Lack of association between *TNF*-308 polymorphism and the clinical and immunological characteristics of systemic lupus erythematosus and primary Sjögren's syndrome

G.J. Tobon, P.A. Correa, L.M. Gomez, J.-M. Anaya

Cellular Biology and Immunogenetics Unit, Corporación para Investigaciones Biológicas (CIB), Universidad del Rosario, Medellín; and Rheumatology Unit, Clínica Universitaria Bolivariana, School of Medicine, Universidad Pontificia Bolivariana (UPB), Medellín, Colombia.

Abstract

Objective

*To investigate the previously reported association of tumor necrosis factor alpha (*TNF*) -308 single nucleotide polymorphism (SNP) with the clinical course and immunological features in patients with systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS).*

Methods

The studied group consisted of 113 consecutive SLE and 65 pSS patients. TNF *-308 SNPwas determined by the polymerase chain reaction-restriction fragment length polymorphism technique. Clinical and immunological characteristics were assessed according to a standard protocol that included disease activity (SLEDAI) and damage (SLICC Damage Index). Serum TNFα levels were measured in samples collected from 32 patients with SLE and 16 with pSS by enzyme-linked immunosorbent assay.*

Results

The TNF2 allele (A) was observed in 46% and 54% of SLE and pSS patients, respectively. We failed to find any significant association between the -308 SNP and disease manifestations, the presence of autoantibodies or cytokine levels in either group.

Conclusion

TNF *-308 SNP (TNF2) does not exhibit a significant influence on the disease course or immunological response in SLE and pSS. Other genetic and/or environmental factors seem to be required and to be more important than TNF2 allele for the progression of these diseases.*

Key words

Tumor necrosis factor, polymorphism, primary Sjögren's syndrome, systemic lupus erythematosus.

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Gabriel J. Tobón, MD: Young Investigator, Cellular Biology and Immunogenetics Unit, CIB; Paula A, Correa, MSc: Assistant Researcher, Cellular Biology and Immunogenetics Unit, CIB, and Assistant Professor, School of Medicine, UPB; Luis M. Gomez, VD: Assistant Researcher, Cellular Biology and Immunogenetics Unit, CIB; Juan-Manuel Anaya, MD: Senior Researcher, Cellular Biology and Immunogenetics Unit, CIB, and Professor of Medicine, School of Medicine, Universidad del Rosario; Medellin, Colombia.

Please address correspondence and reprint requests to: Juan-Manuel Anaya, MD, Corporación para Investigaciones Biológicas, Cra. 72-ANo 78-B-141, Medellín, Colombia.
E-mail address: janaya@cib.org.co

Introduction

Tumor necrosis factor alpha (TNFα) is a pleotropic cytokine produced largely by macrophages and T cells (1, 2). TNFα is synthesized as a 26 kD membrane protein, which is cleaved to produce its soluble 17 kD form. TNFα exerts a range of inflammatory and immunomodulatory activities that are important in host defense. Over-expression of TNFα has been implicated in the pathogenesis of autoimmune diseases including systemic lupus erythematosus (SLE) (3) and primary Sjögren's syndrome (pSS) (4).

The *TNF* gene is located within the class III region of the major histocompatibility complex (MHC) on chromosome 6 (6p21.31), and is highly polymorphic (1, 2). Five microsatellites and numerous single nucleotide polymorphisms (SNP) in the *TNF* promoter have been described, some of which may regulate TNFα expression. One of these SNP represents a guanine (G) to adenine (A) transition at position -308, and has been examined in several autoimmune diseases; however, the results have varied, mainly due to differences in the origin of the studied populations, linkage disequilibrium with other MHC genes (as a part of the 8.1 ancestral haplotype), or insufficient sample size (5,6). We have previously confirmed that the *TNF* -308Aallele is associated with SLE and pSS in our population (7). Few studies, however, have analyzed the influence of this SNP on clinical and immunological variables in diseases with which it is associated (8, 9). Herein we report our analysis of the influence of *TNF* -308 SNP on the clinical course and immunological features in northwestern Colombian patients with SLE and pSS.

Patients and methods

Study population

In this study we analyzed 113 SLE patients (10) and 65 pSS patients (11). Patients were seen in the Rheumatology Unit at the Clinica Universitaria Bolivariana, in Medellin, Colombia. This was a cross-sectional study conducted in compliance with Resolution no. 008430 of 1993 from the Ministry of Health of the Republic of Colombia, and was classified as research with minimal risk. The local Ethics Committee approved the present study.

