, which is located primarily in the nucleus, is a single protein transiently associated with all RNA polymerase III transcripts, the hYRNAs. It carries autoantigenic determinants, and it has been suggested that La/SSB participates in the synthesis, maturation and nuclear export of this class of RNA molecules. In lymphocytes of patients with Sjögren's syndrome three different splicing variants of La/SSB mRNA, exon 1 exon 1' and exon 1'' can be detected. In case of exon 1' the exon 1 was replaced with an alternative 5' end, moreover the difference between the exon 1' and exon 1'' is only four nucleotides.

The aim of this study was to determine whether the three Ro/SSA antigen mRNAs and the different splicing variants of La/SSB mRNAs exist in a transformed human keratinocyte cell line (HaCaT). Moreover our goal was to investigate the effect of UVB and estrogen treatment on the expression of Ro/SSA and La/SSB mRNA forms in HaCaT cells

Inappropriate expression of cytokines is common in autoimmune lesions. The cytokines, which participate in the regulation of the inflammatory response, can be divided functionally into proinflammatory (IL-1, IL-6, TNF) and anti-inflammatory (IL-1RA, IL-10) molecules. Interleukin 10 (IL-10) is a pleiotropic cytokine produced by a number of cell types including lymphocytes, monocytes, macrophages and various tumour cell lines. This cytokine was originally described as cytokine synthesis inhibitory factor because of its ability to inhibit the secretion of cytokines from T helper type 1 (Th1) T cell clones. For example, IL-10 inhibits the secretion of tumor necrosis factor α (TNF α), IL-1, IL-6 and IL-12 from monocytes and macrophages and interferon γ (IFN γ) and IL-2 from T cells. Effects of IL-10 on cytokine production from Th1 cells and macrophages have led to its classification as an

anti-inflammatory cytokine. IL-10 also down-regulates T cell activation by decreasing Major Histocompatibility Complex (MHC) class II expression on antigen presenting cells, but it exerts potent stimulatory effects on B cells by inducing MHC class II expression, cellular proliferation and differentiation. The IL-10 gene maps to chromosome 1. In the 4 kb sized promoter region several polymorphisms have been described: two microsatellites (dinucleotide repeats) in the 5' flanking region of the gene and three single nucleotide polymorphisms (SNP) at positions -1082 (G to A substitution), -819 (C to T substitution) and -592 (C to A substitution). These three polymorphisms occur in three putative haplotypes: GCC. The IL-10 production is controlled by the G at position -1082, GG genotype is the high IL-10 producing, while AA is the lowest producing genotype in the Caucasian population. The rate of IL-10 production is critical in immune regulation, controlling the balance of inflammatory and humoral responses. Differential expression of IL-10 has been implicated in a number of autoimmune disorders including Sjögren's syndrome.

The objective of our study was to analyse the role of IL-10 nt-1082 promoter polymorphism in the clinical and immunologic characteristics of patients with Sjögren's syndrome. We determined the genotype frequencies by PCR-RFLP method and measured the plasma IL-10 concentrations both in Ss patients and healthy controls.

THE AIM OF THIS STUDY

AntiRo/SSA antibodies are the most prevalent specificity among many autoimmune diseases. SLE, homozygous complement deficiency SLE (C2 and C4), Ss/SLE overlap syndrome, subacute cutaneous LE (SCLE), neonatal lupus are frequently observed in association with antiRo/SSA. In contrast, antiLa/SSB antibody is more closely associated with SS. AntiRo/SSA antibodies can be found alone in many sera, while antiLa/SSB antibodies are usually accompanied by antiRo/SSA. Sjögren's syndrome presents usually in middle-aged women, the role of the hormonal milieu is presumable in the etiopathogenesis of this disease. Sex hormones influence both humoral and cell-mediated adaptive immune response, and estrogen is one of the potential factors of this immunologic dimorphism. The effect of estrogen has been suggested to be responsible for the female predominance in autoimmune diseases such as SLE and Ss. The role of estrogen metabolism abnormalities were supported by clinical and experimental results also. It was demonstrated that the binding of Ro/SSA and La/SSB autoantigens was increased to the surface of keratinocytes after estradiol treatment. However it was noticed that the effect of UVB irradiation also results in surface presentation of the Ro and La antigens, so the circulating antibodies can bind to these surface antigens, enhancing the possibility of direct injury of the skin. HaCaT cells were chosen because the binding of the Ro and La antigens to the cell surface can be detectable only on fast reproducing, non-differentiated cells, and this cell-line may consider as an in vitro model of the proliferative basal keratinocytes. We would find the answer for the following question:

