We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK
CITATION
INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Gene Polymorphisms of Immunoregulatory Cytokines IL-10 and TGF-β1 in Systemic Lupus Erythematosus

Irena Manolova, Mariana Ivanova and
Spaska Stanilova

Additional information is available at the end of the chapter

1. Introduction

Systemic lupus erythematosus (SLE) is a complex multifactorial autoimmune disease characterized by loss of immune tolerance and defective immune regulatory mechanisms that leads to B cell hyperactivity and the production of pathogenic autoantibodies directed against a wide range of autoantigens, and particularly nuclear antigens [Liossis et al., 1996; Yurasov et al., 2006; Mandik-Nayak et al., 2008]. In common, the complex interaction of genetic, environmental, as well as immunological factors causes the breaking of self-tolerance of the immune system and leads to development of autoimmunity. The immune system has various mechanisms at the cellular and molecular level that negatively regulate immune responses and counteract establishment of chronic and destructive immunity [Nakken et al., 2012]. A number of circulating cytokine abnormalities have been reported in SLE and recent advances have revealed new insights in cytokine regulation of autoimmune inflammatory responses [Diveu et al., 2008]. In particular, the production of Interleukin-10 (IL-10) and transforming growth factor-β1 (*TGF*-β1), the two main Treg cytokines that suppress the inflammatory response, has been found to be deeply deregulated in SLE patients, so they have been considered essential elements in the etiopathology of the disease.

Given the importance of cytokines in immune system regulation, these molecules are of high interest not only in the "effector phase" of autoimmune disease in which self-tolerance has already been broken, but also in the "initiation phase" of autoimmunity, in which a lasting immune response against self antigens is first generated. Recently, it has been suggested that initial susceptibility to autoimmune disease lies at least partly in the genetics of cytokine regulation, and that many genetic polymorphisms affecting cytokine patterns could alter thresholds for immune responses, resulting in pro-inflammatory presentation of self antigens

and the subsequent misdirection of adaptive immunity against self which is observed in autoimmune disease [Kariuki et al., 2010]

Genetic polymorphisms have emerged in recent years as important determinants of disease susceptibility and severity. Polymorphisms are naturally occurring DNA sequence variations, which differ from gene mutations in that they occur in the normal healthy population and have a frequency of at least 1%. Approximately 90% of DNA polymorphisms are single nucleotide polymorphisms (SNPs) due to single base substitutions. Others include insertion/deletion polymorphisms, minisatellite and microsatellite polymorphisms. Although most polymorphisms are functionally neutral, some have effects on regulation of gene expression or on the function of the coded protein. These functional polymorphisms, despite being of low penetrance, could contribute to the differences between individuals in susceptibility to and severity of disease. Many studies have examined the relationship between certain cytokine gene polymorphism, cytokine gene expression in vitro, and the susceptibility to and clinical severity of diseases [Bidwell et al., 1999; Hollegaard and Bidwell, 2006]. Some genetic polymorphisms at the promoter regions of IL-10 and TGF-β1 genes have been associated with cytokine production. Given that the production of these molecules is controlled at genetic level, functional polymorphisms in their promoters could influence the development and severity of the disease. In the present review, we summarize the information about involvement of IL-10 and TGF-β1 genetic variants on SLE appearance and clinical presentation.

2. Role of IL-10 and *TGF*-β1 in immune regulation and autoimmunity

2.1. IL-10

IL-10 is a pleiotropic cytokine with important immunoregulatory functions, which can be produced by both leukocytes and structural cells within tissues, being produced in particular by Tregs *in vivo* [Wakkach et al., 2008]. It is pivotal in inhibiting inflammation and suppresses Th1-mediated immune response through down regulation of proinflmamatory cytokine secretion from both Th1 and activated macrophages (De Waal Malefyt et al., 1993; Fiorentino et al., 1998]. It also inhibits antigen presenting cells by downregulating major histocompatibility complex class II (MHC-II) and B7 expression [Ding et al., 1993]. In addition to these inhibitory actions, IL-10 promotes B-cell-mediated functions, enhancing survival, proliferation, differentiation, and antibody production [Rousset et al., 1992]. Hence, increased production of IL-10 could thus explain B cell hyperactivity and autoantibody production, two main features of the immune dysregulation in SLE. In fact, high serum levels of IL-10 have been reported in patients with SLE and they correlated positively with the disease activity [Park et al., 1998].

The role of IL-10 in the pathogenesis of lupus remains controversial. The abnormally elevated amounts of IL-10 detected in serum of patients with SLE [Houssiau et al.,1995] and the observation that anti-IL-10 therapy can down-modulate disease in such patients [Llorente and Riehaud-Patin, 2003] suggest that this cytokine may promote disease. In contrast, mouse models have suggested a preventive role for IL-10 in the pathogenesis of lupus [Yin et al.,

2002]. In particular, IL-10 knockout mice were demonstrated to develop severe lupus, with earlier appearance of skin lesions, increased lymphadenopathy, more severe glomerulonephritis, and higher mortality than their IL-10-intact littermate controls [Yin et al., 2002]. Interestingly, the injection of IL-10 could prevent the occurrence of the disease in such models [Yin et al., 2002]. The authors of the study suggested that the contradictory role of IL-10 in lupus could be explained taking into account the phase of the disease. IL-10 deficiency enhanced IFN-γ production in the CD4 and CD8 lineages, and that, in turn, was associated with increased production of IgG2a anti-dsDNA antibodies, especially at the early stages of disease development. In contrast, at later phases of disease, excessive amounts of IL-10 production led to enhanced autoantibody production and subsequent formation of pathogenic autoantibody–antigen complexes [Yin et al., 2002]. However, Llorente and colleagues demonstrated that constitutive IL-10 production by monocytes and B cells in healthy members of multicase families with SLE was significantly higher than that of healthy unrelated controls, but was similar to that of SLE patients, thus suggesting that a genetically controlled high innate IL-10 production may predispose to SLE development. [Llorente et al.,1997]

2.2. *TGF-β1*

Recently, experimental studies have demonstrated an association between *TGF-β*1 and the development of autoimmunity [Aoki et al., 2005]. TGF-β1 belongs to a large family of multifunctional proteins, secreted by a variety of cell types that act as signal molecules in controlling a great number of biological processes. Like IL-10, it is a highly pleiotropic cytokine with an important role in maintaining immune homeostasis [Li et al., 2006]. There are three TGF-β isoforms in mammalian cells, TGF-β1, 2 and 3, which use similar signaling pathways and exert overlapping, albeit not identical biological functions. TGF-β1 is involved in many critical cellular processes, including cell growth, extracellular matrix formation, cell motility, hematopoiesis, apoptosis and immune function (Moustakas et al., 2002; Schuster & Krieglstein, 2002). The cells of immune system, including B, T and dendritic cells as well as macrophages, mostly produce TGF-β1, an isoform that is also found in large amounts in the plasma [Flanders and Roberts, 2001]. TGF-β1 has pronounced anti-inflammatory and immunosuppressive functions, the latter being realized by controlling the activation, proliferation, differentiation and survival of all effector immune cells [Rubtsov et al., 2007; Wahl, 1992]. Importantly, TGF-β1 inhibits maturation of dendritic cells and modulates the functions of antigen-presenting cells, reducing the macrophage production of IL-1, IL-6, and TNF-α [Fainaru et al., 2007]. However, immunosuppressive effect of TGF-β1 was most pronounced for T cells [Rubtsov et al., 2007; Li et al., 2006]. It inhibits T cell proliferation and production of IL-2, differentiation of Th1, Th2, and cytotoxic T lymphocytes (CTL). TGF-β1 suppresses IFN-γ production from Th1, NK cells and CTL, as well as their cytotoxic activity. TGF-β1 plays an essential role in the functioning and survival of regulatory T cells (Treg), and *de novo* generation of Foxp3$^+$ Treg from naive CD4$^+$ CD25$^-$ T lymphocytes in the periphery [Pyzik et al., 2007; Selvaraj et al., 2007; Zheng et al., 2007]. Furthermore, recent study reveals a role for TGF-β1 as effector molecule of Foxp3$^+$ Treg cells [Li et al., 2007]. In contrast to IL-10, TGF-β1 is also an important negative regulator of B cell differentiation and proliferation, inhibiting the production of most immunoglobulin isotypes except IgA [Lebman and Edmiston, 1999].

There are strong evidences to suggest the great importance of this cytokine in the control of autoimmunity [Aoki et al., 2005]. An association between *TGF-β1* and the development of autoimmunity is clearly demonstrated in studies with complete knockout of *TGF-β1* in mice or genetic manipulation of its receptors in T cells. TGF-β1 knockout mice and those with impaired TGF-β1 signaling in T cells develop an autoimmune syndrome with multiple organ involvement and death [Shull et al., 1992; Dang et al., 1995; Gorelik et al., 2000; Marie et al., 2006;]. This syndrome resembles SLE and Sjogren's syndrome in humans [Dang et al., 1995] and is characterized by multifocal inflammatory process affecting the heart, brain, lungs, skeletal muscle, liver, stomach, pancreas, salivary glands and other organs, lymfoproliferation, spontaneous activation of autoreactive T lymphocytes and production of autoantibodies [Dang et al., 1995; Shull et al., 1992].

