EXTENDED REPORT

Differential effect of IL10 and TNFα genotypes on determining susceptibility to discoid and systemic lupus erythematosus

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Objective: To ascertain the possible involvement of functional interleukin 10 (IL10) and tumour necrosis α (TNFα) cytokine promoter polymorphisms on the susceptibility to discoid and systemic lupus erythematosus (DLE, SLE), and their associations with immunological features.
Methods: Single nucleotide polymorphisms of the IL10 (−1082, −819, and −592) and TNFα (−308) genes were determined using allele specific probes in 248 lupus patients and 343 matched controls. To assess functional significance of genotypes, basal mRNA cytokine levels were quantified in 106 genotyped healthy controls by real time RT-PCR. Specific autoantibodies and cutaneous manifestations were analysed in SLE patients and associated with functional genotypes.
Results: After analysing the distribution of IL10 and TNFα transcript levels according to promoter genotypes in healthy individuals, patients and controls were classified into functional single and combined genotypes according to the expected high or low constitutive cytokine production. High TNFα genotypes (−308AA or AG) were associated with SLE independently of IL10 alleles, whereas the risk of developing DLE and the prevalence of discoid lesion in SLE were higher in the high IL10/low TNFα producer group (−1082GG/−308GG). Cytokine interaction also influences the appearance of autoantibodies. Antibodies against Sm are prevalent among low producer patients for both cytokines, a genotype not associated with lupus incidence, whereas low IL10/high TNFα patients have the highest frequency of antibodies to SSa and SSb.
Conclusions: IL10/TNFα interaction influences susceptibility to DLE and the appearance of specific autoantibodies in SLE patients, whereas high TNFα producer genotypes represent a significant risk factor for SLE.

Systemic lupus erythematosus (SLE) is a disorder of immune regulation resulting in a chronic inflammation that affects many organs. Discoid lupus erythematosus (DLE) is a limited skin variant responsible for 50–85% of cutaneous lupus diseases. Although early lesions may be difficult to distinguish from subacute cutaneous lupus erythematosus (SCLE), discoid lesions are characteristic and may also occur in SLE patients. DLE patients rarely have systemic disease, and progression to SLE is uncommon (less than 5%), whereas SCLE patients often fulfil four or more of the criteria used to classify SLE. All these conditions tend to run in families and probably occur in genetically predisposed individuals, but the precise genetic connections of each variant have not been yet determined.

The production of two regulators of the inflammatory reaction—interleukin 10 (IL10) and tumour necrosis α (TNFα)—has been found to be deeply deregulated in SLE, suggesting that these regulators may be involved in the pathogenesis of the disease. High levels of IL10 have been reported in the serum of lupus patients,[1–4] suggesting a high baseline production state. A more controversial issue is the involvement of TNFα in the pathogenesis of lupus, although most investigators have found increased levels in patient sera.[5 6] Genetic polymorphisms at the promoter of IL10 and TNFα genes have been associated with different forms of cytokine production after in vitro lymphocyte stimulation. Although there are no previous reports on the influence of these genes, we believe that variations in basal IL10 and TNFα production may influence disease susceptibility by modulating an initial immune response to in vivo autoantigen encounter.

The IL10 gene promoter has been shown to be very polymorphic. In addition to two microsatellites, there are three single nucleotide polymorphisms (SNPs) at positions −1082(G/A), −819(C/T), and −592(C/A) generating three haplotypes (GCC, ACC, and ATA) associated with variability in IL10 production.[7 8] Various reports have analysed the influence of these polymorphisms on lupus disease, with conflicting results. The IL10.G microsatellite has been associated with SLE incidence in Scottish,[9] Mexican-American,[10] and Italian,[11] but not in Mexican,[12 13] Swedish,[13] or Taiwanese[14] populations. The frequency of −1080G* allele was increased in Vietnamese SLE patients,[15] and carriage of the GCC haplotype has been linked to renal involvement and synthesis of SSa antibodies in white British patients,[16] while the ATA haplotype was overrepresented among white Dutch patients with neuropsychiatric lupus[17] and Chinese patients suffering from renal disorder.[18] However, these haplotypes have not been found to be strong determinants of susceptibility to lupus disease in white populations.[16–22]

