Autoimmunity and Tuberculosis. Opposite Association with *TNF* Polymorphism

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ABSTRACT. ***Objective.*** To examine the influence of the –308 and –238 single nucleotide polymorphisms (SNP) of tumor necrosis factor-α gene (*TNF*) on patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), primary Sjögren's syndrome (SS), and tuberculosis (TB).

Methods. Genomic DNA from patients with RA (n = 165), SLE (n = 100), primary SS (n = 67), and TB (n = 135) and ethnically matched controls (n = 430) was genotyped for *TNF* –308 and –238 SNP by PCR-RFLP.

Results. *TNF* –308A allele was associated with RA (odds ratio, OR 1.8, p = 0.002), SLE (OR 2.6, p < 0.0001), and primary SS (OR 2.9, p < 0.0001). *TNF* –308G was associated with TB (OR 1.8, p = 0.02). The –308 GG genotype was protective for autoimmunity (p < 0.003). *TNF* –238A allele was protective for autoimmunity but represented a susceptibility factor for TB (OR 2.2, p < 0.0001). Haplotype –308A–238G was a protective factor against TB, whereas it carried susceptibility for RA, SLE, and primary SS (p < 0.0001).

Conclusion. The results show an opposite association of *TNF* polymorphism with autoimmunity and TB, and suggest the existence of heterozygote advantage, sustaining the hypothesis that autoimmune diseases are a consequence of natural selection for enhanced TB resistance. Data also provide genetic evidence supporting the common variants/multiple disease hypothesis, which emphasizes that many disease genes may not be disease-specific, and that similar immunogenetic mechanisms underlie autoimmune diseases. (J Rheumatol 2005;32:219–24)

Key Indexing Terms:
TUMOR NECROSIS FACTOR RHEUMATOID ARTHRITIS TUBERCULOSIS
SYSTEMIC LUPUS ERYTHEMATOSUS SJÖGREN'S SYNDROME AUTOIMMUNITY

Tumor necrosis factor-α (TNF-α) is a pleotropic cytokine produced largely by macrophages and T cells[1]. TNF-α is synthesized as a 26 kDa membrane protein that is cleaved to produce its soluble 17 kDa form. TNF-α exerts a range of inflammatory and immunomodulatory activities that are important in host defense. TNF-α has been implicated in the pathogenesis of autoimmune diseases including rheumatoid arthritis (RA)[2], systemic lupus erythematosus (SLE)[3], and primary Sjögren's syndrome (SS)[4]. In addition, TNF-α participates in the physiopathology of several infectious diseases such as tuberculosis (TB), where it plays an important role in the formation and maintenance of the granuloma[5]. Anti–TNF-α therapy has been a breakthrough in the management of RA[6]; however, its use appears to promote reactivation and/or acquisition of TB[7].

The TNF-α gene (*TNF*) is located within the class III region of the major histocompatibility complex (MHC) on chromosome 6 (6p21.31), and is highly polymorphic[8]. Five microsatellites and numerous single nucleotide polymorphisms (SNP) in the *TNF* promoter have been described, some of which may regulate TNF-α expression. Two of these SNP represent a guanine to adenine transition at positions –238 and –308; they have been examined in both autoimmune disease and TB, yielding diverse results, mainly due to differences in the origin of the studied populations, linkage disequilibrium with other MHC genes, or insufficient sample size[8,9].

Given that *TNF* polymorphism may vary among populations[10], that such polymorphism might be associated with some autoimmune diseases[8], and noting that the cytokine also plays an important role in TB[5], a disease favored by the use of TNF-α blockers[7], we evaluated *TNF* –238 and –308 SNP simultaneously in patients with SLE, RA, primary SS, and TB, all in subjects of the same ethnic group.

MATERIALS AND METHODS

Study population. We analyzed 165 consecutive patients with RA[11], 100 with SLE[12], 67 with primary SS[13], and 135 with pulmonary TB. There were 145 women and 20 men with RA; their mean age ± standard deviation was 46 ± 12.7 years, mean duration of disease was 6 ± 5.5 years, and rheuma-

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toid factor (RF) test was positive in 85%. There were 98 women and one man with SLE; their mean age was 37 ± 11 years, mean duration of disease was 6.3 ± 0.7 years, and antinuclear antibodies and anti-DNA antibodies were positive in 98% and 60%, respectively. All patients with primary SS were women, mean age 49 ± 13 years, with mean duration of disease 6.9 ± 5.6 years. Clinical and immunological characteristics of these patients were similar to those previously reported[14-17]; however, they were not considered in this report. None of these patients had previous or current evidence of TB from clinical history and chest radiograph. Since Colombia is an intermediate endemic area for TB and the tuberculin test is not useful for TB diagnosis in our population, we do not systematically apply it.