- what is the basal level of the Ro/SSA and La/SSB antigens mRNAs in HaCaT cells,

- is there any effect of the UVB irradiation and 17- β -estradiol on the expression of the levels of the Ro/SSA and La/SSB antigens mRNAs,
- is there any association between the mRNA expression of Ro/SSA and La/SSB autoantigens and the role of the estradiol and UVB irradiation in the disease susceptibility and development?

Several studies reported from an association between the IL-10 gene polymorphisms and the disease susceptibility and onset. The importance of IL-10 polymorphisms were emphasized in connection with some autoimmune disease, for example autoimmune hepatitis, autoimmune thyroid disease... Beside autoimmune diseases many malignant diseases can be connected with IL-10 promoter polymorphisms such as Hodgkin-lymphoma and Melanoma. The IL-10 production is genetically encoded, and the IL-10 may be an important factor in study of autoimmune and malignant diseases.

The study of the IL-10 promoter nt-1082 polymorphism was required to:

- is there association between the IL-10 gene nt-1082 polymorphism and the disease,
- is there any association between the IL-10 genotypes and the plasma IL-10 levels,
- is there a prognostic role of the IL-10 genotype and plasma levels in the onset and development of the disease?

MATERIALS AND METHODS

mRNA expression of Ro/SSA and La/SSB autoantigens in HaCaT cells

The HaCaT cells were grown in DMEM medium, in CO₂ incubator. The cells were treated with 17- β -estradiol (at a concentration 10⁻⁸ M) and UVB irradiation (at a dose 200 J/m²). The sample processing were done after 12, 24, 48 and 72 hours.

The RNA isolation were made by Chomczynsky's – based on guanidin-thiocyanate-phenol- method, and with the help of SuperScript II Preamplification Kit the RNA were translated into cDNA. We measured the levels of mRNAs using PCR method.

PATIENTS AND METHODS

IL-10 polymorphism

135 healthy subjects and 99 Ss pateints were analyzed for IL-10 nt-1082 polymorphism. DNA were isolated from peripheral lymphocytes of every subjects, the polymorphism were determined using PCR-RFLP method. The plasma IL-10 levels were measured by classical ELISA method.

Comparasion of the IL-10 levels were done by Kruskal-Wallis test, the variance was determine by two-sample Student t-test. The genotype frequencies were compared using chi-square test with Yates' correction.

RESULTS AND DISCUSSION

mRNA expression of Ro/SSA and La/SSB autoantigens in HaCaT cells

Epidermal keratinocytes, especially the basal cells, are the targets of immunological damage in photosensitive lupus, in SCLE and in NLE. HaCaT cells are spontaneously transformed human keratinocytes that show some characteristics of basal epidermal keratinocytes. Our results demonstrate that in HaCaT cells, as in cultured human keratinocytes, one can find the mRNA forms of the 46 kDa, 52 kDa and 60 kDa Ro/SSA protein antigens. Other investigators found that the basal level of the 60 kDa polypeptide was the lowest, and that the calreticulin was the highest. In HaCaT cells, we also found that the mRNA level of 60 kDa form was lower than the levels of calreticulin and the 52 kDa form.