Among the various polymorphisms described at the TNFα promoter, the genetic variant at position −308(G/A) was found to have functional effects on gene transcriptional activity, the uncommon TNF2 allele (−308A*) being a stronger transcriptional activator than −308G* after in vitro lymphocyte stimulation.[23 24] Various studies have analysed the association between TNFα alleles and lupus, with

Abbreviations: ACR, American College of Rheumatology; DLE, discoid systemic lupus erythematosus; RT-PCR, reverse transcriptase polymerase chain reaction; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism

inconclusive results. An increased risk of developing SLE, independent of the HLA-DR genotype, has been reported for carriers of TNF2 allele in Dutch,[25 26] African American,[27] and North American white populations.[28] However, no relation was found in other work analysing mestizo Mexicans,[29] North American whites,[30] and African Americans.[28] A significant association has also been reported between TNF2 and anti-SSa positive SCLE, but not with DLE.[22]

The genetic control of constitutive IL10 and TNFα production and the possible interaction between the two cytokines in influencing lupus susceptibility has not yet been evaluated. Our aim in the present study was to investigate the influence of functional cytokine genetic variants and their interactions in conditioning the occurrence of DLE and SLE in a Spanish population. The relation with autoantibody synthesis in SLE was also examined.

METHODS

Patients

The study population included 248 lupus patients (table 1) and 343 matched healthy individuals, all of white origin. Fifty six patients were diagnosed as having DLE by clinical and histological examination of the characteristic discoid lesion and after excluding features of systemic involvement. In the other 192 patients a diagnosis of SLE was established according to the American College of Rheumatology (ACR) criteria.[31] In 164 of these patients the appearance of cutaneous manifestations during the course of the illness was analysed.

The Hospital Universitario Central de Asturias ethics committee gave ethical approval for the study.

Promoter polymorphisms genotyping

Genomic DNA was extracted from blood samples by standard procedures. SNPs at positions −1082 and −592 of the IL10 gene and at position −308 of the TNFα gene were determined by analysing the Tm of the probe/target duplex after polymerase chain reaction (PCR) amplification and hybridisation with fluorescent labelled probes matched with one sequence variant (LightCycler, Roche Diagnostics, Mannheim, Germany), using previously validated methods.[32] Primers employed were: ATCCAAGACAACACTACTAAGGC and ATGGGGTGGAAGAAGTTGAA for −1082; GGTGAGCACTACCTGACTAGC and GCAGCCCTTCCATTTTACTTTC for −592; and CCTGCATCCTGTCTGGAAGTTA and CTGCA CCTTCTGTCTCGGTTT for −308. Hybridisation probes (designed by TIB Molbiol, Berlin, Germany) were: GGATAGGAGGTCCCTTACTTTCCTCTTACC-F and LC Red 640-CCCTACTTCCCCCTCCCAAA for −1082; AGCCTGGAA CACATCCTGTGACCCC-F and LC Red 640-CCTGTCCTGTAG GAAGCCAGTCTC for −592; and AACCCCGTCCCCATGCCCC-F and LC Red 640-CCAAACCTATTGCCTCCATTTCTTTT GGGGAC for −308. Allele present at −819 IL10 was assigned directly owing to the total linkage with the −592 allele in our population. After analysing alleles at the three IL10 promoter sites we found only six genotypes, which corresponded to the three haplotypes previously described in other white populations.

Table 1 Patient characteristics

All patients	248
DLE	56
Age at diagnosis (years) (median/IQR)	40.0/17.2
Female	40 (71.4%)
SLE	192
Age at diagnosis (years) (median/IQR)	32.0/20.0
Female	179 (93.2%)
Presence of autoantibodies (%):	
anti-SSa	30.6
anti-SSb	14.4
anti-RNP	13.3
anti-Sm	9.4
anti-dsDNA	66.7

