ELSEVIER

Genetic Polymorphisms in Tumor Necrosis Factor (TNF)-α and TNF-β in a Population-Based Study of Systemic Lupus Erythematosus: Associations and Interaction with the Interleukin-1α-889 C/T Polymorphism

Christine G. Parks, Janardan P. Pandey, Mary Anne Dooley, Edward L. Treadwell, E.W. St. Clair, Gary S. Gilkeson, Carol A. Feghali-Bostwick, and Glinda S. Cooper

ABSTRACT: Tumor necrosis factor (TNF) is involved in the pathogenesis of systemic lupus erythematosus (SLE), but the role of TNF polymorphisms in SLE susceptibility remains unclear. Previous studies in different populations report an inconsistent association of the TNF-α −308A allele with SLE, sometimes depending on the presence of HLA-DR3. We examined the association of polymorphisms in TNF-α (−308G/A, −238G/A) and TNFβ (+252A/G) in a population-based study of SLE in the southeastern United States and considered TNF-SLE associations with respect to HLA-DR3 and DR2 and the interleukin (IL)-1α −889C/T polymorphism, previously linked to SLE in this population. Genotypes were analyzed for 230 recently diagnosed SLE patients who met American College of Rheumatology classification criteria and 276 age- and sex-matched controls, randomly selected from driver's license registries. Carriage of the TNF-α −308A allele was significantly associated with SLE in Caucasians (OR = 2.3; 95% CI 1.4, 3.9), but not African Americans. Analyses stratified by IL-1α −889 genotypes (C/C vs C/T or T/T) revealed independent associations of SLE with TNF-α −308A or HLA-DR2 and DR3. This reflected a significant interaction of TNF and IL-1 genotypes in Caucasians, and yielded a strong association (OR = 8.0, $p < 0.00001$) for the combined "HLA-DR3, TNF-α −308A, IL-1α −889C/C" genotype. These findings provide evidence of cytokine gene epistasis in SLE susceptibility. *Human Immunology 65, 622–631 (2004).*

KEYWORDS: TNF; polymorphism; systemic lupus erythematosus; population-based; genetics

ABBREVIATIONS

CI	confidence interval
IL	interleukin
OR	odds ratio
SLE	systemic lupus erythematosus
TNF	tumor necrosis factor

INTRODUCTION

Tumor necrosis factor (TNF) is well known for its role in the regulation of inflammation and apoptosis, two processes involved in the pathogenesis of systemic lupus erythematosus (SLE). Production of TNF is altered in SLE patients and correlated with disease activity [1–3]. Experimental models of SLE show abnormal TNF activity [4–6], which can be manipulated to change patterns of disease onset or severity [7, 8]. In some models,

From the National Institute of Environmental Health Sciences, Durham, NC (C.G.P., G.S.C.); Medical University of South Carolina, Charleston, SC (J.P.P., G.S.G.), University of North Carolina Medical School, Chapel Hill, NC (M.A.D.); East Carolina University School of Medicine, Greenville, NC (E.L.T.); Duke University Medical Center, Durham, NC (E.W.S.C.); University of Pittsburgh, Pittsburgh, PA (C.L.F-B.).

Address reprint requests to: Christine G. Parks, PhD, Epidemiology Branch, A3-05, NIEHS, P0 Box 12233, Durham, NC 27709-12233; Tel: (919) 541-2577; Fax: (919) 541-2511; E-mail: parks@niehs.nih.gov.

Funded in part by an NIH Intramural Research Training Award and the U.S. Department of Energy cooperative agreement DE-FC09-02CH11109. The Carolina Lupus Study was supported by the Intramural Research Program of the NIEHS and the National Center for Minority Health and Health Disparities of the NIH.

Received December 1, 2003; revised March 9, 2004; accepted March 11, 2004.