3. Functional IL-10 and TGF-β1 genetic polymorphisms

3.1. IL-10 genetic polymorphisms and IL-10 production

Human IL-10 is secreted mostly by antigen-presenting cells and Treg lymphocytes subset in response to several activation stimuli. This cytokine could be also constitutively produced at low levels by immune cells, mainly monocytes, macrophages and dendritic cells. IL-10 is a non-covalent homodimer of 36 kDa with two polypeptide chains and its gene, with GeneBank accession number: X78437, is located on chromosome 1 at 1q31- q32 and a number of polymorphisms in the promoter region have been characterized. In contrast to many other cytokines, the synthesis of IL-10 is regulated by the transcription factors Sp1 and Sp3, which are constitutively expressed by different cell types [Moore et al. 2001]. It has been recently shown that c-Jun binds to a highly conserved noncoding sequences (CNS-3) in the IL10 locus, enhancing the expression of IL-10, and AP-1 signaling pathway particularly through c-jun transcription and activity strongly affects IL-10 expression in the Th2 cells and monocytes [Wang Z et al., 2005; Dobreva et al., 2009]. Large interindividual differences in the IL-10 inducibility have been observed, which has shown to have a genetic component of over 70%. The IL-10 gene comprises 5 exons, and to date, at least 49 IL10–associated polymorphisms have been reported, and an even larger number of polymorphisms are recorded in SNP databases (Ensembl Genome Browser, 2006). Promoter polymorphisms have been subject to the most studies, particularly with regard to possible influences on gene transcription and protein production. Three SNPs at -1082(A/G), -819(C/T), -592(C/A) upstream from the transcription start site [D'Alfonso et al., 1995; Turner et al., 1997] have been described as well as additional two microsatellite (CA)n repeats, termed IL10.G (-1.1Kb) and IL10.R (-4Kb) and located at -1151 and -3978 respectively (Eskdale and Galager, 1995; Eskdale et al., 1997).

A complete linkage disequilibrium exists between the alleles present at positions -1082, -819 and -592; so these polymorphisms occurred in tandem These SNPs have been associated with variability in IL-10 production [Eskdale et al., 1998; Turner et al., 1998]. In particular, SNP at position -1082A/G of IL-10 gene has been associated with IL-10 production alone or in haplotypes with other distal SNPs. Turner et al.,1997 have shown that

-1082A allele is associated with lower in vitro IL-10 production by Con A-stimulated PBMC from normal subjects. In our studies, the functional effect of -1082 A/G polymorphism was demonstrated among the Bulgarian population in both healthy volunteers and in patients with sepsis (Stanilova et al., 2006). In Caucasian populations, only three haplotypes have been found (GCC, ACC and ATA), the individuals GCC/GCC being considered as genetically high IL-10 producers [Suarez et al., 2005; Suarez et al., 2003; Turner et al., 1997]. Although the functional effects of polymorphisms in IL-10 have not yet been fully elucidated, obviously that they may play a significant role in modulating susceptibility, development and clinical fetures of autoimmune disease and particularly SLE. The observation of increased circulating levels of IL-10 in active SLE patients which revealed positive correlation with SLEDAI and anti-double-stranded DNA (dsDNA) titer has been reported (Park et al., 1998; Hye-Young et al., 2007). Moreover, a trend toward SLE patients having hypomethylated IL-10 promoter region accompanied by greater disease activity of SLE was recently observed (Lin et al., 2012).

3.2. TGF-β1 genetic polymorphisms and plasma concentrations of TGF-β1

The TGF-β1 gene is located on chromosome 19 (q13.1-13.3) and several SNPs were described so far in promoter region, in the non-translated region (introns), in the coding region (exons), and in the 3'-UTR region of the gene. They include three polymorphisms in the promoter region (-988C/A, -800G/A, and -509C/T), two polymorphisms located in exon 1 +869T/C (codon 10) and +915G/C (codon 25), one on exon 5 +11929C/T (codon 263), and one in 3'-UTR region of the gene at position +72 [Cambien et al., 1996]. Certain inherited variants in the promoter region of the TGF-β gene (-800G/A and -509C/T) have been associated with higher cytokine circulating concentrations. The -800G/A SNP is located in a consensus cyclic AMP response element binding protein (CREB) half site and may cause reduced affinity for CREB transcription factors whose binding is important for transcription control [Grainger at al. 1999]. The -509C/T is located within a YY1 consensus binding site and -509T allele has been associated with increased TGF-β1 plasma level [Grainger at al. 1999] and reduced T-cell proliferation [Meng et al., 2005] and a study of twins estimated that the -509C/T polymorphism explained approximately 8% of the genetic variation in TGF-β1 plasma levels (Grainger at al.,1999). Two SNPs, the +868T/C SNP, and the +915G/C SNP, give rise to amino acid substitutions at positions 10 (Leu10Pro) and 25 (Arg25Pro) in the signal peptide of TGF-β1, respectively (Cambien et al.,1996; Awad et al. 1998). The +868T/C SNP was reported to influence steady-state concentrations of *TGFB1* mRNA in peripheral blood mononuclear cells and serum levels of TGF-β1, and the +915G/C SNP was found to be related to TGF-β1 production in peripheral blood leukocytes (Awad et al., 1998). Another nonsynonymous SNP of *TGFB1*, the +11929C/T SNP (Thr[263]Ile) is located in exon 5 (Cambien et al.,1996; Awad et al.1998). The 11929C/T SNP is located closely to the site where the latency-associated peptide is cleaved from the active part of the protein (Dubois et al., 1995) and therefore, this SNP may be related to the activation process of TGF-β1, as suggested previously (Cambien et al. 1996). A high degree of linkage disequilibrium was observed between pairs of the −509C/T, 868T/C, 913G/C, and 11929C/T SNPs in white populations (Cambien et al.,1996; Grainger at al. 1999).

Although immunosuppressive effects of TGF-β1 have been well established, few studies have investigated serum TGF-β1 levels in autoimmune disorders. In fact, patient with SLE have reduced TGF-β1 production by their peripheral blood lymphocytes (Oshtuka et al., 1998). Hence, reduced TGF-β1 production by immune cells might contribute to the characteristic T cell disregulation, aberrant stimulation of autoreactive B cell, and autoantibody production in SLE patients. Also, it has been reported that decreased serum levels of TGF-β1 in patients with systemic lupus are the most pronounced and constant abnormality in the cytokine levels in these patients [Becker-Merok et al., 2010]. In attempt to elucidate the importance of TGF-β1 for the development of SLE we measured serum levels of TGF-β1 in 53 patients with SLE recruited from 'St Ivan Rilski' University Hospital, Sofia and in 66 healthy controls [unpublished data]. Serum samples were routinely collected and stored frozen at -20 C until assayed. At the time of sampling, neither of the patients and control subjects had clinical signs or symptoms of intercurrent illness. The concentrations of activated TGF-β1 protein in the serum samples of patients and controls were measured by quantitative sandwich ELISA technique, using commercially available kits (Qantakine®, R&D systems, Abingdon, UK). Before assay, the latent TGF-β1 contained in sera was activated to the immunoreactive form using acid activation and neutralization. The results were calculated by reference to the standard curve and expressed as ng/ml. Table 1 presents the serum concentrations of TGF-β1 in patients and healthy controls.

	TGF-β1 concentrations (mean±SD)	Significance
SLE		
total (n=53)	8.88 ± 3.79	
male (n=7)	8.57 ± 2.72	
female (n=46)	8.93 ± 3.95	male *vs* female p = 0.82
Healthy controls		
total (n=66)	32.99 ± 24.84	
male (n=13)	60.98 ± 31.32	
female (n=53)	26.12 ± 17.34	male *vs* female p <0.001
Significance	SLE *vs* HC p<0.001	

Table 1. *TGF-*β1 concentrations (ng/ml 9) in SLE patients and healthy controls.

Our results showed significantly lower levels of active TGF-β1 in lupus patients compared with healthy individuals (p<0.001). These data are in principal agreement with other studies [Jin et al., 2011; Lu et al., 2004]. In addition to the decreased TGF-β1 serum concentrations in SLE patients, the authors of these studies also reported an association between lower TGF-β1 and disease activity, as well as the development of organ damage [Jin et al., 2011; Lu et al., 2004]. Taken together, these observations support the role of TGF-β1 in SLE pathogenesis and modulation of disease expression. In our study, we also observed a large interindividual