Values are n or n (%) unless stated otherwise.
DLE, discoid systemic lupus erythematosus; IQR, interquartile range; SLE,

mRNA isolation and quantification

Sample mRNA (poli-A+) was isolated from whole blood using the mRNA isolation kit for blood/bone marrow (Boehringer Mannheim, Mishawaka, Indiana, USA). Reverse transcription was carried out by standard procedures. Real time reverse transcriptase polymerase chain reaction (RT-PCR) (LightCycler, Roche Diagnostics) was used to quantify cytokine mRNA by monitoring the fluorescence emitted by SYBR Green I dye, using an external standard (cDNA obtained from lipopolysaccharide stimulated peripheral blood mononuclear cells) to generate a calibration curve. β_2 Microblobulin was used as the housekeeping gene, enabling determination of mRNA relative units. Primers employed were: AGCTGAGAACCAAGACCCAGA and GGGCTGGGTCAGCTATCC for IL10; ACAAGCCTGTAGCC CATGT and AAAGTAGACCTGCCCAGACT for TNFα; and CCAGCAGAGAATGGAAAGTC and GATGCTGCTTACATGT CTCG for β_2 microglobulin.

Immunological assessment

Anti-dsDNA antibodies were quantified by radioimmunoassay (Trinity Biotech, Bray, Wicklow, Ireland) or enzyme linked immunosorbent assay (ELISA) (ELIA, Pharmacia, Freiburg, Germany). The presence of anti-Ro/SSa, anti-La/SSb, anti-RNP, or anti-Sm antibodies was detected by ELISA screening (Orgentec, Maintz, Germany) and positive samples were analysed by immunoblotting (Inno-Lia, Innogenetics, Gent, Belgium) or specific ELISA (Orgentec).

Statistical analysis

Genotype frequencies of each SNP were analysed by the χ^2 test to determine that they conformed to Hardy–Weinberg equilibrium based on the observed allele frequencies. Allele and genotype frequencies between patient and control groups and the presence of autoantibodies between patient subpopulations were compared using the χ^2 test and the two tailed Fisher's exact test when the number of expected cases was small. Basal IL10 and TNFα mRNA expression and age at diagnosis were not distributed normally, so non-parametric testing was used throughout (Mann–Whitney U test or Kruskal–Wallis test). The strength of the association between functional single or combined genotypes and disease was assessed by unconditional logistic regression analysis, calculating odds ratios (OR) and 95% confidence intervals (CI). Single locus regression models were run to estimate separately the effects of cytokine polymorphisms, comparing the high producer genotypes with the most common low producers. A combined two loci model was developed including both cytokine polymorphisms to estimate individual effects of each combined genotype, using the common low/low producer genotype as reference. The SPSS 11.0 statistical software package (SPSS Inc, Chicago) was used for all calculations.

RESULTS

IL10 and TNFα promoter polymorphisms

An overrepresentation, at the limit of significance, of the genotype GCC/GCC at the IL10 promoter was observed in the whole lupus population when comparing with controls. The frequency of −308A* allele at the TNFα gene was

Table 2 Distribution of IL10 and TNFα promoter genotypes in healthy Spanish controls and patients with lupus erythematosus

	Controls (n = 343)	All patients (n = 248)		SLE (n = 192)		DLE (n = 56)	
		n (%)	p Value	n (%)	p Value	n (%)	p Value
IL10 (−1082, −819, −592)							
ATA/ATA	31 (9.0)	22 (8.9)	0.944	11 (8.3)	0.782	6 (10.7)	0.688
ATA/ACC	64 (18.7)	35 (14.1)	0.144	31 (16.1)	0.466	4 (7.1)	0.035
ACC/ACC	39 (11.4)	28 (11.3)	0.976	22 (11.5)	0.975	6 (10.7)	0.886
GCC/ATA	58 (16.9)	35 (14.1)	0.357	28 (14.6)	0.482	7 (12.5)	0.407
GCC/ACC	100 (29.2)	76 (30.6)	0.696	58 (30.2)	0.798	18 (32.1)	0.650
GCC/GCC	51 (14.9)	52 (21.0)	0.054	37 (19.3)	0.188	15 (26.8)	0.026
−1082G*	37.90%	43.35%	0.059	41.67%	0.226	49.11%	0.024
−819T/−592A*	26.82%	22.98%	0.134	23.70%	0.262	20.54%	0.151
TNF α (−308)							
AA	7 (2.0)	11 (4.4)	0.095	7 (3.6)	0.272	4 (7.1)	0.054
GA	71 (20.7)	84 (33.9)	<0.0001	72 (37.5)	<0.0001	12 (21.4)	0.901
GG	265 (77.3)	153 (61.7)	<0.0001	113 (58.9)	<0.0001	40 (71.4)	0.340
−308A*	12.39%	21.37%	<0.0001	22.40%	<0.0001	17.86%	0.113