0198-8859/04/$–see front matter
doi:10.1016/j.humimm.2004.03.001

genetic variation in TNF has been shown to be associated with differences in susceptibility [9–11]. However, the role of TNF polymorphisms in human SLE susceptibility remains unresolved. The most frequently studied polymorphism, TNF-α −308G/A, has been inconsistently associated with SLE in different populations (the −308A allele) [12–20]. One explanation for the lack of agreement among studies has been the strong linkage disequilibrium between TNF-α −308A and another SLE risk factor, HLA-DR3 (as part of the conserved ancestral haplotype HLA-A1 B8 DR3), primarily seen in Caucasians [13, 16]. Two recent case control studies, however, have reported a positive association between TNF-α −308A and SLE independent of the HLA-DR3 allele, both in African Americans [19] and in Caucasians [20, 21].

Different patterns of linkage disequilibrium of the TNF-α −308 locus and other relevant polymorphisms in TNF might also account for the apparent inconsistency between studies. Two other well-known polymorphisms, TNF-α −238 and TNFβ +252, have not been studied as extensively as the −308 polymorphism with respect to SLE. There is some evidence that TNF-α −238 may be associated with SLE in Mexican and Italian patients [15, 22], and variation at TNFβ +252 has been associated with SLE in patients from Germany, Korea, and China [23–26]. In addition to HLA-DR3, HLA-DR2 (which represents another conserved major histocompatibility complex [MHC] haplotype, HLA-A3 B7 DR2), is also a risk factor for SLE in some studies [27–29]. MHC class II molecules may regulate the production of TNF *in vitro* [30]; HLA-DR2 has been associated with lower production of TNF compared with DR3 in healthy individuals and was also associated with both the low-producer phenotype and renal involvement in SLE patients [28, 31].

The debate over whether TNF polymorphisms confer susceptibility to SLE has gained increasing relevance with the application of anti-TNF therapy to treat a variety of autoimmune and immune-mediated diseases, including rheumatoid arthritis and inflammatory bowel disease. A substantial percentage of patients treated with TNF antagonists, such as etanercept and infliximab, develop antinuclear antibodies, as well as anti-DNA and anticardiolipin antibodies, and sometimes develop an SLE-like illness [32, 33]. Thus understanding the role of TNF polymorphisms in SLE may provide insights into the mechanisms of this anti-TNF effect in other immune-mediated diseases and may also help in thinking about the application of cytokine-based therapeutics in the treatment of SLE [34].

We examined the role of allelic variation in TNF-α (−308 and −238) and TNFβ (+252) with respect to SLE in both Caucasians and African Americans using a population-based case control design. Newly diagnosed SLE patients were enrolled from both university- and community-based rheumatology clinics. Controls were randomly sampled from the population from which the cases arose to minimize the effects of selection bias [35] and protect against bias from population stratification [36]. Analyses were also conducted to investigate whether the association of SLE and the TNF-α −308 polymorphism was independently observed in the presence or absence of HLA-DR2 and DR3. Genetic susceptibility to SLE is complex, reflecting the interrelations between many factors in the balance of response and regulation of the immune system. Given that TNF and interleukin (IL)-1 have many similar physiologic activities [37], we also considered possible modification of the TNF-SLE association by an IL-1 genotype (IL-1α −889C/T) previously associated with SLE in both African Americans and Caucasians in this study [38].

MATERIALS AND METHODS

Study Sample

The Carolina Lupus Study is a population-based case control study in 60 counties of North Carolina and South Carolina. Cases were SLE patients identified and referred through 30 community-based rheumatologists and four university rheumatology practices. Patients were eligible if they were diagnosed between January 1, 1995, and July 30, 1999; met the 1997 revised American College of Rheumatology classification criteria for SLE; were ≥18 years old; and lived in the study area for ≥6 months before diagnosis. Sex- and state-matched controls, identified through state driver's license records for the 60 study counties, were randomly selected and frequency matched to cases in 5-year age groups. Eligibility criteria for controls were the same as for cases, with the exception that controls must not have been diagnosed with lupus. Sample selection, recruitment, and enrollment were described previously [39]. Study protocols were approved by the institutional review boards of the National Institute of Environmental Health Sciences and other participating institutions. The final sample consisted of 265 cases and 355 controls, with enrollment of 93% of referred cases and 75% of screened and eligible controls. Ninety-percent of cases were female, 60% were African-American, and the mean age at diagnosis was 39 years of age (range 15–81). Thirty percent of controls were African-American, which reflects the racial distribution of the study area. Matching by race can improve the efficiency (cost-wise) of a study, but is not necessary to produce unbiased estimates of association [40].