There were 118 women and 17 men with TB, and they were enrolled for the study at time of treatment for their disease. Their mean age was 40 ± 16 years. TB was diagnosed by the presence of alcohol acid-resistant bacilli in sputum or by isolation of *M. tuberculosis* in culture. In all cases, patients with TB were negative for human immunodeficiency virus (HIV) infection. Patients with autoimmune rheumatic diseases were seen in the Rheumatology Unit at the Clinica Universitaria Bolivariana, and those with TB were seen in the La Maria Hospital, Medellin.

Controls were represented by 430 persons with no inflammatory, autoimmune disease or history of chronic infectious disease, including TB and HIV infection; they were matched to patients by sex, ethnicity, and socioeconomic status and were unrelated to the patients. Their mean age was 49 ± 15 years. This research was conducted in compliance with Resolution 008430 of 1993 of the Ministry of Health of Colombia, and was classified as research with minimal risk. The local Ethics Committee approved the study.

Genotyping for TNF polymorphism. Genomic DNA was extracted from 10 ml of EDTA-anticoagulated blood sample using the standard salting-out technique. Genotyping for the *TNF* SNP at –238 and –308 was by polymerase chain reaction (PCR) on a Bio-Rad thermal cycler iCycler (Bio-Rad, Hertfordshire, UK). For detection of alleles, PCR was followed by restriction enzyme digestion. The *TNF* –308 polymorphism was analyzed using the restriction fragment length polymorphism (RFLP) method as described[18]. Briefly, a 107 bp fragment was amplified with forward primer 5'AGG CAA TAG GTT TTG AGG GCC AT 3' and reverse primer 5'TCC TCC CTG CTC CGA TTC 3' (Invitrogen Life Technologies, Frederick, MD, USA). The PCR conditions were as follows: 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 1 min at 60°C and 1 min at 72°C. A final extension was performed at 72°C for 10 min. PCR products were digested by incubation with *NcoI* enzyme (Promega, Bogotá, Colombia) at 37°C for 12 h. The polymorphism at position –238 of the *TNF* promoter was defined using the PCR-RFLP developed by Gallagher, *et al*[19]. Primers were designed to amplify a 192 bp product as follows: forward primer 5'TTC CTG CAT CCT GTC TGG AAG TAA GAA 3' and reverse primer 5' AGG ATA CCC CTC ACA CTC CCC ATC CTC CCG GAT C 3' (Invitrogen). The PCR conditions for –238 SNP were similar to those described for *TNF* –308 SNP. PCR products were digested by incubation with *BamHI* enzyme (Promega) at 37°C for 2 h. The restriction fragments (87 bp and 20 bp for –308G and 107 bp for –308A; 164 bp and 28 bp for –238G; and 192 bp for –238A) were analyzed on an ethidium-bromide stained 3% agarose gel (Seakem LE Agarose, BioWhittaker, Rockland, ME, USA). Positive and negative controls were included with each batch of samples.

Statistical analysis. Data were managed using SPSS (V9.05 for Windows; SPSS, Chicago, IL, USA). Hardy-Weinberg equilibrium testing and linkage disequilibrium testing were performed using Arlequin software[20] as described[16]. Allele and haplotype frequencies were calculated by direct counting. Differences between allele and genotype frequencies were determined by chi-square and Fisher's exact test as appropriate. Crude odds ratios (OR) as estimates of the relative risk were calculated with 95% confidence intervals (CI). A p value < 0.05 was considered statistically significant.

RESULTS

Allelic and genotype frequencies corresponding to –308 SNP are shown in Tables 1 and 2, and those for –238 SNP in Tables 3 and 4. *TNF* GG –308 homozygosis was shown to be a protective factor for RA, SLE, and primary SS, but a risk factor for TB (Table 1). The –308A allele (TNF2) was associated with RA, SLE, and primary SS, whereas the –308G allele (TNF1) was associated with TB (OR 1.8, 95% CI 1.10–3.0, p = 0.02; Table 2). When the group of patients with autoimmune disease was compared to those with TB, a stronger association between TNF2 and autoimmunity was found in comparison to the control group (OR > 3, p < 0.0001). Similarly, the protective role of TNF1 was stronger in autoimmune disorders than in the TB group (OR < 0.3, p < 0.0001).