variation in serum TGF-β1 levels among healthy subjects. This variation might be partly due to the possible influence of some endogenous factors, such as *gender* and age on the production of *TGF-β1*. Given this supposition, we examined *TGF-β1* levels in the context of age and gender in both healthy and affected individuals. The highest levels of *TGF-β1* were found in men among the healthy subjects. In SLE patients such a correlation between serum levels of *TGF-β1* and gender was not observed (Table 1), which could be explained with fact, that only men individuals with genetic predisposition to lower *TGF-β1* production developed SLE or down regulation of *TGF-β1* by disease progress. Additionally, age was positively correlated with serum *TGF-β1* in healthy controls. *TGF-β1* serum levels were lower in individuals under 45 years and higher in those aged over 45 years (P<0.001) among healthy controls as shown in Figure 1. In SLE patients, there was not a relationship between age and serum *TGF-β1* levels. Thus, healthy individuals showed a pattern in which serum *TGF-β1* was higher in men and elder people. It seems likely that age- or gender-specific cytokine differences could play a role in the observed age- and gender-related incidence patterns observed in SLE [Petri M, 2002].These data allow us to hypothesis that high levels of serum TGF-β1 may protect against autoimmunity in men as well as low levels of serum TGF-β1 may predispose to the onset of autoimmunity in younger individuals.
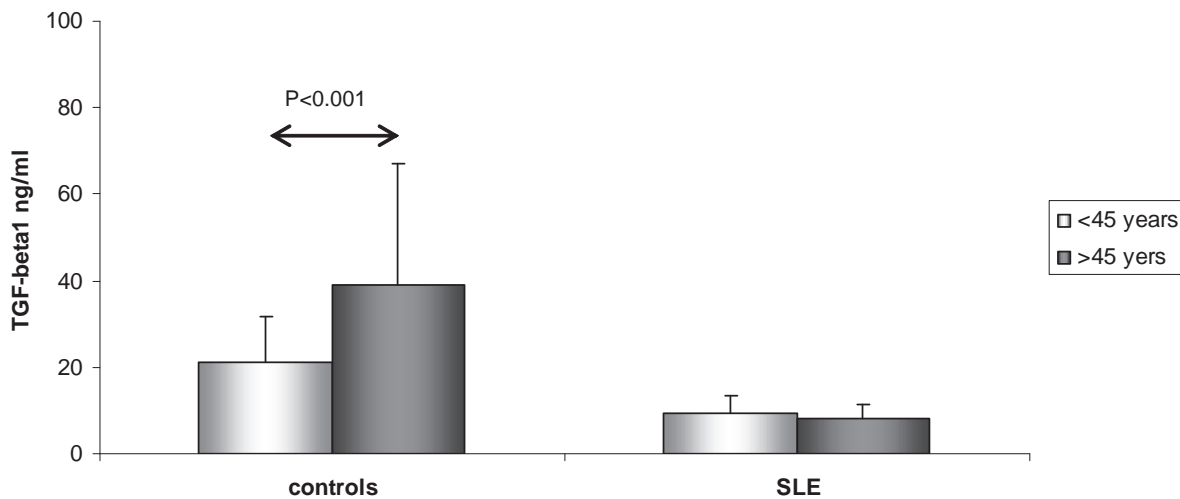

Figure 1. Mean (±SD) serum *TGF-β1* concentrations (pg/ml) in SLE patients and healthy controls according to the age of individuals.

Nowadays, it is considered that the control of TGF-β1 production is complex, but it has been estimated that 54% of its production is under genetic control [Grainger et al., 1999]. In this regard, we analyzed the serum levels of TGF-β1 in relation to various genotypes of -509C/T polymorphism of TGFB1 in 52 healthy controls (n = 21 for CC, 15 for CT, and16 for TT) and in 48 SLE patients (n = 17 for CC, 24 for CT, and 7 for TT). As the mean TGF-β1 levels varied significantly by case-control status (P < 0.001), patients and controls were proceeded seperetly for the genotype-serum levels analysis. The results are presented on Figure 2. Among healthy individuals the highest TGF-β1 concentration was detected in individuals with TT genotype (mean ± SD, 56.9 ± 28.6 ng/ml) compared to those with CC genotype (mean ± SD, 29.5 ± 21.1

ng/ml; p = 0.001) and those with CT genotype (mean ± SD, 25.7 ± 18.8; p = 0.002). Besides the level of serum TGF-β1 in patients was found to be lower than in control subjects (Figure 1), significantly higher TGF-β1 levels were observed in SLE patients having the TT genotype (mean ± SD, 9.8 ± 2.6 ng/ml) compared to patients with CC genotype (mean ± SD, 6.7 ± 2.6 ng/ml; p=0.023). Overall, individuals with TT homozygous genotype had higher serum TGF-β1 concentration in comparison to those with either CC homozygous genotype or CT heterozygous genotype.

The study of Awad and colleagues related the carriage of GG homozygous genotype in position +915 of TGFB1 to significantly higher TGF-β1 production in peripheral blood leukocytes [Awad et al., 1998]. However, Lu et al., 2004 also, analyzing the serum levels of TGF-β1 as well as the TGF-β1 production of unstimulated and stimulated peripheral blood mononuclear cells, did not observe functional correlations with TGF-β1 production and -509 and codon10 alleles. The authors raise the question whether the lower serum TGF-β1 level that cause defective immune regulation in SLE is primarily under genetic control or secondary to the influence of ongoing cellular interactions in the cytokine context. The data from our preliminary study shown that a number of factors such as age, sex, presence of disease, and allele variants of -509 C/T SNP in the gene for TGF-β1 influence the level of this cytokine in the serum. Thus, a genetically controlled low production of TGF-β1 is a predisposing factor for the loss of negative regulation found in SLE and may constitute an important component of the genetically determined susceptibility to this disease and the autoimmune events responsible for its pathogenesis, developed under appropriate environmental stimuli.

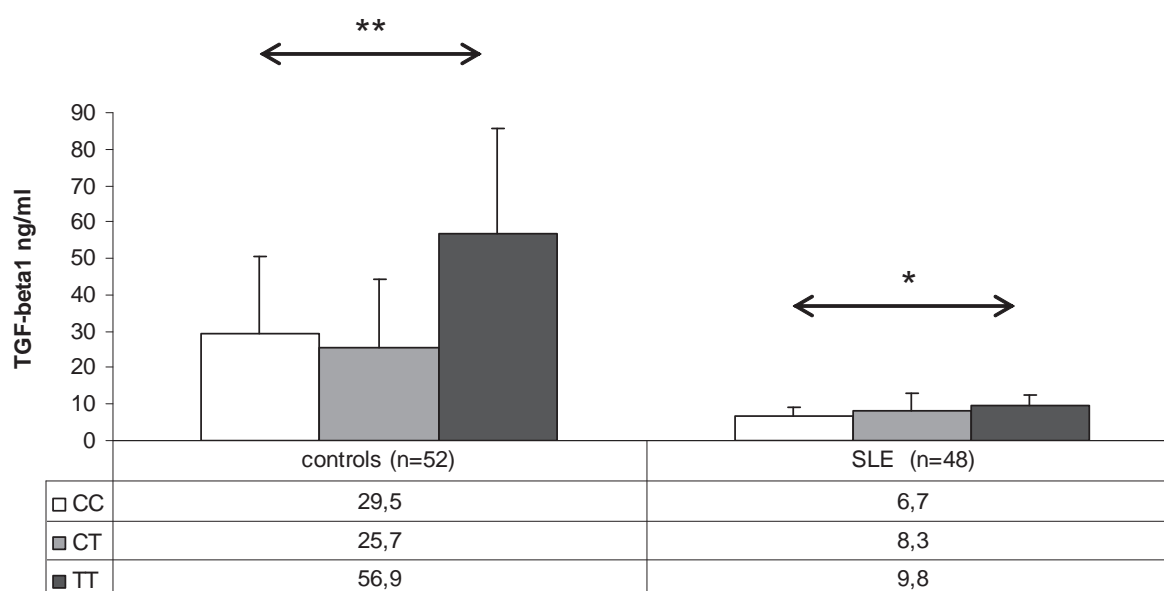|	controls (n=52)	SLE (n=48)
□ CC	29,5	6,7
■ CT	25,7	8,3
■ TT	56,9	9,8

Figure 2. Mean (± SD) serum *TGF*-β1 concentrations (ng/ml) in SLE patients and healthy controls according to the genotypes of -509 C/T polymorphism of *TGFB1*. * p < 0.05, ** p <.01

4. Role of IL-10 and TGF-β1 genotypes as risk factor for appearance of SLE

4.1. Role of IL-10 promoter polymorphism in susceptibility and clinical manifestation of SLE

Several evidences suggest that IL-10 could be a strong candidate gene influencing SLE susceptibility. IL-10 gene has been mapped to chromosome 1q31-32, which is a susceptibility region for SLE (LOD=3.79) [Johanneson et al., 2002]. It is also homologous to a murine SLE susceptibility region [Tsao et al., 1997]. More recently, Gateva et al., 2010 performed a large-scale replication study involving 1,310 cases and 7,859 controls, and identified 21 additional candidate susceptibility loci for SLE. Among the newly identified SLE loci is IL-10.

However, in spite of the considerable number of genetic studies performed, no definitive result about its involvement in SLE susceptibility was achieved. Some works showed significant associations between IL-10 microsatellites or SNPs with SLE susceptibility or with the development of certain clinical or immunological features [Rood et al., 1999; D'Alfonso et al., 2002; Schotte et al., 2004; Chen et al., 2006; Sung et al., 2006; Chong et al., 2006; Rosado et al., 2008] while other studies indicated that these polymorphisms did not appear to have any relevance in the disease [Alarcón-Riquelme et al., 1999; Van der Linden et al., 2000; Dijstel-bloem et al., 2002; Guarnizo-Zuccardi et al., 2007]. The role of IL-10 genotypes has been recently reviewed by Lopez et al., 2010. The more recent association studies dealing IL-10 promoter polymorphisms for SLE susceptibility are summarized on Table 2.