Genotyping

Blood specimens were used to obtain DNA from 243 (92%) of cases and 298 (85%) of controls. DNA speci-

mens of patients and controls were typed for TNF-α −308 (G→A), TNF-α −238 (G→A), and TNF-β +252 (A→G) biallelic restriction fragment length polymorphisms (RFLPs), using polymerase chain reaction (PCR)-based methods previously described [41]. The HLA-DR2 and DR3 alleles were identified by restriction fragment length polymorphism analysis of PCR-amplified products of exon 2 domain of the DRB1 gene [42]. Specimens were defined as positive for HLA-DR3 if they were positive for at least one copy of either the 0301 or 0302 allele, or positive for DR2 if they had at least one copy of 1501, 1502, 1601, or 1602 alleles. Equivocal results were interpreted as missing for three cases and two controls each for DR15 and DR16 genotype in Caucasians. In Caucasians, 3 cases and 1 control were positive for the 0302 allele, compared with 16 cases and 6 controls among African Americans. For DR2, only two cases and one control were positive for the 1502 allele and one control was positive for 1602 in Caucasians, compared with six cases and one control positive for the 1502 allele and five cases and two controls for the 1602 allele in African Americans. Genotypes could not be determined at TNF-β +252 for one African American case, and at HLA-DR for four cases (three African-American, one Caucasian) and two controls (one African-American, one Caucasian). Genotyping of IL-1α −889 (C→T) was performed as previously described [38].

Analyses

Race-stratified analyses were conducted in parallel using genotype results from 230 cases (144 African-American and 86 Caucasian) and 276 controls (73 African-American and 203 Caucasian). Racial differences in allele frequencies and allelic association between the three TNF polymorphisms and HLA-DR2 and DR3 alleles were examined among controls by bivariate analyses. For each locus, the chi-square statistic (or Fisher's exact test statistic for small cell size, $n < 5$) was calculated to compare the frequency of genotypes in cases and controls.

Odds ratios (OR) and 95% confidence intervals (CI) were estimated by unconditional logistic regression, a parametric method that allows adjustment for confounders and analysis of potential interactions in diseases with complex genetic etiologies [43]. Single-locus regression models were run to estimate the effects of each of the three TNF polymorphisms with respect to SLE: for each locus we examined each genotype and carriage of the rare allele (either heterozygous or homozygous) compared with the common homozygous genotype. A combined model was developed including all three loci, thereby taking into account potential confounding by the effects of adjacent polymorphisms. Including the −238 and +252 polymorphisms made little change on the −308 estimated OR; therefore, single-locus models were run to assess the effects of TNF-α −308 stratified by presence or absence of HLA-DR2 and DR3. Interactions of carriage of the TNF-α −308A allele and HLA-DR2 and DR3 were evaluated by including product terms in race-stratified models. The statistical significance of interactions was evaluated based on the difference in the −2 log likelihoods for the models with and without the interaction term. Neither the TNF308*DR3 or TNF308*DR2 interactions were significant; thus, an overall model was constructed including TNF-α −308, HLA-DR3, and HLA-DR2 to estimate their individual effects while controlling for all three factors. The model including TNF-α −308, HLA-DR3, and HLA-DR2 was subsequently run in analyses stratified by IL-1α −889 genotype (CC vs CT or TT). Interactions between IL-1 and TNF genotypes or HLA-DR2 and DR3 were assessed as previously described.

Single-locus regression models were used to estimate the association in cases between TNF polymorphisms and age at diagnosis, proteinuria, and anti-dsDNA antibodies. Proteinuria was defined as two or more urine samples containing ≥3 mg/ml albumin reported in medical records up to 6 months after diagnosis, and anti-dsDNA antibodies were assessed in specimens collected at the time of participant enrollment as previously described [44].