By contrast, *TNF* GA –238 heterozygosis was a protective factor for RA, SLE, and primary SS, but was a risk factor for TB (Table 3). *TNF* –238A was associated with presence of TB but was a protective factor for RA, SLE, and primary SS (Table 4).

In the control group, heterozygosis in proportions of 20% and 22% was found for the –308 and –238 SNP, respectively. With the exception of primary SS and SLE, all patient populations and controls were in Hardy-Weinberg equilibrium. There were no significant differences between alleles and genotypes when men and women were compared.

Haplotype *TNF* –308A–238G was a risk factor for RA, SLE, and primary SS, but was a protective factor for TB (Table 5). Most of the patients with primary SS and RA had been previously examined for HLA-DRB1 polymorphism[15,16]. Analysis of these patients showed no linkage disequilibrium between *TNF* –308 and –238 SNP and HLA-DRB1 (by likelihood-ratio test, with empirical distribution by permutation[16,20]).

DISCUSSION

We simultaneously examined *TNF* –308 and –238 single nucleotide polymorphisms in patients with autoimmune rheumatic diseases and TB, as well as in clinically healthy individuals, all from the same ethnic group. Our data reveal important results. First, they show an opposite association of the *TNF* polymorphism with autoimmunity and TB. *TNF* –308A–238G haplotype was shown to be a risk factor for autoimmunity (SLE, RA, and primary SS), but conferred protection to TB (Table 5). Second, our data suggest the occurrence of an overdominant selection in our population (a heterozygote advantage), in which heterozygotes for a specific allele (–308A) are resistant to one disease (TB) but susceptible to another (autoimmunity; Table 2), providing a genetic insight into the inverse relationship between occurrence of TB and autoimmune diseases (i.e., RA)[21,22]. Natural selection for resistance to a pathogen can lead to increase in the frequency of alleles that are otherwise deleterious[23]. If protection from infection is a stronger selective force than the negatively selected phenotype, the deleterious allele will accumulate in the population as long as the infec-

Table 1. Genotype frequencies of the *TNF* –308 SNP. Comparisons were done between each patient group and healthy controls.

Genotype	RA, n = 165	SLE, n = 100	Primary SS, n = 67	TB, n = 135	Controls, n = 430
GG, n (%)	109 (66)*	53**	32 (48)†	118 (87)††	338 (78)
AA, n (%)	4 (2)	2	1 (1)	1 (1)	5 (1)
GA, n (%)	52 (32)	45	34 (51)	16 (12)	87 (20)

* OR 0.53, 95% CI 0.35–0.78, p = 0.002. ** OR 0.30, 95% CI 0.19–0.48, p < 0.0001. † OR 0.25, 95% CI 0.15–0.42, p < 0.0001. †† OR 1.9, 95% CI 1.10–3.30, p = 0.02.

Table 2. Allele frequencies of the *TNF* –308 SNP. Comparisons were done between each patient group and healthy controls.

Allele	RA, n = 330	SLE, n = 200	Primary SS, n = 134	TB, n = 270	Controls, n = 860
G, n (%)	270 (82)	151 (76)	98 (73)	252 (93)	763 (89)
A, n (%)	60 (18)*	49 (24)**	36 (27)†	18 (7)††	97 (11)

* OR 1.8, 95% CI 1.26–2.54, p = 0.002. ** OR 2.6, 95% CI 1.77–3.83, p < 0.0001. † OR 2.9, 95% CI 1.90–4.57, p < 0.0001. †† OR 0.6, 95% CI 0.34–0.93, p 0.03.

Table 3. Genotype frequencies of the *TNF* –238 SNP. Comparisons were done between each patient group and healthy controls.

Genotype	RA, n = 165	SLE, n = 100	Primary SS, n = 67	TB, n = 135	Controls, n = 430
GG, n (%)	165 (100)	99	67 (100)	75 (56)	333 (77)
AA, n	0	0	0	0	1
GA, n (%)	0*	1**	0†	60 (44)††	96 (22)

* OR 0.01, 95% CI 0–0.16, p < 0.0001. ** OR 0.04, 95% CI 0–0.26, p < 0.0001. † OR 0.03, 95% CI 0–0.41, p < 0.0001. †† OR 2.8, 95% CI 1.85–4.19, p < 0.0001.