With respect to microsatellite variants, different alleles of IL10.G have been reported to be associated with SLE incidence in various populations. Thus, frequency of IL10.G9 allele (21 CA repeats) was significantly decreased in European [D'Alfonso et al., 2000, D'Alfonso et al., 2002, Eskdale et al., 1997] and Mexican-American [Mehrian et al., 1998] SLE patients, whereas the long alleles IL10.G10, G11 and G13 (with a CA repeat number greater than 21) were significantly increased in Mexican-American [Mehrian et al., 1998], Italian [D'Alfonso et al., 2000, D'Alfonso et al., 2002] and British [Eskdale et al., 1997] patients respectively. On the contrary, an increase in IL10.G4 (short allele) was reported in Chinese patients [Chong et al., 2004] whereas no significant differences in IL10.G alleles were detected in other cohorts [Schotte et al., 2004, Alarcon-Riquelme et al., 1999; Johansson et al., 2002]. Recently, a large meta-analysis summarized the results focused on the role of IL-10 promoter polymorphisms for SLE susceptibility from 16 published case-control studies involving a total of 2391 SLE patients and 3483 controls (Nath et al., 2005). The results of the meta-analysis performed by Nath et al., 2005 showed a significant association between SLE and the G11 allele of IL10.G (OR=1.279, 95% CI; 1.027±1.593, P=0.028) in whole populations, and IL-10 promoter -1082G allele was associated with SLE in Asians (OR=1.358, 95% CI; 1.015±1.816, P=0.039). It has been reported that LPS-stimulated cells from individuals carriers of the IL10.G allele with 26 CA repeats presented higher IL-10 production than those from carriers of short alleles [Eskdale et al., 1998], suggesting that long alleles might be responsible for a high IL-10 production. Thus, accordingly to these data, high IL-10 producer genotypes (with more than 21 CA repeats) could be associated with SLE susceptibility, while presence of short alleles could confer a protective effect [Chen et al., 2006; D'Alfonso et al., 2002].

Polymoprhisms	Association	SLE/contr.	Population	References
IL10G, IL10R -1082; -819; -592	Association of IL10.G11 allele in whole populations; Association of -1082G allele with SLE in Asians	2391/3483	Meta-analysis	Nath et al., 2005
-592 A/C	No association with susceptibility Association of -592C with SLE activity	350/330	Korean	Sung et al., 2005
IL10G	Increased G9 and decreased G8 in SLE Association of G13 with ACLA-IgM Association of G8 with CNS lupus	237/304	Taiwanese	Chen et al., 2006
-1082, -819, -592	Association of ACC/ACC with susceptibility to SLE	195/159	Thai	Hirankarn et al., 2006
-1082; -819; -592	No association	120/102	Colombian	Guarnizo-Zuccardi et al., 2007
IL10G, IL10R -1082; -819; -592	No association of microsatellites Increased GCC in SLE	116/51	Spanish	Rosado et al., 2008
-1082; -819; -592	Increased GCC in SLE	103/300	Polish	Sobkowiak et al., 2009
-1082; -819; -592 -592 A/C	No association	110/138	Chinese	Yu et al., 2010
-1082; -819; -592	Increased ATA haplotype in sLE	172/215	Taiwanese	Lin et al., 2010
-1082	No association	157/126	Bulgarian	Miteva et al., 2010

Table 2. Association of IL-10 promotor polymorphisms - IL10G, IL10R, -1082G/A (rs1800896), -819C/T (rs1800871), -592A/C (rs1800872), with SLE. Association studies published after 2005 years are given only.

Conflicting results were also obtained after examining the possible association between SLE susceptibility and SNPs at -1082, -819, and -592 positions of IL-10 gene in the different populations in which they were investigated. The frequency of high IL-10 producers (carriers of -1082G allele or GCC haplotype) has been found to be increased in several works with Asian [Nath et al., 2005; Hirankarn et al., 2006] or European [Rosado o et al., 2008; Sobkowiak et al., 2009] patients, although most of the studies performed in Caucasian populations did not show significant associations [Lazarus et al. 1997, Guarnizo-Zuccardi et al, 2007;, Koss et al., 2000; Suárez et al., 2005; Dijstelbloem et al., 2002; Van der Linden et al., 2000; Crawley et al., 1999].

Rosado et al., 2008 found that the GCC haplotype frequency was significantly higher in Spanish patients with SLE. To assess the functional role of genotypes, they also quantified serum IL-10 levels from patients and controls and found higher serum IL-10 levels in patients. On the basis of these data, they suggest that the IL-10 promoter haplotype that produces higher levels of cytokine is associated with SLE in Spanish population. Similar are the results and final conclusion of the study performed by Sobkowiak et al., 2009; despite the higher prevalence of the GCC/GCC, GCC/ATA and ATA/ATA genotypes in SLE patients than in controls, they

observed that only GCC/GCC genotype was significant more frequent in SLE. Hence, the conclusion suggests the GCC/GCC promoter genotype may contribute to SLE incidence in Polish patients. A very recent study investigated the association between of IL-10 promoter polymorphisms (-1082, -819 and -592) with SLE in a total of 172 Taiwanese patients and 215 controls reported an association of IL-10 ATA haplotype with SLE in Taiwanese population [Lin et al., 2010]. In another Asian population, an association of ACC/ACC haplotype of IL-10 in susceptibility to SLE has been observed by Hirankarn et al., 2006.

In this regard, we investigated the role of -1082A/G promoter polymorphism of IL-10 gene as risk factor for development and clinical manifestations of SLE in Bulgarian population. Our preliminary results did not reveal a significant association of -1082 SNP in IL-10 with SLE [Miteva et al., 2010]. New data for the distribution and the frequencies of the -1082A/G alleles and genotypes among the SLE patients and healthy controls are presented on Table 3. The results of our case-control study based on 157 patients with SLE and 166 unaffected control individuals showed that the genotype distribution is consistent with those published for other Caucasian type control cohorts [Lopez et al., 2010). We also found the prevalence of homozygous GG genotype in SLE cases (27%) compared to the controls (13%) with OR = 1.185 (95% CI = 0.58÷2.45), although the difference did not reach statistical significance (p=0.548). In addition, we observed an increased frequency of GG genotype compared to the reference AA genotype in patients with antiphospholipid synrom (APS) (27%) compared with patients without APS (14%) with OR = 2.750, 95% CI = 0.910 ÷ 8.347, p = 0.074). This suggests that carriage of a higher IL-10 producing genotype is a risk factor for antiphospholipid autoantibody production and APS appearance, thus having a modifying effect on the clinical presentation of the disease.

rs1800896-10 82 A/G IL10	Genotype						Allele	
	AA n (%)	AG n (%)	AG+AA n (%)	AG+GG n (%)	GG n (%)		A n (%)	G n (%)
SLE (n=157)	56 (36%)	74 (47%)	130 (73%)	101 (74%)	27 (27%)		186 (59%)	128 (41%)
						χ^2=0.203		
HC (n=166)	59 (36%)	83 (50%)	142 (86%)	107 (64%)	24 (14%)	p=0.934	201 (61%)	131 (39%)
OR (95% CI)	0.844 (0.41±1.72)	0.793 (0.40±1.56)	0.814 (0.43±1.54)	-	1 ref.		0.947 (0.68±1.31)	1 ref.
p		0.614	0.471	0.5	-			0.735
OR (95% CI)	1. ref	0.939 (0.56±1.57)	-	0.994 (0.61±1.61)	1.185 (0.58±2.42)		1 ref.	1.056 (0.76±1.47)
p		0.799	-	0.981	0.614			0.735

Table 3. Genotypic and allelic frequency of gene polymorphism at position -1082 A/G in IL-10 gene in SLE patients and controls

The presence of autoantibodies, mainly directed against nuclear antigens (ANAs), is one of the most characteristic features of SLE. The effect of IL-10 genotypes did not seem to be especially relevant, although it has been reported an increased prevalence of antibodies against several extractable nuclear antigens (anti-ENA) in patients with the allele IL-10.G9 [Eskdale, et al., 1997], and the presence of anti-Sm antibodies was found significantly overrepresented among patient carriers of G14 and G15 alleles and R2-G15 and R2-G14 haplotypes [Schotte et al., 2004]. An association of the carriage of low IL-10 producer alleles such as IL10.G13 allele with the presence of anticardiolipin IgM antibodies and IL10.G8 allele with neurological affectation has been reported in Taiwanese patients with SLE [Chen et al., 2006], results very similar to the data from our study.

Considering increased circulating levels of IL-10 have been consistently reported in the sera of patients with SLE, it is possible that different cytokine production may not only influence the autoantibody production, but also the clinical presentation of the disease. However, there were no definitive data on the association of IL-10 polymorphisms and specific clinical manifestations, probably due to the heterogeneity of the disease. For instance, renal involvement has been associated with both high (GCC) [Lazarus et al., 1997, Zhu et al., 2005] and low (ATA) [Mok et al., 1999] IL-10 producer genotypes. High prevalence of neuropsychiatric [Rood et al., 1999; Chen et al. 2006] and cardiovascular disorders [Fei et al., 2004] has been reported in patients with low genetic production whereas high IL-10 production has been linked to an increased incidence of serositis, hematological disorder [Chong et al.], SLICC/ACR Damage Index [Sung et al., 2006] and presence of discoid or mucocutaneous lesions [Suárez et al., 2005; Alarcón-Riquelme et al.,1999]. This last association was supported by the increased frequency of the high producer -1082G allele observed in patients with discoid lupus erythematosus [Suárez et al., 2005; Van der Linden et al., 2000] and by the fact that cutaneous manifestations improved in SLE patients under anti IL-10 monoclonal antibody treatment [Llorente et al., 2000]. In conclusion IL-10 promoter SNPs alone have not exhibits strong association with SLE susceptibility, but their role cant' be excluded. Each polymorphism in regulatory regions of gene, may either directly influence gene expression or indirectly via tight linkage with other polymorphisms occurring elsewhere in the same or in other cytokine gene. A particular combination of SNPs on cytokine genes in individual genotype has different impacts on induced cytokine production. Miteva and Stanilova investigate the combined effect of -1082A*G in IL10 and +16974A*C in IL12B SNPs on induced cytokine production by stimulated peripheral blood mononuclear cells isolated from healthy donors (Miteva, Stanilova, 2008). Results demonstrated that the production of IL-10 from PBMC depended on both, -1082A*G in IL10 and +16974A*C in IL12B polymorphisms and the presence of high producer IL-12p40 genotype led to diminished production of IL-10 determined by -1082*G-allele of SNP in IL10. In the same vein, we suppose that individuals with genotype which combine SNPs responsible for higher production of IL-10 simultaneously with lower production of TGF-β1 should be more susceptible to SLE.