RESULTS

Association of TNF Genotype and SLE

Table 1 shows the frequency of TNF-α and TNF-β genotypes in SLE cases and controls. Genotype frequencies were in Hardy-Weinberg equilibrium. Some allele frequencies differed by race: the TNF-α −238A allele and the TNF-β +252A alleles were significantly ($p < 0.05$) more common in Caucasian compared with African American controls, but there was no difference in allele frequency at TNF-α −308. Allelic associations were also observed across the three loci examined: carriage of TNF-α −308A was significantly ($p < 0.05$) associated with TNF-α −238G in Caucasian controls and with TNF-β +252A in Caucasian cases and controls and African American cases. In Caucasians, the TNF-α −308A/G and A/A genotypes were more common in SLE patients than controls: overall, TNF-α −308 genotype was associated with SLE (χ^2 10.8, 2 DF, $p = 0.0045$). The TNF-α −308A/G genotype was significantly associated with SLE compared with the G/G genotype (OR = 2.3; $p = 0.002$). The association was somewhat stronger for the A/A genotype (OR = 3.3, $p > 0.05$), but we observed no significant dose response for number of copies of the A allele. The combined A/G or A/A genotype was significantly associated with SLE in Caucasians in both single- (OR = 2.3, $p = 0.001$) and

TABLE 1 Frequency of TNF genotypes in African Americans and Caucasians in the Carolina Lupus Study[a]

Genotypes	Caucasian			African Americans		
	Cases $n = 86$ n (%)	Controls $n = 203$ n (%)	OR (95% CI)[b]	Cases $n = 144$ n (%)	Controls $n = 73$ n (%)	OR (95% CI)
TNF-α –308						
G/G	42 (49)	140 (69)	1.0 (referent)	108 (75)	52 (71)	1.0 (referent)
G/A	40 (46)	59 (29)	2.3 (1.3–3.9)[c]	31 (22)	20 (27)	0.7 (0.4–1.3)
A/A	4 (5)	4 (2)	3.3 (0.8–13.9)	5 (3)	1 (1)	2.4 (0.3–21.1)
G/A or A/A (single-locus model)			2.3 (1.4–3.9)[d]			0.8 (0.4–1.6)
G/A or A/A (three-locus model)			2.1 (1.3–3.7)[e]			0.9 (0.5–1.7)
TNF-α –238						
G/G	79 (92)	175 (86)	1.0 (referent)	136 (94)	71 (97)	1.0 (referent)
G/A	7 (8)	28 (14)	0.5 (0.2–1.3)	8 (6)	2 (3)	2.1 (0.4–10.1)
G/A (single-locus model)			0.5 (0.2–1.3)			2.1 (0.4–10.1)
G/A (three-locus model)			0.7 (0.3–1.7)			2.0 (0.4–9.5)
TNF-β +252						
A/A	15 (17)	21 (10)	1.0 (referent)	31 (22)	20 (27)	1.0 (referent)
A/G	43 (50)	84 (41)	0.6 (0.3–1.3)	71 (50)	32 (44)	1.4 (0.7–3.0)
G/G	28 (32)	98 (48)	0.5 (0.2–1.0)	41 (29)	21 (29)	1.5 (0.7–3.3)
A/G or G/G (single-locus model)			0.5 (0.3–1.1)			1.4 (0.7–2.6)
A/G or G/G (three-locus model)			0.7 (0.3–1.6)			1.2 (0.7–2.5)

[a] Frequencies based on n shown except as follows for TNF-β +252, which was based on 143 cases in African Americans.

[b] OR and 95% CI were estimated using logistic regression models for each locus separately (single-locus model) and adjusting for genotypes at all three loci (three-locus models) as indicated.

[c] $p = 0.002$.

[d] $p = 0.001$.

[e] $p = 0.005$.

Abbreviations: CI = confidence interval; OR = odds ratio; TNF = tumor necrosis factor.

three-locus (OR = 2.1, $p = 0.005$) models. In African Americans, the −308A/A genotype was more common in cases (3%) than in controls (1%) (OR = 2.4, $p > 0.05$), but there was no overall association between carriage of the TNF-α −308A allele and SLE (OR = 0.8). Viewed in terms of allele frequency, the −308A allele was significantly more common in Caucasian cases than controls (0.28 vs 0.17, $p = 0.002$), but this was not seen in African Americans (0.14 vs 0.15). Allelic variation at TNF-α −238 and TNFβ +252 was not associated with SLE in either Caucasians or African Americans. The apparent inverse association (OR = 0.5, $p = 0.058$) between the TNFβ +252 G/G genotype and SLE in Caucasians was less pronounced after adjusting for TNF-α −308 (OR = 0.7, $p = 0.417$).