Genotyping for TNFα polymorphism

The *TNF* -308 polymorphism was analyzed in the genomic DNA by restriction fragment length polymorphism (RFLP) as described by Wilson *et al*. (12). The presence of G defines the TNF1 allele and A defines TNF2 allele.

Clinical variables

Information on patient demographics and cumulative clinical and laboratory manifestations over the disease course were obtained either by verification during discussion with the patient or by chart review, and were recorded in a standard data-collection for that purpose. Each clinical and laboratory variable was registered as "present" or "absent" for every specific patient during the course of the disease and at the time of blood sample collection.

The clinical and laboratory variables associated with SLE, including each feature of the revised American College of Rheumatology criteria, were evaluated and defined as follows: 1) arthritis: nonerosive arthritis involving 2 or more peripheral joints; 2) malar rash; 3) photosensitivity; 4) alopecia; 5) discoid lupus; 6) Raynaud's phenomenon; 7) renal involvement, as evidenced by a renal biopsy result showing the World Health Organization's (WHO) class II-V histopathology, active urinary sediment, or proteinuria ≥ 500 mg/24 h. Nephrotic syndrome was defined as ≥3.5 g/d of proteinuria, hypoalbuminemia (<2.8 g/dL), hyperlipidemia, and edema. Lupus nephritis was defined as present or absent according to the abnormalities of the previous tests. 8) Neurologic involvement, as evidenced by seizures or psychosis without any other definable cause, or other conditions such as peripheral neuropathy, stroke, transverse myelitis, chorea, or other central nervous system lesions directly attributable to SLE in the absence of other causes; 9) pleuritis: pleural rub and/or effusion and/or typical pleuritic pain; 10) pericarditis: documented by electrocardiogram, rub, or evidence of pericardial effusion; 11)

autoimmune haemolytic anemia, with a hematocrit ≤ 35%, reticulocyte count ≥ 4%, and positive Coombs test; 12) leukopenia, white cells ≤ 4000/mm3; 13) thrombocytopenia, platelets ≤ 100,000/mm^3; 14) arterial or venous thrombosis diagnosed on clinical grounds and confirmed by appropriate tests (3). The clinical variables associated with pSS were classified according to Oxholm *et al.* (13).

Disease scores

SLE activity was evaluated according to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (14). The severity of the disease and the organic damage were evaluated using the systemic lupus international collaborating clinics (SLICC) damage index (SDI) (15).

TNFα levels

Measurements of TNFα in frozen serum samples collected from 32 patients with SLE and 16 with pSS were performed by solid-phase sandwich enzyme-linked immunosorbent assay using the OptEIA kits (Pharmingen, BD Biosciences, San Diego, CA), according to the manufacturer's instructions.

Autoantibodies

Antinuclear antibodies were determined by indirect immunofluorescence using Hep-2 cells as susbtrate. Serum levels of IgG anti-DNA, anti Ro, anti La, anti-RNP and anti-Sm antibodies were studied by enzyme-linked immunosorbent asay (QUANTA-Lite, Inova, San Diego, CA), according to the manufacturer's instructions. Rheumatoid factor levels were determined by turbidimetry, according to the manufacturer's instructions. Autoantibodies and cytokine levels were simultaneously determined in the same serum samples.

Statistical analysis

Data were managed and stored using the SPSS program (V9.05 for Windows, Chicago, IL). Differences in frequencies were determined by X^2 and Fisher exact test as appropriate and those between means were determined by Mann-Whitney U test. A p value of < 0.05 was considered as statistically significant.

Results

TNF2 allele (A) was observed in 46% and 54% of SLE and pSS patients, respectively. We previously reported that in the control group Hardy-Weinberg Equilibrium was found for the -308 SNP, while in SLE and pSS Hardy-Weinberg Equilibrium was not observed. In healthy individuals (419 controls) TNF2 was previously found in 21% (7). The distribution of different alleles in healthy controls was as follow: TNF1/TNF1: 77%; TNF1/TNF2 21% and TNF2/TNF2 (A/A) in 1.4% (7). In the SLE group, 60 patients (53%) were homozygous for TNF1, 51 (45.1%) were heterozygous and only 2 patients (1.7%) were homozygous for the TNF2 genotype. In pSS patients, 30 (46.1%) were homozygous for TNF1, 35 (54%) were heterozygous and none patient was homozygous for the TNF2 genotype.