In peripheral blood lymphocytes from patient with primary Sjögren's syndrome, two alternative types of La/SSB mRNA were identified. These were the results of promoter switching combined with an alternative splicing mechanism. In HaCaT cells we also found the two alternative mRNA types of La/SSB (La exon 1', and exon 1'') as well the common variant. The mRNA level of La exon 1' was much lower in HaCaT cells than in lymphocytes of patients with Sjögren's syndrome.

Investigators suggested that Ro/SSA antigens and La/SSB antigens, normally present in the nucleus and cytoplasm of human keratinocytes could be induced to appear on the cell surface following exposure to UV radiation. UV irradiation of cultured keratinocytes induces apoptotic changes and the clustering of autoantigens at the cell surface with smaller blebs containing the Ro antigen, calreticulin, ribosomes and endoplasmic reticulum and larger blebs containing Ro, La and nucleosomal DNA. However, there may be another way for Ro and La proteins to appear on the cell surface. Physiological UV doses, especially UVB, result in the production by epidermal keratinocytes of a number of molecules that can influence cutaneous

inflammation without apoptotic changes. Some of these molecules may be directly induced by UV radiation after activating transcription factors by singlet oxygen-dependent mechanisms. It is possible that Ro and La proteins appear on the cell surface following exposure to UV radiation as a result of induced transcriptional activation of their regulatory transcription factors. It has been suggested but not yet proven, that genetic polymorphism within the regulatory pathways for these molecules could be a predisposing factor for photosensitive cutaneous lupus erythematosus.

When keratinocytes are exposed to UVB radiation, it is not clear whether all genes encoding Ro/SSA proteins or only a limited subset of this complex are upregulated. Therefore, we examined the expression of the mRNA of the three Ro/SSA polypeptides that have been cloned and sequenced (60, 52 and 46 kDa) after UVB irradiation. After a physiologically relevant dose of UVB radiation, a significantly higher level of mRNA of the calreticulin was observed in HaCaT cells after 24 h, while no changes in the mRNA levels of the 52 and 60 kDa Ro/SSA were found. Although there was a difference in the mRNA levels in the 24 h and 48 h control samples, the UV-irradiated cells had higher levels than any of the control samples. The variation in the basal mRNA levels of calreticulin can not easily be explained since these levels can be induced by several different factors such as cell stress (e.g. heat shock), perturbation in the normal endoplasmic reticulum, heavy metals etc.

In accordance with our results, other studies have reported that only calreticulin, and not the 60 and 52 kDa polypeptides, is upregulated in total cellular and cell surface expression in A431 cells after UVB irradiation. Autoantibodies against the Ro/SSA and La/SSB antigens are frequently produced by patients with particular autoimmune disease. Several lines of evidence indicate that these autoantibodies play not only diagnostic, but also pathogenic role in these diseases (especially in SCLE and NLE). The strongest evidence comes from observations of patients with NLE. In the NLE syndrome the development of skin disease

corresponds to the presence of maternal IgG antiRo/SSA antibodies in the neonate, and the development of heart block is highly associated with antibodies to the same antigen. The pattern of cutaneous IgG deposition in SCLE has been reproduced by the infusion of purified antiRo/SSA autoantibodies into human skin-grafted mice. AntiLa/SSB antibodies may also be involved because antibodies against this antigen are usually detectable in antiRo/SSA-positive serum and there are patients with SCLE with high antiLa/SSB titers but with very low titers of antiRo/SSA antibodies.

The display of Ro/SSA and La/SSB autoantigens on the surface of normal human keratinocytes following UVB irradiation and the resulting binding of the circulating antiRo/SSA and antiLa/SSB autoantibodies to their antigens could result in tissue injury through complement-mediated lysis or antibody-dependent cell-mediated cytotoxicity. Our results correlate with those of an earlier study and calreticulin could be a critical component of this Ro/SSA complex that appears on the cell surface of keratinocytes after UVB irradiation.