Table 4. Allele frequencies of the *TNF* –238 SNP. Comparisons were done between each patient group and healthy controls.

Allele	RA, n = 330	SLE, n = 200	Primary SS, n = 134	TB, n = 270	Controls, n = 860
G, n (%)	330 (100)	199 (99.5)	134 (100)	210 (78)	762 (89)
A, n (%)	0*	1 (0.5)**	0†	60 (22)††	98 (11)

* OR 0.01, 95% CI 0–0.19, p < 0.0001. ** OR 0.04, 95% CI 0–0.28, p < 0.0001. † OR 0.03, 95% CI 0–0.46, p < 0.0001. †† OR 2.2, 95% CI 1.56–3.17, p < 0.0001.

tious agent remains prevalent. If heterozygous individuals are resistant to disease and homozygous individuals are susceptible, heterozygote advantage is observed, as in our population. This theory proposes that individuals heterozygous at a locus may have a more effective immune response to a wider diversity of pathogens[23]. Our results are consistent with the chromosomal exchange of these SNP, since the –308A and –238A exchange in the *TNF* promoter never occurs in the same chromosome[24]. Third, this study, carried out on a Northwestern Colombian population, a group primarily derived from Spaniards that did not mix in significant proportions with Amerindian or Black populations[25,26], provides new data that could assist future comparisons on allelic and genotype frequencies, which in turn may elucidate the history of human populations, since *TNF* polymorphism varies among populations[10].

Studies have revealed that –308A allele is associated with RA both as a susceptibility and a severity factor[27-30]. In

Table 5. Estimated haplotype frequencies. Comparisons were done between each patient group and healthy controls.

	RA, n = 330	SLE, n = 200	Primary SS, n = 134	TB, n = 270	Controls, n = 860
–308G–238G, %	82	76	73	73	79
–308A–238A, %*	0	0	0	2	2
–308G–238A, %	0	0.5	0	21	10
–308A–238G, %	18**	23.5***	27†	4 ††	9

* There was no single homozygote individual for this haplotype. ** OR 2.2, 95% CI 1.53–3.16, $p < 0.0001$. *** OR 3, 95% CI 2.04–4.53, $p < 0.0001$. † OR 3.6, 95% CI 2.32–5.67, $p < 0.0001$. †† OR 0.46, 95% CI 0.24–0.85, $p = 0.01$.

our population, we observed that this allele was a predisposing factor for RA (OR 1.8), while *TNF* –238A was protective (OR 0.01). The *TNF* GG –238 genotype has been associated with RA severity independently of the presence of HLA-DR4[27,31,32]. In our population the genotype as well as the *TNF* –308A–238G haplotype was associated with RA.

Similarly, the –308A allele has been associated with susceptibility to SLE in Caucasian populations, in linkage disequilibrium with HLA-DR3[33,34], as well as in an HLA-independent manner[35,36]. This was not observed in populations with high Amerindian ancestry[37] or in Africans[34]. A study on California families failed to link –308A allele to SLE[38]. Our findings support the association of this allele with disease (OR 2.4), while *TNF* –238A was protective (OR 0.04). Other studies have reported discordant results concerning *TNF* –238[34,37,39]. Ethnicity and sample size could account for these discrepancies.

In primary SS, both TNF-α mRNA and its protein are significantly expressed in ducts and in mononuclear cells of salivary gland infiltrates[4], and TNF-α has been suggested to participate in the proteolysis of glandular acini[40]. Our results show that, as it occurs in RA and SLE, the *TNF* –308A–238G haplotype is a risk factor for disease (OR 3.6, $p < 0.0001$).

Autoimmune diseases are observed in genetically susceptible individuals in whom clinical expression is modified by permissive and protective environments over time. From a genetic point of view, these are complex diseases — their inheritance does not follow a single-gene dominant or single-gene recessive Mendelian law, and thus they are polygenic. Our results indicate that the *TNF* –308A–238G haplotype constituted a common susceptibility factor for autoimmune diseases (RA, SLE, and primary SS) in our population. This finding sustains the common variants/multiple disease hypothesis, which emphasizes that many disease genes may not be disease-specific, and that similar immunogenetic mechanisms underlie these diseases[41-44].