4.2. Role of TGB-β1 genetic polymorphisms in susceptibility and clinical manifestation of SLE

Regarding the association between decreased *TGF-β1* serum levels and the development of autoimmunity, the mechanisms which control the concentration of *TGF-β1* in plasma are under extensive investigations. The concentration of both latent complex and active *TGF-β1* in plasma has been shown to be predominantly under genetic control [Grainger et al., 1999]. In light of these findings TGF-β1 gene is a functional candidate gene for genetic predisposition in systemic lupus erythematosus.

The presence of polymorphisms in the *TGFB1* locus may indicate predisposition to diseases, such as systemic lupus erythematosus that have been linked here and elsewhere [Caserta et al., 2004; Lu et al., 2004] to the circulating levels of TGF-β1. To test this hypothesis, we performed a population based case-control study to investigate the association of -509C/T polymorphism of the *TGFB1* gene with susceptibility to SLE [Manolova et al., 2012]. In this investigation, the change at position -509C/T in the *TGFB1* gene (rs1800469) was studied using RFLP-PCR among 147 cases with SLE and 134 normal Bulgarian subjects. The genotype distribution and allele frequencies of -509C/T SNP in gene promoter of *TGFB1* among SLE patients and healthy donors are presented in Table 4.

rs1800469 -509C/T TGFB1	Genotype						Allele	
	CC n (%)	CT n (%)	CT+CC n (%)	CT+TT n (%)	TT n (%)		C n (%)	T n (%)
SLE (n=149)	48 (32.2%)	79 (53%)	127 (85.2%)	101 (67.8%)	22 (14.8%))		175 (58.7%)	123 (41.3%)
HC (n=134)	48 (35.8%)	57 (42.5%)	105 (78.3%)	86 (64.1%)	29 (21.6%)	$\chi^2=3.735$ p=0.155	153 (57.1%)	115 (42.9%)
OR (95% CI)	1.318 (0.63-2.77)	1.827 (0.91±3.69)	1.59 (0.83±3.07)	-	1 ref.		1.069 (0.76±1.5)	1 ref.
p	0.428	0.068	0.133	-			0.694	
OR (95% CI)	1 ref.	1.386 (0.79±2.43)	-	1.17 (0.7±1.98)	0.759 (0.36±1.59)		1 ref.	0.935 (0.66±1.33)
p		0.223	-	0.522	0.428			0.694

Table 4. Genotypic and allelic frequency of gene polymorphism at position -509 in *TGFB1* gene in SLE patients and controls

The genotype distribution for TGFB1 -509C/T polymorphism was in agreement with Hardy-Weinberg equilibrium among cases ($\chi2$=2.00; p=0.367) and controls ($\chi2$=2.237; p=0.326). Homozygous CC genotype was found in 32.2% of patients and 36.8% of control subjects, heterozygous CT genotype was observed in 53% of SLE patients and 42.5% of controls, homozygous TT genotype was detected in 14.8% of cases and 21.6% of controls. There were no significant differences in the genotype (p=0.155) and allele (p=0.694) frequencies of -509C/T polymorphism of the TGFB1 gene between SLE patients and controls. However, we observed a higher frequency of heterozygous CT genotype (OR = 1.827; 95% CI 0.91±3.69; p = 0.068) and lower frequency of TT genotype (OR = 0,759; 95% CI: 0.36±1.59; p=0.428) in SLE patients compared to healthy controls. In logistic regression analysis the presence of allele C in the genotype (CT + CC versus TT) was associated with a 1.6 times higher risk of developing systemic lupus erythematosus.

Genotype and allele frequencies of -509C/T polymorphism in *TGFB1* which we established in Bulgarian population were comparable to those found in other populations. However, the available data in the literature reveal the existence of ethnic differences in frequencies of the allele variants of this polymorphic marker (Table 5). C allele had the higher representation among Europeans in French [Cambien et al., 1996], German [Wu et al., 2008] and British [Awad et al., 1998] studies with a frequency of 65 to 76 percent, while among healthy individuals from different Asian ethnicities T allele occurs more frequently or almost equally with the C allele [Amirghofran Z, 2009; Zhang et al., 2009; Chung et al.,, 2007]. In Bulgarian population allele C is slightly more common and was found in 57% of healthy subjects and in 59% of cases with SLE.

Locus	Allele	Frequency (%) among healthy controls				
		Bulgarian study Data from this study	French study Cambien et al., 1996	British study Awad et al., 1998	German study Wu et al., 2009	Asian study Lu et al., 2004
-509	C	57	66	76	70	48
	T	43	34	24	30	52

Table 5. Interethnic differences in allele frequencies of -509C/T SNP in *TGFB1* in healthy controls.

According to our knowledge there are only a limited number of studies aiming to evaluate the possible role of polymorphisms in the TGF-β1 gene as predisposing factors for SLE, but the results of these studies are contradictory. The polymorphisms in of -509C/T SNP in TGF-β1 have been explored in SLE only by three research teams [Lu et al., 2004; Caserta et al., 2004; Vuong et al., 2010]. In overall, the contradictory results in the literature could be explained by the genetic heterogeneity of SLE in different populations and possible sample stratification. Table 6 summarizes the association studies dealing TGF-β1 gene polymorphisms for SLE susceptibility.

Polymoprhisms	Association	SLE/contr.	Population	References
+915G/C (rs1800471)	No association	203/158	German	Schotte et al., 2003
-988C/A (rs2241712), -800G/A (rs1982072), -509C/T (rs1800469), +869T/C (rs1982073), +915G/C (rs1800471)	No association	138/182	Taiwanese	Lu et al., 2004
-509C/T (rs1800469)	No association	23/32	North America	Caserta et al., 2004
+869T/C (rs1982073), +915G/C (rs1800471)	Association of +869C allele with SLE; decreased high TGF-β1 producers haplotypes in SLE (codon10 T allele/25 G allele) No association with clinical manifestations	120/102	Colombian	Guarnizo-Zuccardi et al., 2007
+869T/C (rs1982073)	No association with susceptibility +869TT associated with aseptic necrosis and anti-Ro antibodies	196/102	Japan	Wang et al., 2007
-509C/T (rs1800469) +869T/C (rs1982073) intron G/T (rs2241715) 3'-UTR A/G (rs6957)	No association	272/307	Sweden	Vuong et al., 2010
-509C/T (rs1800469)	Decreased frequency of TT (OR = 0,759; 95% CI: 0.36±1.59) allele C (CT+CC vs TT) risk factor for SLE (OR=1.59; 95% CI: 0.83±3.07)	149/134	Bulgarian	Manolova et al. 2012

Table 6. Association of TGF-β1 gene polymorphisms with SLE.

Lu et al. conducted a case-control study involving 134 patients and 182 healthy individuals of Taiwanese origin to evaluate the association of -509C/T SNP in *TGFB1* with susceptibility to systemic lupus erythematosus [Lu et al., 2004]. In the same study, authors investigated also association of some others TGFβ1 single nucleotide polymorphisms, including -988C/A, -800G/A, +869T/C (Leu10Pro), and +915 G/C (Arg25Pro) with susceptibility to SLE and shown that none of the TGFβ1 SNPs was strongly associated with SLE in Taiwanese patients. They conclude that these polymorphisms do not represent a genetic predisposition to SLE. In another study, Schotte and colleagues investigated the +915G/C polymorphism at codon25 and did not found any association between this polymorphism and SLE in German population [Schotte et al., 2003]. In addition, authors found no association of major disease manifestations or specific autoantibodies with TGFB1 genotypes or alleles. The authors conclude that +915G/C polymorphism in TGFB1 neither significantly contributes to the disease susceptibility, nor predisposes to clinical and immunological manifestations typical of SLE. Also, there were no significant associations between several SNPs from the TGFβ1 including -509C/T, +869T/C,

intronic G/T (rs2241715), and3'-UTR A/G(rs6957) with SLE or with lupus nephritis in Sweden population [Vuong et al., 2010].

In contrast to these data are the results obtained by Guarnizo-Zuccardi et al., 2007 for several cytokine gene polymorphisms in Colombian patients with SLE. They analyze the relation between the +868T/C and the +915G/C SNP in *TGFB1* with the development and clinical manifestations of SLE. The authors found a strong association of SLE with the TGF-β1 codon25 C allele, associated with decreased TGF-β1 production and found lower rates of higher-producing GG genotype and a higher frequency of heterozygous genotypes in this polymorphic marker in patients with SLE. As for the +868T/C polymorphism at codon10, Guarnizo-Zuccardi et al., 2007 did not observe association between SLE and codon10 when analyzing independently, but they found a significant association when the haplotypes codon10/20 were evaluated, which could be because of the linkage disequilibrium between the two SNPs. This extended genotypic analysis revealed a lower frequency of high TGF-β1 producers – haplotype 10/25 T/T-G/G in Colombian patients with SLE. Unlike the relationship of +868T/C and +915G/C SNPs of the *TGFB1* gene to disease susceptibility, they found no association between clinical features of the disease and the polymorphisms studied. As opposed to this report, Wang et al., 2007 did not find an impact of +869T/C polymorphism in TGF-β1 gene on disease susceptibility in population-based case-control study involving 196 patients with SLE and 106 healthy controls in Japan. However, they found an association between +869T/C *TGFB1* polymorphism and several clinical features of SLE. The carriage of TT genotype of +869T/C polymorphism which is associated with a lower serum TGF-β1 level was related to the occurrence of aseptic necrosis and higher incidence of anti-SSA/Ro antibodies in SLE patients. Consistent with the last finding, the children with the TT +869T/C genotype of *TGFB1* gene have been reported to be more susceptible to anti-SSA/Ro antibody-associated congenital hear block [Clancy et al., 2003].