The hormonal milieu may also play an important role in Ro/SSA and La/SSB associated disease. A female predominance in SCLE, SLE, NLE and SS is well documented. The disease activity increases during menses and during use of oral contraceptives. Moreover, androgen treatment delays the onset and severity of autoimmune phenomena in female NZB/NZW F1 mice. Estrogen treatment of cultured keratinocytes results in the increased expression of Ro/SSA and La/SSB antigens on the cell surface. Our results from the 17-beta-estradiol stimulation differ from those of an investigation which found that at concentrations of 10^{-8} to 10^{-7} M, 17- β -estradiol induces both 52 kDa and 60 kDa Ro/SSA mRNAs in human keratinocytes by up to fivefold compared with untreated cells. In HaCaT cells 17-beta-estradiol at a concentration of 10^{-8} M, did not cause significant changes in the levels of mRNA of calreticulin or that of the 60 kDa and 52 kDa Ro/SSA proteins. It is difficult to

understand why HaCaT cells show a different behavior compared to cultured human keratinocytes under estrogen stimulation, while after UVB irradiation they react similarly. However, this duality in HaCaT cells has also been observed by Lehman who demonstrated that HaCaT cells can be used in studies of the vitamin D3 pathway and its relationship to proliferation. Differences in calcitriol synthesis and catabolism from those of cultured primary keratinocytes, however, must be considered.

Concerning the different mRNA forms of the La/SSB protein, we found no change in the levels of La exon 1' mRNA after UVB irradiation. Estrogen stimulation, however, resulted in a decrease in the exon 1' mRNA levels, but the significance of this finding is not yet clear. NF-kappaB element is presented in the regulatory region of exon 1' variant and in turn could play a role in the regulation of this mRNA. This NF-kappaB element could be influenced by active oxygen species produced as a result of, for example, UV irradiation, and this element could also be controlled by different hormones. Other recent results, emphasizing the importance of this topic, also show differences in the expression of the two La mRNAs as a function of chemicals and mitogens.

In conclusion, we detected the mRNA forms of all three different Ro/SSA antigens and that of three La/SSB species produced by alternative splicing in transformed human keratinocytes. After UVB irradiation the mRNA levels of calreticulin increased and treatment of the cells with 17-beta-estradiol resulted in a gradual decrease in the levels of La exon 1' mRNA. Our results strengthen the belief that Ro and La antigens participate in the pathogenesis of different autoimmune disease, but further studies are required to elucidate the exact pathomechanism involved.

IL-10 polymorphism

Differences of more than 3 million nucleotides can be seen comparing the genomes of two members of the same population as a result of single nucleotide polymorphism (SNP). There are other types of genetic differences (e.g. other types of polymorphisms, insertions, deletions, duplications, etc.) as well, but one of the more important genetic alterations is the SNP.

SNPs can be found in different parts of the genome, such as in coding or non-coding regions, with or without any effects on the gene product. Sometimes an SNP in the non-coding region (e.g. in a promoter) influences the transcription of the gene, but in other instances even an SNP in the coding exon has no effect on the protein structure and/or function. Synonym SNPs do not change the encoded amino acids, but non-synonym SNPs induce new ones. The resulting altered proteins could possess different features comparing to the non-altered, common forms. In the cases of altered proteins the difference could be seen e.g. in conformational alteration that eventually could lead to a change in enzyme activities, in certain cases even the entire function of the whole protein could be diminished. Therefore, individuals with different SNPs could have slightly different metabolic pathways. After having sequenced the entire human genome there is a possibility to determine the individual differences. Some of the different approaches concerning the SNPs are: a whole chromosome, a part of the genome, and a complex disease, or a single gene. During the determination of all of the SNPs one faces at least such difficulties as one had by sequencing the human genome. However the methods used in sequencing were further enhanced, and by solving the problems of automation and theoretical approaches we could further accelerate the speed of the determination of SNPs.

Knowledge on the genetic variability could lead to the resolutions of different biological problems. Evolutionary alterations and the trait of selection can be followed,

among others, since most of the changes occur in highly recombinant regions: the evolution of species and that of interspecies can be traced. SNPs have another important role, for example, in pharmacology. It is well known that individuals tolerate drugs and environmental effects differently. There are people with strong adverse reactions to certain medicaments: more than 100,000 people die yearly in the USA because of anaphylactic reactions, nevertheless the knowledge on SNPs in metabolic genes could result even in a better formulation of medicaments too.