The role of TNF-α in autoimmunity may vary between diseases. While there is compelling evidence indicating a pathogenic role of this cytokine in RA[1,2], a protective role has been suggested in SLE[3], and incomplete knowledge exists concerning its function in primary SS. The question is how to relate these genetic findings with cytokine function in disease. The premise of studies of *TNF* promoter SNP is that gene variants with a significant role in pathology will lead to greater understanding of the regulatory mechanisms in both health and disease, and may provide knowledge for identifying and allowing early intervention in at-risk individuals[9]. However, although some studies have associated *TNF* SNP with cytokine synthesis[8], recent work indicates that the –308 and –238 polymorphisms are not functional[9]. We failed to find a significant association between these SNP and disease manifestations, clinical activity, the presence of autoantibodies, and circulating TNF-α levels in SLE and primary SS patients[45]. Regulation of *TNF* includes both epigenetic and posttranscriptional mechanisms, none of which have been considered so far[1,9]. Thus, *TNF* polymorphism contributes to susceptibility to autoimmune rheumatic diseases; however, the relative magnitude of its non-HLA-DR/DQ effects is uncertain due to the extraordinary linkage disequilibrium that extends over the MHC and to the regulatory mechanisms that control its expression.

With regard to TB, there is evidence that TNF-α acts as both a protective molecule and a mediator of disease manifestations[5]. It is considered that this cytokine is necessary for control of the acute infection caused by *M. tuberculosis* since it is of great importance in granuloma formation in TB, as well as in other diseases caused by mycobacteria[5]. To some extent this explains the reactivation of TB in patients with RA treated with TNF-α blockers[7]. Although the role of TNF-α is considered important in TB, there are no data about its polymorphism in American populations. Two studies in Asia failed to establish an association between –238 and –308 SNP and pulmonary TB[46,47]. Our results provide insight into the role of *TNF* polymorphism in TB in our population.

The *TNF* polymorphism has been considered a predisposing factor to TNF blocker therapy in patients with RA. *TNF* GG –308 homozygosity was associated with a better response to infliximab[48], and together with interleukin 10 GG –1087 homozygosity was associated with a superior

response to etanercept[49]. It is notable that *TNF* GG –308 homozygosity was a protective factor for RA in our population. Our goal was not to predict the risk of TB in patients with RA; further studies are needed to address this.

We describe an opposite association between *TNF* polymorphism and autoimmunity and TB. Studies are under way to evaluate the polymorphism of other genes located within the ~3.5 MB region comprising the MHC to elucidate whether this differential association with autoimmunity and TB is primary or secondary, depending on linkage disequilibrium with other loci incriminated in the immune response. These results support the hypothesis that autoimmune diseases are present-day manifestations of the selective genetic pressure exerted by infectious diseases (i.e., TB epidemics) of the recent past[21].