The significance was evaluated by χ^2 test or two tailed Fisher's exact test.
DLE, discoid systemic lupus erythematosus; IL10, interleukin 10; SLE, systemic lupus erythematosus; TNFα, tumour necrosis factor α.

significantly increased in patients compared with controls (table 2). When lupus subtypes were analysed separately, DLE was associated with −1082G* IL10 but not with TNFα alleles, whereas SLE remained highly associated with −308A* allele but not with IL10 SNPs.

To determine the possible influence on disease susceptibility of variations in constitutive IL10 and TNFα production, we quantified basal cytokine mRNA levels in 106 healthy genotyped subjects and analysed the distribution of transcript levels according to promoter genotypes. Despite important interindividual variations, we found that individuals homozygous for the GCC haplotype are the highest producers of basal IL10, showing significant differences when compared with individuals who were homozygous or heterozygous for the ACC or ATA haplotypes, indicating that the −1082 position was relevant for constitutive IL10 production (fig 1A). Similarly, individuals carrying the genotype −308GG at the TNFα gene have reduced transcript levels compared with the other two genotypes (fig 1B).

Functional genotypes and lupus susceptibility

On the basis of these findings, patients and controls were classified into high and low functional genotypes according to the expected constitutive mRNA levels (table 3). Additionally, in order to analyse putative IL10/TNFα interactions, individuals were classified into the four possible functional combined genotypes. The single locus model study showed that high IL10 producer genotype was significantly overrepresented in DLE, whereas a predisposition to develop SLE was found among those carrying the high TNFα producer allele. Analysis of the combined IL10/TNFα genotypes yielded a different distribution in patients and controls ($p = 0.00008$, 4×2 contingency table). When analysing the influence of combined genotypes on the appearance of lupus subtypes, we found that carriage of the TNF2 allele was associated with SLE independently of the IL10 genotype ($p = 0.394$, 2×2 contingency table). However, a significant influence of IL10/TNFα interaction was detected in determining DLE susceptibility. Logistic regression modelling for combined genotypes showed that high IL10/low TNFα producers had a stronger association with DLE than the whole high IL10 group.

On the basis of these results, we studied the possible involvement of IL10 in the appearance of skin disease in SLE patients. We analysed the influence of IL10 genotypes on the appearance of phosensitivity, malar rash, and discoid and subacute cutaneous lesions in patients with low TNFα genotype. Table 4 shows that carriage of the high IL10 genotype increased the prevalence of discoid lesions, but not other cutaneous manifestations. No significant effect of IL10 genotype on skin lesions was found among high TNFα patients (data not shown).