Table I shows the SLE characteristics as function of *TNF* –308 genotype. We did not observe a significant influence of -308 SNP on clinical and immunological characteristics of patients. No association between *TNF* SNP and the level of cytokine was observed, regardless of the activity of disease (Table I). Similar results were observed in patients with pSS (Table II).

Discussion

In the present study we examined simultaneously the *TNF* -308 polymorphism in patients with SLE and pSS, all from the same ethnic group primarily derived from Spaniards which did not mix in significant proportions with Amerindian or Black populations (16, 17). Although previously reported to be a risk factor for susceptibility towards these autoimmune rheumatic diseases (7), this SNPseems not to influence the clinical course of SLE. Table III summarizes previous studies in which the association between TNFα polymor-

Table I. Characteristics of Northwestern Colombian patients with SLE in function of *TNF* -308 SNPgenotype.

Characteristic	-308 GG N = 60	-308 GA N = 51
Sex, m:f	2:58	1:50
Age, years	32.5 ± 11.3	34.9 ± 11.9
Duration of SLE, years	5.5 ± 5.8	6.5 ± 7.4
Damage index (SDI) > 1, %	27	21
Activity index (SLEDAI)*	6.9 ± 7.5	6.5 ± 5.4
Clinical manifestations, %		
Musculoskeletal involvement	96	92
Cutaneous involvement	86	88
Raynaud's phenomenon	39	42
Cardiopulmonar	26	29
Nephritis	42	44
Neurologic involvement	48	37
Hematologic	72	77
Autoantibodies, %		
Antinuclear	99	98
Anti-DNA	69	79
Anti-Ro	35	30
Anti-La	9	17
Anti-Sm	33	39
Anti-RNP	55	56
TNF-α, pg/ml	19.2 ± 51.5 (n = 32)	23.9 ± 60.7 (n = 10)

Significant differences were not found.
SDI: SLICC Damage Index; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index, TNF-α: tumor necrosis factor alpha.
*SLEDAI was registered at the same time the serum sample was obtained for cytokines and autoantibodies measurement.
There were two patients homozygous for -308AA.

Table II. Clinical and laboratory characteristics of patients with pSS as a function of *TNF* -308 genotype.

Characteristic	-308 GG N = 30	-308 GA N = 35
Age, years	50.4 ± 13.3	53 ± 13.7
Duration of disease, years	6.9 ± 6.6	6.3 ± 4.4
Inflammatory – vascular *	57	66
Internal organ **	23	17
Raynaud's phenomenon	20	14
Immune-mediator	20	14
Anti-Ro/SSAantibodies #	68	84
Anti-La/SSB antibodies #	29	39
Total rheumatoid factor †	53	51
TNFα, pg/ml	41.8 ± 86 (n = 7)	15.2 ± 28 (n = 9)

Except where indicated, data are in percentages. Significant differences were not found.
* Includes at least one of the following: arthralgias, arthritis, cutaneous and nervous system involvement (13).
** Includes exocrine disease affecting at least one of the following: lungs, pancreas, gastric mucosa, kidneys, and liver (13). This term is attached to epithelial involvement that characterizes Sjögren's syndrome in these organs.
# By ELISA. Anti-DNAand anti-Sm antibodies were not found.
† By turbidimetry , (+) > 40 U/ml.
Immune-mediator: Presence of anemia, leucopenia or fever.
TNFα levels did not significantly differ with genotype.

phisms and SLE has been investigated (8, 9, 18-31). The TNF2 allele was originally shown to be associated with SLE in Caucasians (30). However, the results derived from other ethnicities are contradictory. For example, Zuniga *et al*. (19) failed to show association between *TNF* -308 polymorphism and SLE in Mexican population, contrary to Sullivan *et al*. (23) who demonstrated that TNF2 polymorphism was associated with an increased risk of SLE in African-Americans. These controversies may be explained mainly on the differences in the origin of the studied populations, linkage disequilibrium with other MHC genes (as a part of 8.1 ancestral haplotype), or on insufficient sample size (5,6). *TNF* polymorphism has been shown to influence neonatal lupus (8), but not neuropsychiatric lupus (9). Although we have previously confirmed that TNF2 allele is associated with SLE and pSS in our population (7), in the present analysis we failed to find an association between this allele and the clinical and immunological features of SLE and pSS patients.