Systemic lupus erythematosus is a heterogeneous disease with diverse clinical manifestations that range could be due to genetic factors. In this regard, we also analyzed the effect of the -509C/T polymorphism in TGFB1 the clinical manifestations evolved in the course of the disease [Manolova et al., 2012]. The results of our study demonstrated a weak association of -509C/T polymorphism of TGFB1 with clinical manifestations of SLE. The carriage of the heterozygous genotype was associated with about 2-fold higher risk for the occurrence of hematological manifestations (OR=2.41; 95%CI: 1.10±5.32; p=0.016) and antibodies against dsDNA (OR = 2.0; 95% CI: 0.96±4.2, p = 0.045) in lupus patients, while the CC genotype is a protective factor for these events. Based on our and others data, we could assume the TGFβ1 gene polymorphisms as one of the genetic factors that explain the heterogeneity seen in SLE.

5. Conclusions

In recent years, efforts have been made to identify genes involved in the genetic predisposition and severity of SLE. During the last two decades, many of the 'candidate' cytokine genes

implicated in SLE development have been identified and was summarized in this review. SLE is clinically heterogeneous and genetically complex, and we expect that individual genes and cytokine patterns will be more or less important to different disease manifestations and subgroups of patients. Defining these genotype-cytokine-phenotype relationships will increase our understanding of both initial and progression disease pathogenesis.

In ours and others studies have been analyzed the association of IL-10 and TGF-β1genetic variants with susceptibility to and outcome of SLE, showing variable results in most cases. However, it is known that the actions of cytokines may be profoundly conditioned by the presence of other cytokines, this being particularly true in the case of IL-10 and TGF-β1. New studies of particular combinations of SNPs on these cytokine genes in individual genotype and their impacts on the induced cytokine production could reveal the relation with susceptibility and clinical presentation of autoimmunity diseases.

Author details

Irena Manolova[1], Mariana Ivanova[2] and Spaska Stanilova[3]

1 Department of Health Care, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

2 Clinic of Rheumatology, University Hospital, Medical University, Sofia, Bulgaria

3 Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

References

[1] Alarcón-Riquelme ME, Lindqvist AK, Jonasson I, Johanneson B, Sandino S, Alcocer-Varela J, Granados J, Kristjánsdóttir H, Gröndal G, Svenungsson E, Lundberg I, Steinsson K, Klareskog L, Sturfelt G, Truedsson L, Alarcón-Segovia D, Gyllensten UB. Genetic analysis of the contribution of IL10 to systemic lupus erythematosus. J Rheumatol 1999;26(10):2148-52.

[2] Amirghofran, Z, Jalali, S. A, Ghaderi, A, & Hosseini, S. V. Genetic polymorphism in the transforming growth factor beta1 gene (-509 C/T and-800 G/A) and colorectal cancer. Cancer Genet Cytogenet (2009). , 190(1), 21-5.

[3] Aoki, C. A, Borchers, A. T, Li, M, Flavell, R. A, Bowlus, C. L, Ansari, A. A, & Gershwin, M. E. Transforming growth factor beta (TGF-beta) and autoimmunity. Autoimmun Rev (2005). , 4(7), 450-9.

[4] Awad, M. R, Gamel, A, Hasleton, P, Turner, D. M, Sinnott, P. J, & Hutchinson, I. V. Genotypic variation in the transforming growth factor-beta1 gene: association with

transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. Transplantation (1998). , 66(8), 1014-20.

[5] Becker-merok, A, Eilertsen, G. Ø, & Nossent, J. C. Levels of transforming growth factor-beta are low in systemic lupus erythematosus patients with active disease. J Rheumatol (2010). , 37(10), 2039-2045.

[6] Bidwell, J, Keen, L, Gallagher, G, Kimberly, R, Huizinga, T, Mcdermott, M. F, Oksenberg, J, Mcnicholl, J, & Pociot, F. Hardt C & D'Alfonso S. Cytokine gene polymorphism in human disease: on-line databases. Genes and immunity (1999). , 1, 3-19.

[7] Cambien, F, et al. Polymorphisms of the transforming growth factor-beta 1 gene in relation to myocardial infarction and blood pressure. The Etude Cas-Témoin de l'Infarctus du Myocarde (ECTIM) Study. Hypertension (1996). , 28(5), 881-7.

[8] Caserta, T. M, Knisley, A. A, Tan, F. K, Arnett, F. C, & Brown, T. L. Genotypic analysis of the TGF beta-509 allele in patients with systemic lupus erythematosus and Sjogren's syndrome. Ann Genet (2004). , 47(4), 359-63.

[9] Chen, J. Y, Wang, C. M, Lu, S. C, Chou, Y. H, & Luo, S. F. Association of apoptosis-related microsatellite polymorphisms on chromosome 1q in Taiwanese systemic lupus erythematosus patients. Clin Exp Immunol (2006). , 143(2), 281-7.

[10] Chong, W. P, Ip, W. K, Wong, W. H, Lau, C. S, Chan, T. M, & Lau, Y. L. Association of interleukin-10 promoter polymorphisms with systemic lupus erythematosus. Genes Immun (2004). , 5(6), 484-92.

[11] Chung, S. J, Kim, J. S, Jung, H. C, & Song, I. S. Transforming growth factor-[beta]1-509T reduces risk of colorectal cancer, but not adenoma in Koreans. Cancer Sci (2007). , 98(3), 401-4.

[12] Clancy, R. M, Backer, C. B, Yin, X, Kapur, R. P, Molad, Y, & Buyon, J. P. Cytokine polymorphisms and histologic expression in autopsy studies: contribution of TNF-alpha and TGF-beta 1 to the pathogenesis of autoimmune-associated congenital heart block.J Immunol. (2003). Sep 15;, 171(6), 3253-61.

[13] Crawley, E, Woo, P, & Isenberg, D. A. Single nucleotide polymorphic haplotypes of the interleukin-10 5' flanking region are not associated with renal disease or serology in Caucasian patients with systemic lupus erythematosus. Arthritis and Rheumatism (1999). , 42(9), 2017-2018.

[14] Alfonso, D, Giordano, S, Mellai, M, Lanceni, M, Barizzone, M, Marchini, N, Scorza, M, Danieli, R, Cappelli, M. G, Rovere, M, Sabbadini, P, & Momigliano-richiardi, M. G. P. Association tests with systemic lupus erythematosus (SLE) of IL10 markers indicate a direct involvement of a CA repeat in the 5' regulatory region. Genes Immun (2002). , 3(8), 454-63.

[15] Alfonso, D, Rampi, S, Bocchio, M, Colombo, D, Scorza-smeraldi, G, & Momigliano-richardi, R. P. Systemic lupus erythematosus candidate genes in the Italian popula-

tion: evidence for a significant association with interleukin-10. Arthritis Rheum (2000). , 43(1), 120-8.

[16] Alfonso, D, Rampi, S, Rolando, M, Giordano, V, & Momigliano-richiardi, M. New polymorphisms in the IL-10 promoter region. Genes Immun 2000;1:231-233., 2000.

[17] Dang, H, Geiser, A. G, Letterio, J. J, Nakabayashi, T, Kong, L, Fernandes, G, & Talal, N. SLE-like autoantibodies and Sjögren's syndrome-like lymphoproliferation in TGF-beta knockout mice. J. Immunol (1995).

[18] De Waal Malefyt RDYssel H, de Vries JE. Direct effects of IL-10 on subsets of human CD4+ T cell clones and resting T cells. Specific inhibition of IL-2 production and pro-liferation. J. Immunol (1993). , 150(11), 4754-65.

[19] Dijstelbloem, H. M, Hepkema, B. G, et al. The R-H polymorphism of Fcγ receptor IIa as a risk factor for systemic lupus erythematosus is independent of single-nucleotide polymorphisms in the interleukin-10 gene promoter. Arthritis and Rheumatism (2002). , 46(4), 1125-1126.

[20] Ding, L, Linsley, P. S, Huang, L-Y, Germain, R. N, & Shevach, E. M. IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. Journal of Immunology (1993). , 151(3), 1224-1234.

[21] Diveu, C, Mcgeachy, M. J, & Cua, D. J. Cytokines that regulate autoimmunity. Curr Opin Immunol (2008). , 20(6), 663-8.

[22] Dobreva, Z. G, Miteva, L. D, & Stanilova, S. A. The inhibition of JNK and MAPKs downregulates IL-10 and differentially affects c-Jun gene expression in human mono-cytes. Immunopharmacol Immunotoxicol (2009). , 38.

[23] Dubois, C. M, Laprise, M-H, Blanchette, F, Gentry, L. E, & Leduc, R. Processing of transforming growth factor β1 precursor by human furin convertase. J Biol Chem (1995). , 270, 0618-10624.

[24] Eskdale J & Galager GA polymorphic dinucleotide repeat in the human IL-10 pro-moter region. Immunogenetics (1995). , 42, 444-445.