One of the well known parts of the SNP-theme is the disease susceptibilities. Series of publications support the idea that the presence of certain SNPs is in connection with the emergence of a disease since individuals with defined SNP suffer from a disease more frequently if one compare them to the healthy population. Only a few instances in a row: Alzheimer disease, sickle cell anemia, hyperlipidemia, schizophrenia, different malignancies. Obviously not only single gene disorders can play a role in the pathogenesis of different diseases but several other factors as well (multiple genes, environmental factors, etc.).

A genetic contribution to the etiology of Ss is evidenced by the increased frequency of Ss in relatives and siblings of primary Ss patients. Multiple studies investigated the possible associations between IL-10 promoter polymorphisms in patients with SLE or rheumatoid arthritis. Studies have shown high levels of IL-10 in peripheral blood mononuclear cells and salivary glands in patients with primary Ss, and other recent studies have analyzed the IL-10 promoter polymorphisms in patients with primary Ss. An increased frequency of the GCC haplotype and a decreased frequency of the ACC haplotype were observed in Ss patients but there was no correlation between extraglandular manifestations and IL-10 haplotypes and no significant differences were found in clinical and immunologic features of Ss patients.

Some recent studies indicate that SNPs can be rendered to individuals living a longer life than the average. Perhaps these results will not directly lead to the lengthening of the

maximal life span; however, genes that play an important role in the aging process could be identified. In this respect SNPs are important factors in determining the information level of the cells of individuals, which determines the maximal life span, in turn SNP is one of the factors that determine the aging process. The frequency of diseases increases with age, so Ss, similarly to different malignant diseases, could have common roots with senescence. A polymorphism, the -1082G/A, in the promoter region of the gene was extensively studied with respect to the Ss and to aging as well. These results indicate that the -1082G haplotype is significantly higher in old men than in young controls while the number of the GCC/ATA genotype is significantly higher in women suffering from Ss. Sjögren's syndrome is basically a women's disease with a higher than 90% involvement of women. According to our own results the AA homozygote type in the -1082 position is more elevated in Ss than in the age-matched controls, whereas the GG homozygote women are underrepresented compared to the AA homozygote women with Ss (although in the control group the frequency of the GG and AA types are similar). It is obvious that the extremely high age and a disease susceptibility are usually not determined by a single SNP, although the -1082G allele can be rendered to the possibility of high age (in men) and the -1082A one to the elevated Ss disease susceptibility (in women).

In this study we analyzed the -1082 polymorphism of the IL-10 gene promoter and found that the frequency of the GG genotype was similar and the frequency of the AA genotype was increased to a lesser degree in primary Ss patients compared with the control subjects. These results are in agreement with a Japanese study where the GCC haplotype, which is predominant in white subjects, was less common in Japanese Ss patients, while the frequency of the AA genotype was higher. Moreover, we measured the levels of IL-10 in plasma and found that the presence of the disease is associated with high plasma levels of IL-10, which is in agreement with the results observed by other investigator in mononuclear cell

cultures and with results presented by Llorente et al., in which elevated levels of IL-10 mRNA and IL-10 protein were found in peripheral blood mononuclear cell cultures in primary Ss. Although results of a Finnish investigation showed higher IL-10 protein production in subjects who were negative for A at position -1082 compared to A positive individuals, we found that the plasma IL-10 levels were elevated considerably in those who carry the A allele at position -1082 compared to those who were negative for A allele nt-1082. The level of IL-10 is an important factor, because IL-10 and IL-6 play central roles in the maturation of plasma cells and activation of immunoglobulin synthesis. We did not find a direct correlation between IL-10 levels and IgG levels or presence of SSA/B antibodies, but it is possible that the increased levels of IL-10 contribute to the development of hypergammaglobulinemia, which is characteristic of Ss, and promotes autoantibody production associated with primary Ss. Moreover patients with Ss have an increased risk of developing lymphomas, and it is possible that persistently high levels of IL-10 may contribute to this conditions as well.