Although *TNF* –308 G/ASNPhas been the most extensively studied polymorphism in the *TNF* gene, and has been suggested to be associated with the synthesis of protein, *in vitro* stimulation of TNFα production by cells from –308 G/G homozygous individuals and G/A heterozygote individuals has produced conflicting results. For example, few studies have revealed higher TNFα production by cells from G/A donors than by G/G cells (1) while others have reported no significant effect (32-33).

In pSS, both TNFα mRNAand its protein are significantly expressed in ducts

Table III. TNFα polymorphism and SLE (review of literature).

Ref.	Patients / controls (n)	Year	Comments
8	88 children	2003	TNF2 association with neonatal lupus
9	64 patients	2002	Lack of association of TNF2 SNPwith neuropsychiatric SLE
18	91 / 253	2001	HLA-independent association with TNF2 allele
19	51 / 55	2001	Lack of association with *TNF* -308 SNP
20	91 SLE families	2001	Lack of association with *TNF* -308 SNP
21	31 SCLE patients and 20 discoid lupus	2000	TNF2 association with subacute SLE
22	99 / 177	2000	HLA-independent association with TNF2 allele
23	88/64	1997	TNF2 association with SLE in African-Americans.
24	105 / 115	1997	SLE patients had a marginally higher percentage of TNF2 than controls, which was in linkage disequilibrium with HLA-DR3
25	91 / 109	1997	*TNF* microsatellites (a2, b3 and d2) were associated with SLE, particularly with photosensitivity and Raynaud's phenomenon
26	100 / 107	1997	Lack of association with *TNF*-308 SNP
27	49 SLE white patients and 49 SLE black patients	1996	*TNF*-238 and *TNF*-308 SNPdid not confer susceptibility to SLE
28	67 / 89 (Chinese)	1996	Lack of association with -308 SNP.
29	40 patients	1994	TNF2 association.
30	81 / 168	1994	TNF2 association
31	20 / 23	1993	TNF2 association

SNP: single nucleotide polymorphism; SCLE: subacute cutaneous lupus erythematosus; SLE: systemic lupus erythematosus.

and in mononuclear cells of the salivary glands' infiltrate (4) with TNFα inducing proteolysis of the glandular acini (34). Our previous results showed that, as it occurs in SLE, the TNF2 allele is a risk factor for pSS (7), but has not influence on the clinical and immunological characteristics of disease, as shown here. Several studies had found high levels of Th1 and Th2 cytokines in pSS patients with strong expression of TNFα in salivary glands (35-37). Nonetheless, only few studies have evaluated the *TNF* –308 polymorphism in this disease (38-40). A recent study showed an association between TNF2 and interstitial nephritis in patients with pSS (38). A different report showed association between TNF2 and rheumatoid factor (36). In French patients, TNF2 was associated with anti-SS-B antibodies (40).

In summary, *TNF* -308 SNP does not exert a significant influence on disease course or immunological response in northwestern Colombian patients with SLE or pSS. The involvement of other genetic and/or environmental factors seems to be required and to be more important than TNF2 allele for the progression of these diseases. The premise of studies of promoter SNP is that gene variants with a significant role in pathology will lead to a greater understanding of the regulatory mechanisms involved in both health and disease and may provide useful knowledge for identifying, and allowing early interventions in at-risk individuals. However, a recent study indicates that *TNF* -308 polymorphism might be not functional (41). Regulation of TNF gene includes both epigenetic and post-transcriptional mechanisms (2,41), none of which have been considered so far. Thus, *TNF* polymorphism might contribute to susceptibility to autoimmune rheumatic diseases; however, the relative magnitude of its non-HLA-DR/DQ effects is uncertain due to both the extraordinary linkage disequilibrium that extends over the MHC and to the regulatory mechanisms that control its expression. Finally, autoimmune diseases are entities frequently observed in genetically susceptible individuals in whom their clinical expression is modified by permissive and protective environments occurring over time. From a genetic point of view, these are complex diseases, meaning that their inheritance do not follow a single-gene dominant or single-gene recessive Mendelian law, and thus that they are polygenic. Besides *TNF* several other genes may influence their susceptibility and clinical course (42).

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