Analyses Stratified by HLA-DR3 and IL-1 Genotype

Table 2 shows the association between SLE and carriage of the TNF-α −308A allele relative to the presence or absence of HLA-DR2 and DR3. In Caucasians, carriage of TNF-α −308A was significantly associated with HLA-DR3 in both controls (OR = 6.9, $p < 0.0001$) and cases (OR = 13.4, $p < .0001$). In contrast, HLA-DR2 was not associated with TNF-α −308 genotype in Caucasians and neither DR2 nor DR3 was associated with TNF-α −308 genotype in African Americans. Among Caucasians, carriage of TNF-α −308A was significantly associated with SLE in the absence of HLA-DR2 (OR = 2.8, $p = 0.0014$), and nonsignificantly associated with SLE in the presence of HLA-DR3 (OR = 2.6, $p > 0.05$). Among African Americans, carriage of TNF-α −308A was not associated with SLE either in the absence of DR2 (OR = 0.9) or presence of DR3 (OR = 0.5). The statistical interactions between HLA-DR2 or DR3 and TNF-α −308A were not significant ($p > 0.20$), so their independent effects could be estimated in a model including all three terms.

Table 3 shows ORs for a model including TNF-α −308A, HLA-DR2, and HLA-DR3, to simultaneously estimate their independent effects overall and in subgroups stratified by IL-1α −889 genotype. Overall, associations were seen between SLE and HLA-DR3 in Caucasians (OR = 2.1, $p = 0.02$) and HLA-DR2 in African Americans (OR = 2.0, $p = 0.03$). Stratified analyses in Caucasians revealed that the TNF-α 308G/A or A/A genotypes were significantly associated with SLE in those with the IL-1α −889C/C genotype (OR = 3.4, $p = 0.013$), but not in those with the C/T or T/T

TABLE 2 Frequency of TNF-α –308A/G or A/A genotype and association with SLE in the presence or absence of HLA-DR2 or DR3

Genotypes	Caucasians			African Americans		
	Cases	Controls	OR (95% CI)[a]	Cases	Controls	OR (95% CI)[a]
DR2 Present	$n = 27$	$n = 51$		$n = 66$	$n = 22$	
TNF-α –308 G/A or A/A	37%	29%	1.4 (0.5–3.9)	25%	27%	0.9 (0.3–2.7)
DR2 Absent	$n = 56$	$n = 150$		$n = 77$	$n = 49$	
TNF-α –308 G/A or A/A	55%	31%	2.8 (1.5–5.3)[b]	25%	28%	0.9 (0.4–2.0)
DR3 Present	$n = 35$	$n = 43$		$n = 37$	$n = 14$	
TNF-α –308 G/A or A/A	82%	65%	2.6 (0.9–7.6)	27%	43%	0.5 (0.1–1.8)
DR3 Absent	$n = 49$	$n = 159$		$n = 104$	$n = 58$	
TNF-α –308 G/A or A/A	26%	21%	1.3 (0.6–2.8)	25%	25%	1.0 (0.5–2.0)

[a] Odds ratio for TNF –308A/G or A/A versus G/G genotype estimated from logistic regression models stratified by the presence or absence of DR2 (at least one copy of 1501, 1502, 1601, or 1602 alleles) or HLA-DR3 (at least one copy of 0301 or 0302 alleles). HLA-DR2 and DR3 genotypes could not be determined for four cases (three African-American and one Caucasian) and two controls (one African-American, one Caucasian).

[b] $p = 0.0014$.