[25] Eskdale, J, Gallagher, G, Verweij, C. L, & Keijsers, V. Westendorp RGJ, and. Huizinga TWJ. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci U S A (1998). , 95(16), 9465-70.

[26] Eskdale, J, Kube, D, Tesch, H, & Gallagher, G. Mapping of the human IL10 gene and further characterization of the 5' flanking sequence. Immunogenetics (1997). , 46, 120-128.

[27] Eskdale, J, Wordsworth, P, Bowman, S, Field, M, & Gallagher, G. Associations be-tween polymorphism at the human IL-10 locus and systemic lupus erythematosus. Tissue Antigens (1997). , 49, 635-9.

[28] European Bioinformatics InstituteSanger Institute. SNP database: Ensembl Genome Browser. (2006). Available at: http://www.ensembl.org/index.html.

[29] Fainaru, O, Shay, T, Hantisteanu, S, Goldenberg, D, Domany, E, & Groner, Y. TGFbeta-dependent gene expression profile during maturation of dendritic cells. Genes Immun (2007). , 8(3), 239-244.

[30] Fei, G-Z, Svenungsson, E, Frostegård, J, Padyukov, L, & The, A. IL-10 allele is associated with cardiovascular disease in SLE. Atherosclerosis (2004). , 177(2), 409-414.

[31] Fiorentino, D. F, Bond, M. W, & Mosmann, T. R. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. Journal of Experimental Medicine (1993). , 170(6), 2081-95.

[32] Flanders, K. C, & Roberts, A. B. TGF-β. In:Ippenheim JJ, Feldmann M (eds). Cytokine Reference, Academic Press: San Diego, CA, (2001). , 1

[33] Gateva et alA large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. Nat Genet (2009). , 41(11), 1228-33.

[34] Gorelik L Flavell RAAbrogation of TGFbeta signalling in T cells leads to spontaneous T cell differentiation and autoimmune disease. Immunity (2000). , 12(2), 171-181.

[35] Grainger, D. J, Heathcote, K, Chiano, M, Snieder, H, Kemp, P. R, Metcalfe, J. C, Carter, N. D, & Spector, T. D. Genetic control of the circulating concentration of transforming growth factor type β1. Hum Mol Genet (1999). , 8(1), 93-7.

[36] Guarnizo-zuccardi, P, Lopez, Y, Giraldo, M, Garcia, N, Rodriguez, L, Ramirez, L, Uribe, O, Garcia, L, & Vasquez, G. Cytokine gene polymorphisms in Colombian patients with systemic lupus erythematosus. Tissue Antigens (2007). , 70(5), 376-82.

[37] Hirankarn, N, Wongpiyabovorn, J, Hanvivatvong, O, Netsawang, J, Akkasilpa, S, Wongchinsri, J, Hanvivadhanakul, P, Korkit, W, & Avihingsanon, Y. The synergistic effect of FC gamma receptor IIa and interleukin-10 genes on the risk to develop systemic lupus erythematosus in Thai population.Tissue Antigens (2006). , 68(5), 399-406.

[38] Hollegaard MV & Bidwell JL(2006). Cytokine gene polymorphism in human disease: on-line databases, Supplement 3. Genes Immun 2006;, 7, 269-76.

[39] Houssiau, F. A, Lefebvre, C, Vanden, B. M, Lambert, M, Devogelaer, J-P, & Renauld, J-C. Serum interleukin 10 titers in systemic lupus erythematosus reflect disease activity. Lupus (1995). , 4(5), 393-395.

[40] Hye-young, C, Jae-wook, C, Hyoun-ah, K, Jeong-moon, Y, Ja-young, J, Young-min, Y, Seung-hyun, K, Hae-sim, P, Chang-hee, S, & Cytokine, I. L. and IL-10 as Biomarkers in Systemic Lupus Erythematosus. Journal of clinical immunology (2007). , 27, 461-466.

[41] Jin, T, Almehed, K, & Carlsten, H. Forsblad-d'Elia H. Decreased Serum Levels of TGF-β1 are associated with Renal Damages in Female Patients with Systemic Lupus Erythematosus. Lupus (2011). , 21(3), 310-8.

[42] Johanneson, B, Lima, G, Von Salomé, J, & Alarcón-segovia, D. Alarcón-Riquelme ME; Collaborative Group on the Genetics of SLE, The BIOMED II Collaboration on the Genetics of SLE and Sjögrens syndrome. A major susceptibility locus for systemic lupus erythemathosus maps to chromosome1q31. Am J Hum Genet (2002). , 71(5), 1060-71.

[43] Kariuki, S. N, & Niewold, T. B. Genetic regulation of serum cytokines in systemic lupus erythematosus. Transl Res (2010). , 155(3), 109-17.

[44] Koss, K, Fanning, G. C, Welsh, K. I, & Jewell, D. P. Interleukin-10 gene promoter polymorphism in English and Polish healthy controls. Polymerase chain reaction haplotyping using 3′ mismatches in forward and reverse primers. Genes and Immunity (2000). , 1(5), 321-324.

[45] Lazarus, M, Hajeer, A. H, Turner, D, Sinnott, P, & Worthington, J. Ollier WER, and Hutchinson IV, Genetic variation in the interleukin 10 gene promoter and systemic lupus erythematosus. Journal of Rheumatology (1997). , 24(12), 2314-2317.

[46] Lebman, D. A, & Edmiston, J. S. The role of TGF-beta in growth, differentiation, and maturation of B lymphocytes. Microbes Infect (1999). , 1(15), 1297-1304.

[47] Li MoWan YY, Flavell RA. T cell-produced transforming growth factor-beta1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. Immunity (2007). May;, 26(5), 579-91.

[48] Li, M. O, Wan, Y. Y, Sanjabi, S, Robertson, A. K, & Flavell, R. A. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol (2006). , 24, 99-146.

[49] Lin, Y. J, Wan, L, Huang, C. M, Sheu, J. J, Chen, S. Y, Lin, T. H, Chen, D. Y, Hsueh, K. C, Lai, C. C, Tsai, F. J, & Tnf-alpha, I. L. promoter polymorphisms in susceptibility to systemic lupus erythematosus in Taiwan. Clin Exp Rheumatol (2010). , 28(3), 318-24.

[50] LinS-Y et al., A whole genome methylation analysis of systemic lupus erythematosus: hypomethylation of the IL10 and IL1R2 promoters is associated with disease activity. Genes and Immunity (2012). , 13, 214-220.

[51] Liossis, S. N, Kovacs, B, Dennis, G, Kammer, G. M, & Tsokos, G. C. B cells from patients with systemic lupus erythematosus display abnormal antigen receptor-mediated early signal transduction events. J Clin Invest (1996). , 98(11), 2549-57.

[52] Llorente, L, Richaud-patin, Y, & Richaud-patin, Y. Dysregulation of interleukin-10 production in relatives of patients with systemic lupus erythematosus. Arthritis and Rheumatism (1997). , 40(8), 1429-1435.

[53] Llorente, L, Richaud-patin, Y, Garcia-padilla, C, Claret, E, Jakez-ocampo, J, & Cardiel, M. H. Clinical and biologic effects of anti-interleukin-10 monoclonal antibody administration in systemic lupus erythematosus. Arthritis and Rheumatism (2000). , 43, 1790-1800.

[54] Llorente, L, & Richaud-patin, Y. The role of interleukin-10 in systemic lupus erythematosus. J Autoimmun (2003). , 20(4), 287-9.

[55] López, P, Gutiérrez, C, & Suárez, A. IL-10 and TNFalpha genotypes in SLE. J Biomed Biotechnol (2010).

[56] Lu, L. Y, Cheng, H. H, Sung, P. K, Yeh, J. J, Shiue, Y. L, & Chen, A. Single-nucleotide polymorphisms of transforming growth factor-β1 gene in Taiwanese patients with systemic lupus erythematosus. J Microbiol Immunol Infect (2004).

[57] Mandik-nayak, L, Ridge, N, Fields, M, Park, A. Y, & Erikson, J. Role of B cells in systemic lupus erythematosus and rheumatoid arthritis. Curr Opin Immunol (2008). , 20(6), 639-45.

[58] Manolova, I, Ivanova, M, Aleksandrova, E, Miteva, L, Stoilov, R, Rashkov, R, & Stanilova, S. Association of transforming growth factor promoter polymorphism with systemic lupus erythematosus. Revmatologia (Sofia) (2012).

[59] Marie JC Liggitt DRudensky AY. Cellular mechanisms of fatal early-onset autoimmunity in mice with the T cell-specific targeting of transforming growth factor-beta receptor. Immunity (2006). , 25(3), 441-454.

[60] Mehrian, R, & Quismorio, F. P. Jr, Sstrassmann G, et al. Synergistic effect between IL-10 and bcl-2 genotypes in determining susceptibility to systemic lupus erythematosus. Arthritis Rheum (1998). , 41, 596-602.

[61] Meng, J, Thongngarm, T, Nakajima, M, Yamashita, N, Ohta, K, Bates, C. A, Grunwald, G. K, & Rosenwasser, L. J. Association of transforming growth factor-beta1 single nucleotide polymorphism C-509T with allergy and immunological activities. Int Arch Allergy Immunol (2005). , 138(2), 151-60.

[62] Miteva, L, & Stanilova, S. The combined effect of IL-10 and IL-12 polymorphisms on induced cytokine production. Human Immunology (2008). , 69, 562-566.