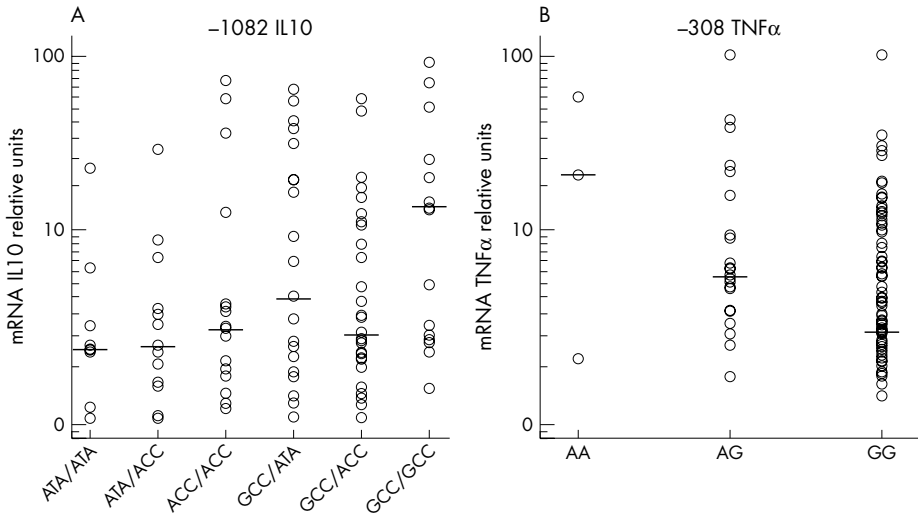

Figure 1 Relation between IL10 and TNFα genotypes and constitutive cytokine expression. Interleukin 10 (IL10) (panel A) and tumour necrosis factor α (TNFα) (panel B) basal mRNA levels were quantified in peripheral blood cells of 106 genotyped healthy subjects by a highly sensitive real time RT-PCR technique, as described in Methods. The distribution of transcript levels according to promoter genotypes was analysed. Horizontal bars are median values. Significance was evaluated by the Mann–Whitney U test: IL10 GCC/GCC *v* other genotypes, $p = 0.026$; TNFα GG *v* other genotypes, $p = 0.021$.

Table 3 IL10 and TNFα genotypes and lupus erythematosus susceptibility

	Controls, n (%)	All patients			SLE			DLE		
		n (%)	OR (95% CI)*	p Value*	n (%)	OR (95% CI)*	p Value*	n (%)	OR (95% CI)*	p Value*
−1082 IL10										
Low (AA/AG)	292 (85.1)	196 (79.0)	Reference	0.041	155 (80.7)	Reference	0.214	41 (73.2)	Reference	0.028
High (GG)	51 (14.9)	52 (21.0)	1.61 (1.02 to 2.55)		37 (19.3)	1.38 (0.83 to 2.30)		15 (26.8)	2.11 (1.08 to 4.11)	
−308 TNFα										
Low (GG)	265 (77.3)	153 (61.7)	Reference	<0.0001	113 (58.9)	Reference	<0.0001	40 (71.4)	Reference	0.337
High (AA/GA)	78 (22.7)	95 (38.3)	2.18 (1.48 to 3.20)		79 (41.1)	2.63 (1.72 to 4.02)		16 (28.6)	1.36 (0.72 to 2.58)	
Combined IL10/TNFα										
Low/low	224 (65.3)	117 (47.2)	Reference		90 (46.9)	Reference		27 (48.2)	Reference	
Low/high	68 (19.8)	79 (31.9)	2.23 (1.46 to 3.39)	0.0002	65 (33.9)	2.46 (1.55 to 3.89)	0.0001	14 (25.0)	1.70 (0.84 to 3.44)	0.139
High/low	41 (12.0)	36 (14.5)	1.70 (0.99 to 2.90)	0.052	23 (12.0)	1.27 (0.69 to 2.32)	0.442	13 (23.2)	2.62 (1.24 to 5.51)	0.012
High/high	10 (2.9)	16 (6.5)	3.87 (1.57 to 9.55)	0.003	14 (7.3)	5.62 (2.04 to 15.5)	0.001	2 (3.6)	1.76 (0.36 to 8.54)	0.485
Trend test*			<0.0001			<0.0001			0.072	

*Calculated by unconditional logistic regression modelling adjusted for sex.
CI, confidence interval; IL10, interleukin 10; OR, odds ratio; TNFα, tumour necrosis factor α.

Table 4 Influence of IL10 polymorphism on the appearance of skin disease in SLE patients with low TNFα genotype

	Low IL10 (n = 72)	High IL10 (n = 21)	p Value
Photosensitivity	40 (55.6%)	13 (61.9)	0.605
Malar rash	36 (54.2%)	11 (52.4)	0.885
Discoid lesions	9 (12.5%)	7 (33.3)	0.044
Subacute cutaneous lesions	12 (16.7%)	2 (9.5)	0.729

Values are the n (%). Significance was evaluated by χ^2 test or two tailed Fisher's exact test.
IL10, interleukin 10; SLE, systemic lupus erythematosus.