Abbreviations: SLE = systemic lupus erythmatosus; TNF = tumor necrosis factor.

genotype (OR = 1.1, $p > 0.05$). This reflected a significant interaction ($p = 0.05$). In contrast, the association with HLA-DR3 was only seen in Caucasians with IL-1α −889C/T or T/T genotypes (OR = 3.0, $p = 0.014$). In African Americans, the associations with HLA-DR2 (OR = 2.9, $p = 0.006$) and DR3 (OR = 2.6, $p = 0.035$) were only seen in those with the IL-1α −889C/T or T/T genotype. No association was seen between TNF α −308 and IL-1 α −889 genotypes among DR3-negative SLE patients (data not shown). Overall, the most common combined genotype, "HLA-DR3 negative, TNF-α −308G/G, IL-1α −889C/T or T/T," was less common in SLE patients in both Caucasians (21% cases vs 40% controls, $p = 0.0025$) and African Americans (28% cases vs 43% controls, $p = 0.0180$). In comparison, the strongest associations were seen for the combined "HLA-DR3 positive, TNF-α −308A, IL-1α−889C/C" genotype in both Caucasians (19% cases vs 4% controls; OR = 8.0) and African Americans (4% cases, 0% controls; OR not calculated).

Associations with SLE Phenotype

There was no association between TNF genotype and proteinuria (data not shown). Caucasian cases with the TNF-α −308A allele were more likely to be older than cases with the homozygous G/G genotype (median age

TABLE 3 Association of SLE with HLA-DR and TNF-α –308 genotype overall and stratified by IL-1α –889 genotype

Genotype	Odds ratios and 95% confidence intervals[a]				
	Overall	Stratified by IL-1α −889 C/T polymorphism			
		IL-1α –889C/C	p Value	IL-1α –889C/T or T/T	p Value
In Caucasians	82 cases/201 controls	42 cases/68 controls		41 cases/144 controls	
HLA-DR2 present	1.5 (0.9–2.8)	1.2 (0.5–2.9)		1.9 (0.9–4.2)	
HLA-DR3 present	2.1 (1.1–3.7)[b]	1.1 (0.4–3.0)		3.0 (1.2–7.1)	0.014
TNF-α –308G/A or A/A	1.7 (1.0–3.2)	3.4 (1.3–8.8)	0.013	1.1 (0.5–2.7)	
In African Americans	140 cases/71 controls	61 cases/18 controls		79 cases/53 controls	
HLA-DR2 present	2.0 (1.1–3.6)[c]	1.1 (0.4–3.4)		2.9 (1.4–6.3)	0.006
HLA-DR3 present	1.8 (0.9–3.8)	1.2 (0.3–4.3)		2.6 (1.1–6.2)	0.035
TNF-α –308G/A or A/A	0.9 (0.4–1.6)	1.5 (0.4–5.8)		0.9 (0.4–1.8)	

[a] Odds ratios derived from logistic regression models run separately for African Americans and Caucasians and including three variables: DR3 (0301 or 0302), DR2 (1501, 1502, 1601, 1602), and TNF –308 (G/A or A/A). Three models were run for each racial subgroup: one overall model, and one model each for carriers of the IL-1α –889C/C, and IL-1α –889C/T or T/T genotypes.

[b] $p = 0.02$.

[c] $p = 0.03$.

Abbreviations: see Table 2.

46 years vs 34 years): older Caucasian cases were more likely to carry TNF-α −308A (OR = 3.7; $p = 0.005$). This was not seen in African Americans (median 35 and 35.5 years, respectively). There was no association among either TNF, IL-1, or the combined genotypes and the presence of anti-dsDNA antibodies in Caucasians or African Americans (data not shown).

DISCUSSION

In this population-based case control study, we observed an association between carriage of the TNF-α −308A allele and SLE in Caucasians, which was seen only in those with the IL-1α −889 C/C genotype, providing new evidence of an interaction between TNF −308A and an allelic variant in another proinflammatory cytokine. One explanation for the lack of agreement among the previous studies of TNF-α −308 and SLE has been the strong linkage disequilibrium between TNF-α −308A and HLA-DR3 (as part of the conserved ancestral haplotype HLA-A1 B8 DR3) in Caucasians [13, 16]. A few studies have shown an association of TNF-α −308A and SLE in the absence of an association with HLA-DR3 [17] or otherwise independent of the HLA-DR3 allele in Caucasians [20] and African Americans [19]. We saw a tendency toward independent effects of TNF and DR3 in the overall adjusted model in Caucasians. However, when stratified by the IL-1α −889 genotype, the effects of TNF and HLA-DR2 or DR3 were clearly independent of one another, depending on the presence of the variant IL-1α −889T allele. In Caucasians, this reflected a statistically significant interaction, providing evidence of epistasis between TNF-α −308 and IL-1α −889. When effect modification is present, it may not be appropriate to combine subgroups in which the effects significantly differ. Thus, although the overall combined models are presented in the first column in Table 3 to enable comparisons with earlier studies, the stratified results more accurately reflect our findings with respect to the underlying relationships in these data. Epistasis between genes on chromosomes 6 and 2 has also been described with respect to the production of anticentromere antibodies in scleroderma (HLA and KM) [45]. Interestingly, the associations with HLA-DR2 and DR3 alleles and SLE were seen primarily in those with the IL-1α −889 C/T or T/T genotype, independent of variation at TNF-α −308. Although the interaction of HLA-DR2 and DR3 with IL-1α −889 was not significant, it is noteworthy that the same pattern occurred in both African Americans and Caucasians.