[63] Miteva, L, Manolova, I, Ivanova, M, Stoilov, R, Rashkov, R, & Stanilova, S. Lack of association between promoter polymorphism-1082A/G in Interleukin-10 gene and genetic predisposition to systemic lupus erythematosus. Revmatologia (Sofia) (2010). , 18(3), 33-38.

[64] Mok, C. C, Lanchbury, J. S, Chan, D. W, & Lau, C. S. Interleukin-10 promoter polymorphism in southern Chinese patients with systemic lupus erythematosus. Arthritis Rheum (1998). , 41, 1090-5.

[65] Moore, K. W. de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. (2001). , 19, 683-765.

[66] Moustakas, A, Pardali, K, Gaal, A, & Heldin, C. H. Mechanisms of TGF-β signaling in regulation of cell growth and differentiation. Immunol Lett (2002).

[67] Nakken, B, Alex, P, Munthe, L, Szekanecz, Z, & Szodoray, P. Immune-regulatory mechanisms in systemic autoimmune and rheumatic diseases. Clin Dev Immunol (2012).

[68] Nath, S. K, Harley, J. B, & Lee, Y. H. Polymorphisms of complement receptor 1 and interleukin-10 genes and systemic lupus erythematosus: a meta-analysis. Hum Genet (2005). , 118(2), 225-34.

[69] Ohtsuka, K, Gray, J. D, Stimmler, M. M, Toro, B, & Horwitz, D. A. Decreased production of TGF-b by lymphocytes from patients with systemic lupuis erythematosus. The Journal of Immunology (1998). , 160, 2539-2545.

[70] Park, Y. B, Lee, S. K, Kim, D. S, Lee, J, Lee, C. H, & Song, C. H. Elevated interleukin-10 levels correlated with disease activity in systemic lupus erythematosus. Clinical and Experimental Rheumatology (1998). , 16(3), 283-288.

[71] Petri, M. Epidemiology of systemic lupus erythematosus. Best Pract Res Clin Rheumatol (2002). , 16(5), 847-58.

[72] Pyzik M Piccirillo CATGF-beta1 modulates Foxp3 expression and regulatory activity in distinct CD4+ T cell subsets. J Leukoc Biol (2007). , 82(2), 335-346.

[73] Rood, M. J, Keijsers, V, & Keijsers, V. Neuropsychiatric systemic lupus erythematosus is associated with imbalance in interleukin 10 promoter haplotypes of the. Annals Rheumatic Diseases (1999). , 58(2), 85-89.

[74] Rosado, S, Rua-figueroa, I, Vargas, J. A, Garcia-laorden, M. I, Losada-fernandez, I, Martin-donaire, T, Perez-chacon, G, Rodriguez-gallego, C, Naranjo-hernandez, A, Ojeda-bruno, S, Citores, M. J, & Perez-aciego, P. Interleukin-10 promoter polymorphisms in patients with systemic lupus erythematosus from the Canary Islands. Int J Immunogenet (2008). , 35(3), 235-42.

[75] Rousset, F, Garcia, E, & Garcia, E. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proceedings of the National Academy of Sciences of the United States of America (1992). , 89(5), 1890-1893.

[76] Rubtsov, Y. P, & Rudensky, A. Y. TGFbeta signalling in control of T-cell-mediated self-reactivity. Nat Rev Immunol (2007). , 7, 443-453.

[77] Schotte, H, Gaubitz, M, Willeke, P, Tidow, N, Assmann, G, Domschke, W, & Schlüter, B. Interleukin-10 promoter microsatellite polymorphisms in systemic lupus erythematosus: association with the anti-Sm immune response. Rheumatology (Oxford) (2004). , 43(11), 1357-63.

[78] Schotte, H, Willeke, P, Rust, S, Assmann, G, Domschke, W, Gaubitz, M, & Schlüter, B. The transforming growth factor-beta1 gene polymorphism (G915C) is not associated with systemic lupus erythematosus.Lupus. (2003). , 12(2), 86-92.

[79] Schuster, N, & Krieglstein, K. Mechanisms of TGF-hmediated apoptosis. CellTissue Res (2002). , 307(1), 1-14.

[80] Selvaraj, R. K, & Geiger, T. L. A kinetic and dynamic analysis of Foxp3 induced in T cells by TGF-beta. J Immunol (2007). , 179(2), 7667-7677.

[81] Shull, M. M, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature (1992). , 359(6397), 693-699.

[82] Sobkowiak, A, Lianeri, M, Wudarski, M, Lacki, J. K, & Jagodzinski, P. P. Genetic variation in the interleukin-10 gene promoter in Polish patients with systemic lupus erythematosus. Rheumatol Int (2009). , 29(8), 921-5.

[83] Stanilova, S. A, Miteva, L. D, Karakolev, Z. T, & Stefanov, C. S. Interleukin-10-1082 promoter polymorphism in association with cytokine production and sepsis susceptibility. Intensive Care Med (2006). , 32(2), 260-6.

[84] Suarez, A, Castro, P, Alonso, R, Mozo, L, & Gutierrez, C. Interindividual variations in constitutive interleukin-10 messenger RNA and protein levels and their association with genetic polymorphisms. Transplantation (2003). doi:TP. 0000055216.19866.9A., 75, 711-717.

[85] Suarez, A, Lopez, P, Mozo, L, & Gutierrez, C. Differential effect of IL10 and TNF{alpha} genotypes on determining susceptibility to discoid and systemic lupus erythematosus. Ann Rheum Dis (2005). doi:ard.2004.035048., 64, 1605-1610.

[86] Sung, Y. K, Park, B. L, Shin, H. D, Kim, L. H, Kim, S. Y, & Bae, S. C. Interleukin-10 gene polymorphisms are associated with the SLICC/ACR Damage Index in systemic lupus erythematosus.Rheumatology (Oxford). (2006). , 45(4), 400-4.

[87] Tsao, B. P, et al. Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus. J Clin Invest (1997). , 99(4), 725-31.

[88] Turner, D. M, Williams, D. M, Sankaran, D, Lazarus, M, Sinnott, P. J, & Hutchinson, I. V. An investigation of polymorphism in the interleukin-10 gene promoter. European Journal of Immunogenetics (1997). , 24(1), 1-8.

[89] Van Der Linden, M. Westendorp RGJ, Sturk A, Bergman W, and Huizinga TWJ. High interleukin-10 production in first-degree relatives of patients with generalized but not cutaneous lupus erythematosus. Journal of Investigative Medicine (2000). , 48(5), 327-334.

[90] Vuong, M. T, Gunnarsson, I, Lundberg, S, Svenungsson, E, Wramner, L, Fernström, A, Syvänen, A. C, Do, L. T, Jacobson, S. H, & Padyukov, L. Genetic risk factors in lu-

pus nephritis and IgA nephropathy--no support of an overlap.PLoS One. (2010). e10559

[91] Wahl, S. M. Transforming growth factor-beta (TGF-bet) in inflammation: a cause and a cure. J Clin Immunol 12, (1992).

[92] Wakkach, A, Augier, S, Breittmayer, J. P, Blin-wakkach, C, & Carle, G. F. Characterization of IL-10-secreting T cells derived from regulatory CD4+CD25+ cells by the TIRC7 surface marker. Journal of Immunology (2008). , 180(9), 6054-6063.

[93] Wang, B, Morinobu, A, Kanagawa, S, Nakamura, T, Kawano, S, Koshiba, M, Hashimoto, H, & Kumagai, S. Transforming growth factor beta 1 gene polymorphism in Japanese patients with systemic lupus erythematosus. Kobe J Med Sci (2007).

[94] Wang, Y. Q, Ugai, S, Shimozato, O, Yu, L, Kawamura, K, Yamamoto, H, Yamaguchi, T, Saisho, H, & Tagawa, M. nduction of systemic immunity by expression of interleukin-23 in murine colon carcinoma cells. Int J Cancer (2003). , 105(6), 820-4.

[95] Wu, G. Y, Hasenberg, T, Magdeburg, R, Bönninghoff, R, Sturm, J. W, & Keese, M. Association between EGF, TGF-beta1, VEGF gene polymorphism and colorectal cancer. World J Surg (2009). , 33(1), 124-9.

[96] Yin, Z, Bahtiyar, G, Zhang, N, Liu, L, Zhu, P, Robert, M. E, Mcniff, J, Madaio, M. P, & Craft, J. IL-10 regulates murine lupus. J Immunol (2002). , 169(4), 2148-55.

[97] Yurasov, S, Tiller, T, Tsuiji, M, Velinzon, K, Pascual, V, Wardemann, H, & Nussenzweig, M. C. Persistent expression of autoantibodies in SLE patients in remission. J Exp Med (2006). , 203(10), 2255-61.

[98] Zhang, Y, Liu, B, Jin, M, Ni, Q, Liang, X, Ma, X, Yao, K, Li, Q, & Chen, K. Genetic polymorphisms of transforming growth factor-beta1 and its receptors and colorectal cancer susceptibility: a population-based case-control study in China. Cancer Lett (2009). , 275(1), 102-8.

[99] Zheng, S. G, et al. IL-2 is essential for TGF-beta to convert naive CD4+CD25- cells to CD25+Foxp3+ regulatory T cells and for expansion of these cells. J Immunol (2007). , 178(4), 2018-2027.

[100] Zhu, L-J, Liu, Z-H, Zeng, C-H, Chen, Z-H, Yu, C, & Li, L-S. Association of interleukin-10 gene-592 A/C polymorphism with the clinical and pathological diversity of lupus nephritis. Clinical and Experimental Rheumatology (2005). , 23(6), 854-860.