Cytokine polymorphisms and autoantibody production in SLE patients

When combined genotypes were studied in relation to the presence of autoantibodies in SLE patients (table 5), we found the highest prevalence of anti-Sm antibodies among low IL10/low TNFα producers, but no relation was detected when analysing each cytokine individually. A significant influence of combined genotypes was also observed on the production of antibodies against SSb and, especially, SSa. High TNFα genotype was associated with SSa synthesis (44.3% *v* 22.1%, p = 0.001), but significance was not reached with low IL10 producers (34.2% *v* 18.9%, p = 0.072). Analysis of the combined genotypes showed a strong association with low IL10/high TNFα (49.2% *v* 22.1%, p = 0.00012), indicating that both cytokine genes contribute to the SSa phenotype. Anti-dsDNA antibodies were more abundant among high/high producers, although this finding was not statistically significant (85.7% *v* 64.6%, p = 0.108). Curiously, this combined genotype, infrequent in our population (7.3% in SLE), was carried by three of the 13 male patients included in the study, suggesting a different genetic component of male SLE.

DISCUSSION

We have found a relation between polymorphisms at the promoter region of TNFα and IL10 genes and the appearance of lupus disease in white Spaniards. Genetic variants at the promoter regions of these cytokine genes have been correlated with variations in mitogen induced transcriptional activity and cytokine synthesis in vitro,[7 23 24] thus modulating the immune response after antigen challenge. Various cell types are constitutively capable of producing detectable amounts of these cytokines—mainly cells of myeloid origin and less abundantly T and B lymphocytes. Individual steady state levels of IL10 and TNFα may deviate an initial immune response towards different forms of T cell activation, influencing the susceptibility to transform a limited autoimmune response into an autoimmune disease. It is not known whether constitutive levels of IL10 and TNFα are genetically controlled, and their putative influence on the appearance of lupus is also unknown. To this end, we first measured basal IL10 and TNFα mRNA in blood cells from healthy subjects using a sensitive RT-PCR technique, and analysed their association with single nucleotide polymorphisms. Correlation with cytokine protein levels could not be undertaken, as many serum samples were below the detection limit of the assay. An association was found between the GG genotype at the −1082 IL10 position and high transcript levels, whereas large amounts of TNFα mRNA were associated with carriers of the −308A* allele. These findings allowed us to define functional constitutive genotypes and to classify patients and controls as high or low IL10 and TNFα producers. We found that the presence of the high producer TNF2 allele caused a substantial increase in the occurrence of SLE, whereas the incidence of DLE in a

Table 5 Prevalence of autoantibodies in patients with systemic lupus erythematosus, distributed in functional genotypes

	Combined IL10/TNFα genotypes			
	Low/low (n = 90)	Low/high (n = 65)	High/low (n = 23)	High/high (n = 14)
Age at onset (years) (median/IQR)	34/22.0	30/22.5	35/13.0	24/20
Male patients	4 (4.4%)	5 (7.7%)	1 (4.3%)	**3 (21.4%)**§
Autoantibodies to:				
dsDNA	57 (63.3%)	41 (63.1%)	17 (73.9%)	12 (85.7%)
SSa	21 (23.3%)	**32 (49.2%)**†	4 (17.4%)	3 (21.4%)
SSb	8 (8.9%)	**14 (21.5%)**‡	1 (4.3%)	4 (28.6%)
RNP	15 (16.7)	7 (10.8)	2 (8.7)	2 (14.3)
Sm	**12 (13.3)***	3 (4,6)	1 (4.3)	1 (7.1)

Values are n (%) unless specified. Significance was evaluated by χ^2 test or two tailed Fisher's exact test.
*p = 0.040; †p = 0.00012; ‡p = 0.033; §p = 0.024 (each genotype *v* other genotypes).
IL10, interleukin 10; IQR, interquartile range; TNFα, tumour necrosis factor α.

single cytokine analysis seemed not to be influenced by TNFα genotypes. The opposite occurred when analysing IL10 genotypes. Interestingly, we found for the first time a significant correlation of the high IL10 genotype with the appearance of DLE, but not SLE.