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REFERENCES

1. Correa PA, Anaya JM. Bases moleculares para el estudio del TNF alfa en la artritis reumatoidea [Molecular understanding of TNF alpha in rheumatoid arthritis]. Rev Colomb Reumatol 2001;8:236-50. Internet [cited 30 September 2004]. Available from: http://www.encolombia.com/medicina/reumatologia/reuma82-01-bases.htm
2. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. Annu Rev Immunol 1996;14:397–440.
3. Gomez D, Correa PA, Gomez LM, Cadena J, Molina JF, Anaya JM. Th1/Th2 cytokines in patients with systemic lupus erythematosus: Is tumor necrosis factor alpha protective? Semin Arthritis Rheum 2004;33:404-13.
4. Fox RI, Kang HI, Ando D, Abrams J, Pisa E. Cytokine mRNA expression in salivary gland biopsies of Sjogren's syndrome. J Immunol 1994;152:5532-9.
5. Flynn JL, Chan J. Immunology of tuberculosis. Annu Rev Immunol 2001;19:93–129.
6. Feldmann M. Development of anti-TNF therapy for rheumatoid arthritis. Nat Rev Immunol 2002;2:364-71.
7. Gardam MA, Keystone EC, Menzies R, et al. Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. Lancet Infect Dis 2003;3:148-55.
8. Hajeer AH, Hutchinson IV. Influence of TNFα gene polymorphisms on TNFα production and disease. Hum Immunol 2001;62:1191-9.
9. Bayley JP, Ottenhoff TH, Verweij CL. Is there a future for TNF promoter polymorphisms? Genes Immun 2004;5:1-15.
10. Baena A, Leung JY, Sullivan AD, et al. TNF-alpha promoter single nucleotide polymorphisms are markers of human ancestry. Genes Immun 2002;3:482-7.
11. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
12. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
13. Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61:554-8.
14. Anaya JM, Correa PA, Mantilla RD, Jiménez F, Kuffner T, McNicholl JM. Rheumatoid arthritis in African Colombians from Quibdó. Semin Arthritis Rheum 2001;31:191-8.
15. Anaya JM, Correa PA, Mantilla RD, Arcos-Burgos M. Rheumatoid arthritis association in Colombian population is restricted to HLA-DRB1*04 QRRAA alleles. Genes Immun 2002;3:56-8.
16. Anaya JM, Correa PA, Mantilla RD, Arcos-Burgos M. TAP, HLA-DQB1 and HLA-DRB1 polymorphisms in Colombian patients with primary Sjögren's syndrome. Semin Arthritis Rheum 2002;31:296-305.
17. Correa PA, Molina JF, Pinto LF, Arcos-Burgos M, Herrera M, Anaya JM. TAP1 and TAP2 polymorphism in northwestern Colombian patients with systemic lupus erythematosus. Ann Rheum Dis 2003;62:363-5.
18. Wilson AG, di Giovine FS, Blakemore AI, Duff GW. Single base polymorphism in the human tumor necrosis factor alpha (TNFα) gene detectable by NcoI restriction of PCR product. Hum Mol Genet 1992;1:353.
19. Gallagher G, Eskdale J, Oh HH, Richards SD, Campbell DA, Field M. Polymorphism in the TNF gene cluster and MHC serotypes in the West of Scotland. Immunogenetics 1997;45:188-94.
20. Schneider S, Roessli D, Excoffier L. Arlequin: software for population genetics data analysis. Ver 2.000. Geneva: Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva; 2000.
21. Mobley JL. Is rheumatoid arthritis a consequence of natural selection for enhanced tuberculosis resistance? Med Hypotheses 2004;62:839-43.
22. Rothschild BM, Rothschild C, Helbling M. Unified theory of the origins of erosive arthritis: conditioning as a protective/directing mechanism? J Rheumatol 2003;30:2095-102.
23. Dean M, Carrington M, O'Brien SJ. Balanced polymorphism selected by genetic versus infectious human disease. Annu Rev Genomics Hum Genet 2002;3:263–92.
24. Hohler T, Grossmann S, Stradmann-Bellinghausen B, et al. Differential association of polymorphisms in the TNFα region with psoriatic arthritis but not psoriasis. Ann Rheum Dis 2002;61:213–8.
25. Carvajal-Carmona LG, Soto ID, Pineda N, et al. Strong Amerind/white sex bias and a possible Sephardic contribution among the founders of a population in northwest Colombia. Am J Hum Genet 2000;67:1287-95.
26. Correa PA, Whitworth WC, Kuffner T, McNicholl J, Anaya JM. HLA-DR and DQB1 gene polymorphism in the North-western Colombian population. Tissue Antigens 2002;59:436-9.
27. Brikman BM, Huizinga TW, Kurban SS, et al. Tumor necrosis factor α gene polymorphisms in rheumatoid arthritis: association with susceptibility to, or severity of, disease? Br J Rheumatol 1997;36:516-21.
28. Cvetkovic JT, Wallberg-Jonsson S, Stegmayr B, Rantapaa-Dahlqvist S, Lefvert AK. Susceptibility for and clinical manifestations of rheumatoid arthritis are associated with polymorphisms of the TNF-α, IL-1ß, and IL-1Ra genes. J Rheumatol 2002;29:212-9.
29. Waldron-Lynch F, Adams C, Amos C, et al. Tumor necrosis factor 5' promoter single nucleotide polymorphisms influence susceptibility to rheumatoid arthritis (RA) in immunogenetically defined multiplex RA families. Genes Immun 2001;2:82-7.
30. Vinasco J, Beraun Y, Nieto A, et al. Polymorphism at the TNF loci in rheumatoid arthritis. Tissue Antigens 1997;49:74-8.
31. Fabris M, Di Poi E, D'Elia A, Damante G, Sinigaglia L, Ferraccioli G. Tumor necrosis factor-α gene polymorphism in severe and mild-moderate rheumatoid arthritis. J Rheumatol 2002;29:29-33.
32. Kaijzel EL, van Krugten MV, Brinkman BM, et al. Functional analysis of a human tumor necrosis factor α promoter