Although a recent study found that the appearance of the disease was earlier in Ss patients carrying the IL-10 GCC haplotype, we could not find this kind of difference when we compared the age at onset of the three genotypes. Nevertheless, differences in the clinical and immunologic Ss features were observed in our patients with respect to the three different IL-10 promoter polymorphisms. We found an increased frequency of systemic involvement in GG genotype carriers, however, we found no correlation between immunologic (antiRo/SSA, antiLa/SSB) features and IL-10 genotypes. Thus, the IL-10 promoter polymorphism at nt-1082 seems to have some measurable influence on the clinical expression of primary Ss.

In conclusion we describe a high level of plasma IL-10 in patients with Sjögren's syndrome compared with healthy controls, and show that the -1082 GG genotype is not responsible for this phenotype. We found an increased frequency of the A allele and AA genotype in Ss patients similarly to the results of a Japanese study. IL-10 polymorphism may

be an important component of the genetic background, the susceptibility, and clinical expression of primary Ss patients, but more extensive genetic tests should be done including the examination of other IL-10 polymorphisms.

SUMMARY

Autoimmune diseases have a very complex clinical picture, several factors may play a role in the onset of the autoimmune disease and the disease susceptibility such as genetic polymorphisms (e.g. interleukins), autoantibodies (antiRo/SSA, antiLa/SSB, etc.) and aging may play a role in it as well, since the onset of several autoimmune diseases is age-dependent.

Antibodies produced against the Ro/SSA and La/SSB autoantigens are not only of diagnostic value but they may even play a role in the pathogenesis of several autoimmune diseases (Sjögren's syndrome, subacute cutaneous lupus erythematosus, neonatal lupus erythematosus and systemic lupus erythematosus). Among other factors, such as ultraviolet (UV) radiation and also the hormonal milieu are well-known cofactors in the pathogenesis of these autoimmune diseases. The goal of our research was to study the possible alterations in mRNA levels of three different Ro antigens and that of two La species produced by alternative splicing in transformed human keratinocytes (HaCaT cells) after UVB irradiation as well as after 17- β -estradiol treatment. The polymerase chain reaction technique was used to determine the mRNA levels of the Ro and La species after 24, 48, and 72 hours of irradiation. mRNA levels of calreticulin increased as a function of time after UV irradiation but mRNA levels of Ro 52 kDa and 60 kDa Ro mRNAs were unaltered. After treating the cells with 17- β -estradiol, there was no change observed in the levels of Ro mRNAs or La exon1 mRNA, but a gradual decrease was noted in the mRNA levels of La exon1'. The importance of alterations in the ratio of La exon1 to exon1' is supported by the observations in patients with Sjögren's syndrome, and our results strengthen their role in the pathogenesis of different autoimmune diseases.

A further aim of our study was to investigate the frequency of the -1082 polymorphism of the interleukin-10 (IL-10) gene and soluble IL-10 levels in Hungarian

primary Sjögren's syndrome (Ss) patients. Ninety-nine Ss patients and 135 healthy volunteers were examined. Samples were analyzed by the PCR-RFLP method and IL-10 plasma levels were assessed by a commercial ELISA assay. IL-10 plasma levels were higher in the primary Ss patients (36.4 ± 57.5 pg/ml $n=99$) compared with healthy subjects (9.9 ± 20.3 pg/ml, $n=135$, $p=10^{-6}$). The elevated IL-10 phenotype of Ss patients was not associated with increased G allele frequency as reported earlier, while in the control group, we found higher IL-10 levels among the subjects who were carriers of the GG genotype (17.7 ± 23.2 pg/ml) as compared to the other two genotype carriers (AA 8.98 ± 16.5 and GA 8.5 ± 21.1 pg/ml, $p=0.01$). Our data do not support some previous observations that indicated an association between deregulated IL-10 secretion in Ss and higher G allele frequency. However, our results clearly demonstrate that GG homozygosity is associated with elevated IL-10 levels in apparently healthy subjects, but this can not account for the IL-10 related specific disease features observed in Ss. Thus, other genetic factors contribute to the clinical spectrum of this heterogeneous disease at least in the Hungarian population.