Various mechanisms may be involved in the detrimental effect of TNFα in SLE. Because of its marked proinflammatory properties, it is quite plausible that high genetically sustained production, acting on an autoimmune-prone genetic background, resulted in the maintenance and amplification of an initial immune response to unknown self peptides. TNFα also has numerous effects on T and B lymphocytes, involving several immune functions. In a murine model, TNFα participates in autoimmune processes linked to inappropriate lymphocyte survival[33]; however, this cytokine is also a highly active promoter of the apoptotic process. It has been reported that sera from SLE patients react with proteins phosphorylated during apoptosis,[34] probably by recognising new epitopes generated by phosphorylation or proteolysis.[35] Because of the proapoptotic properties of TNFα, probably enhanced in SLE patients by their raised concentrations of IFNα,[36 37] high levels of this cytokine may trigger an autoimmune response to antigenically modified autoproteins generated during the apoptotic process. An alternative to the pathogenic role of TNF2 in SLE is that it could be a marker for the extended haplotype HLA-A1 B8 DR3, which is strikingly associated with SLE and other autoimmune conditions. Nevertheless, carriage of TNF2 allele—in the presence or absence of other loci—leads to an increase in TNFα production, thus modifying cytokine homeostasis in favour of the development of pathogenic situations. In addition, several studies have shown that both TNF2 and HLA-DR3 contribute independently to SLE susceptibility.[21 25–27]

No previous studies have reported an association of IL10 alleles with susceptibility to DLE. van der Linden *et al*[21] found higher frequencies of the −1082G* allele in DLE than in SLE patients (57% *v* 45%), although the difference was not statistically significant, probably because of the reduced number of patients included in the study. Interestingly, Alarcon-Riquelme *et al*[12] found an association tendency between mucocutaneous manifestations in SLE patients and the IL10.G locus, which is in a weak linkage disequilibrium with the −1082 polymorphism.[38] These findings indicate a probable role of IL10 in triggering discoid lesions. IL10 is a highly pleiotropic lymphokine that may act as a potent suppressor of systemic inflammatory responses while, on the other hand, inducing inflammation when overexpressed locally. This cytokine is constitutively expressed in a range of cellular populations,[39] including keratinocytes, in which IL10 production is incremented in response to ultraviolet irradiation.[40] Moreover, it has been shown that following UVB exposure, IL10 stimulates dermal endothelial cells to produce proinflammatory cytokines and chemokines,[41] suggesting that genetically high IL10 production could be responsible for discoid lesions. As support for this hypothesis, it is worth noting the finding that cutaneous manifestations improved in all SLE patients soon after initiating anti-IL10 monoclonal antibody treatment.[42]

The isolated assessment of cytokine genotypes, though of relevance, may not provide a realistic picture of their influence on lupus disease. The actions of cytokines may be profoundly conditioned by the presence of other cytokines, particularly in the case of IL10 and TNFα, which have complex and predominantly opposing roles in the systemic inflammatory responses. We have evaluated the interaction between IL10 and TNFα in promoting the appearance of lupus disease. Our results show a strong association between susceptibility to develop SLE and the high TNFα genotype, independently of IL10 production. However, the highest risk of developing DLE was found among individuals with the combined genotype high IL10/low TNFα, probably because of the influence of IL10 levels which cannot be modulated by the low production of TNFα. Furthermore, the high prevalence of discoid lesion observed in SLE patients with this genotype supports this hypothesis. In addition, our results suggest that carriage of the TNF2 allele decreases the risk of developing DLE, favouring the development of SLE or SCLE among those individuals with a high IL10 producer genotype.

Another interesting finding is the association of autoantibody production with cytokine genotypes. Curiously enough, the highest percentage of anti-Sm antibodies was found among patients genotyped as low IL10/low TNFα, a genotype negatively associated with lupus susceptibility. It is known that anti-Sm antibody synthesis is genetically regulated, appearing more often in non-white ethnic groups and in childhood lupus,[43 44] suggesting that SLE susceptibility and anti-Sm synthesis are regulated by different genetic elements. The highest percentage of antibodies to SSa and SSb was found among carriers of the low IL10/high TNFα genotype. This is in accordance with the higher frequency of TNF2 allele found in patients with subacute cutaneous lupus erythematosus,[22 45] congenital heart block,[46] and cutaneous neonatal lupus,[47] pathologies linked to the presence of anti-SSa antibodies.

In conclusion, our observations suggest that alleles that regulate constitutive high levels of TNFα may be a risk factor for SLE, whereas combined IL10/TNFα genotypes affect susceptibility to DLE and could influence different SLE phenotypes.

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