polymorphism related to joint damage in rheumatoid arthritis. Mol Med 1998;4:724-33.
33. Wilson AG, Gordon C, di Giovine FS, et al. A genetic association between systemic lupus erythematosus and tumor necrosis factor alpha. Eur J Immunol 1994;24:191-5.
34. Rudwaleit M, Tikly M, Khamashta M, et al. Interethnic differences in the association of tumor necrosis factor promoter polymorphisms with systemic lupus erythematosus. J Rheumatol 1996;23:1725-8.
35. Rood MJ, van Krugten MV, Zanelli E, et al. TNF-308A and HLA-DR3 alleles contribute independently to susceptibility to systemic lupus erythematosus. Arthritis Rheum 2000;43:129-34.
36. Van der Linden MW, van der Slik AR, Zanelli E, et al. Six microsatellite markers on the short arm of chromosome 6 in relation to HLA-DR3 and TNF-308A in systemic lupus erythematosus. Genes Immun 2001;2:373-80.
37. Zuniga J, Vargas-Alarcón G, Hernandez-Pacheco G, Portal-Celhay C, Yamamoto-Furusho JK, Granados J. Tumor necrosis factor-alpha promoter polymorphisms in Mexican patients with systemic lupus erythematosus. Genes Immun 2001;2:363-6.
38. Tsuchiya N, Kawasaki A, Tsao BP, Komata T, Grossman JM, Tokunaga K. Analysis of the association of HLA-DRB1, TNF alpha promoter and TNFR2 (TNFRSF1B) polymorphisms with SLE using transmission disequilibrium test. Genes Immun 2001;2:317-22.
39. D'Alfonso S, Colombo G, Della Bella S, Scorza R, Momigliano-Richiardi P. Association between polymorphisms in the TNF region and systemic lupus erythematosus in the Italian population. Tissue Antigens 1996;47:551-5.
40. Azuma M, Motegi K, Aota K, Hayashi Y, Sato M. Role of cytokines in the destruction of acinar structure in Sjögren's syndrome salivary glands. Lab Invest 1997;77:269-80.
41. Anaya JM, Talal N. Sjögren's syndrome comes of age. Semin Arthritis Rheum 1999;28:355-9.
42. Heward J, Gough SCL. Genetic susceptibility to the development of autoimmune disease. Clin Sci 1997;93:479–91.
43. Becker KG, Simon RM, Bailey-Wilson JE, et al. Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. Proc Natl Acad Sci USA 1998;95:9979-84.
44. Becker KG. The common variants/multiple disease hypothesis of common complex genetic disorders. Med Hypotheses 2004;62:309-17.
45. Tobon G, Correa PA, Gomez LM, Pineda-Tamayo, Anaya JM. Lack of association between TNF alpha -308 polymorphism and clinical and immunological characteristics of systemic lupus erythematosus and primary Sjögren's syndrome [abstract]. Ann Rheum Dis 2004;63 Suppl:237.
46. Selvaraj P, Sriram U, Mathan Kurian S, Reetha AM, Narayanan PR. Tumor necrosis factor α (-238 and -308) and ß gene polymorphisms in pulmonary tuberculosis: haplotype analysis with HLA-A, B and DR genes. Tuberculosis 2001;81:335–41.
47. Delgado JC, Baena A, Thim S, Goldfeld AE. Ethnic-specific genetic associations with pulmonary tuberculosis. J Infect Dis 2002;186:1463-8.
48. Mugnier B, Balandraud N, Darque A, Roudier C, Roudier J, Reviron D. Polymorphism at position -308 of the tumor necrosis factor alpha gene influences outcome of infliximab therapy in rheumatoid arthritis. Arthritis Rheum 2003;48:1849-52.
49. Padyukov L, Lampa J, Heimburger M, et al. Genetic markers for the efficacy of tumor necrosis factor blocking therapy in rheumatoid arthritis. Ann Rheum Dis 2003;62: 526-9.