ÖSSZEFOGLALÁS

Az autoimmun betegségek igen összetett kórképek, számos tényező befolyásolja a kialakulásukat: különböző genetikai eltérések, például az SNP-k (pl: IL-10), a különböző autoantitestek (többek között: antiRo/SSA, antiLa/SSB), s az öregedés is szerepet játszhat, hiszen sok autoimmun betegség megjelenése életkorhoz köthető.

A Ro/SSA és La/SSB autoantigének ellen termelődő antitesteknek nemcsak a diagnosztikában, hanem az autoimmun betegségek patomechanizmusában is szerepük van. Több más tényező mellett az UVB besugárzás és a hormonális hatások is ismert kofaktorai ezen betegségeknek. A célunk az volt, hogy tanulmányozzuk a háromféle Ro/SSA és a kétféle La/SSB alternatív splicing formáinak különböző mRNS szintjeit transzformált humán keratinocitákban UVB besugárzás és 17-béta-ösztadiol kezelés hatására. PCR technika segítségével határoztuk meg a különböző mRNS szinteket 24, 48, 72 órás UVB besugárzás után. A kalretikulin mRNS szintje megnövekedett, míg az 52 és 60 kDa Ro/SSA mRNS-ek szintje nem változott. Ösztadiol kezelés hatására nem volt változás a Ro/SSA és az La/SSB exon1 mRNS szintekben, de csökkent az La/SSB exon1' mRNS szint. A két variáns (exon1 és exon1') arányának szerepe már bizonyított Sjögren szindrómában, s a mi eredményeink is megerősítik azt a feltételezést, miszerint a Ro és La antigének részt vesznek a különböző autoimmun betegségek pathogenezisében, így a Sjögren szindrómában is.

Az IL-10 promóterének -1082-es pozíciójában levő polimorfizmust és a szolubilis IL-10 szinteket vizsgáltuk Sjögren szindrómás betegek és egészségesek csoportjában. PCR-RFLP módszert alkalmaztunk a genotípusok meghatározásához, a szérum IL-10 szintjét ELISA módszerrel mértük. Az IL-10 szignifikánsan magasabb volt a Ss betegek ($36,4 \pm 57,5$ pg/ml) esetén, mint a kontroll személyekben ($9,9 \pm 20,3$ pg/ml; $p=10^{-6}$). A magas citokintermelő képességgel jellemezhető fenotípus és a nagyobb G allél gyakoriság között

nem találtunk összefüggést a Ss betegekben szemben a korábbi vizsgálatok eredményeivel, viszont a kontroll csoportban emelkedett IL-10 szinteket detektáltunk azok között, akik GG genotípus hordozók voltak ($17,7 \pm 23,2$ pg/ml), összehasonlítva a másik két genotípust hordozó csoporttal (AA: $8,98 \pm 16,5$ pg/ml; GA: $8,5 \pm 21,1$ pg/ml; $p=0,01$). Vizsgálataink nem támasztják alá a korábbi vizsgálatokat, miszerint Ss-ben összefüggés lenne a magas G allél gyakoriság és a szabályozatlan IL-10 szekréció között. Az eredményeink szerint viszont a GG homozigótaság kapcsolatban van az emelkedett IL-10 szinttel az egészséges populációban, de ez nem magyarázza az IL-10-zel összefüggésbe hozható azon specifikus betegség paramétereit, melyeket általában vizsgálnak Ss-ben. Valószínűleg más genetikai faktorok is részt vesznek a betegség klinikai spektrumának kialakításában a magyar beteg populációban.

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