There is considerable evidence linking the biologic activities of TNF, IL-1, and HLA-DR. Both TNF and IL-1 can act through nuclear-factor-κB [37], and variation in one may affect activities of the other. For instance, TNF can augment the production of IL-1α in stimulated macrophages [46], and TNF-α −308A has been associated with increased production of IL-1α in the absence of differences in TNF-α levels [14]. Anti-TNF therapies (*e.g.,* of rheumatoid arthritis) also downregulate the systemic production of IL-1 [47, 48]. The IL-1α −889α C/T polymorphism has been associated with differences in both IL-1α and IL-1β production [49–51], with the T allele linked to increased transcription and plasma levels. We previously reported that IL-1α −889 genotype was associated with SLE in the Carolina Lupus Study [38], with lower frequencies of the variant allele in cases (0.31 Caucasians, 0.37 African Americans) than controls (0.39 Caucasians, 0.46 African Americans). Other studies relate HLA-DR2 to lower production of TNF-α compared with DR3 [28], and there is evidence that MHC class II molecules can regulate the production of both TNF and IL-1 *in vitro* [30]. In sum, our findings suggest a plausible epistatic relationship between TNF and IL-1 in Caucasians whereby carriage of TNF-α −308A (associated with higher IL-1α levels [14]) contributes to risk of SLE only in the context of the IL-1α −889α C/C genotype (associated with lower IL-1 production). These observations illustrate the need to better understand the complex interactions of multiple cytokines that contribute to genetic susceptibility in SLE.

In experimental models of SLE, both TNF and IL-1 are important in the induction and pathology of disease, but their effects depend on dose, timing, and other factors such as the relative production of IL-10 [4, 52, 53]. The role of genetic polymorphisms in SLE may be challenging to dissect, given the multiple genes implicated in a recent microarray study that described unique clustering of SLE patients [54]: many of the 20 genes identified had not previously been identified as SLE risk factors, but include members of the TNF and IL-1 cytokine families. Given the network properties of cytokines, it would make sense to focus future analyses on groups of cytokines involving both pro- and anti-inflammatory feedback mechanisms [55]. For example, in SLE, the role of TNF and IL-1 polymorphisms could partly depend on allelic variation in IL-10, which has been also associated with SLE [56–59]. We are examining the distribution of IL-10 polymorphisms in the Carolina lupus study, and will consider whether racial differences in IL-10 polymorphism could explain the different pattern for TNF polymorphisms and SLE among African Americans.

Our results do not replicate the findings of another case control study reporting a positive association of the TNF-α −308A allele in African Americans [19]. We observed a higher number of African American cases with the homozygous −308A/A genotype, but saw no association for the combined G/A or A/A genotype de-

spite having adequate statistical power to detect a minimum OR of 2.4 ($\beta = 0.20$, $\alpha = 0.05$). We did not observe independent associations between SLE and the other two polymorphisms, TNF-α −238 and TNFβ +252, which had previously been associated with SLE [15, 22–26]. The one apparent association for the TNFβ +252 G/G genotype in Caucasians (OR = 0.5) was diminished after controlling for variation at TNF-α −308 and −238.

Studies indicate that variation at both TNF-α −308 and TNFβ +252 can affect TNF production levels [60, 61], but it is not clear which polymorphisms are most functionally relevant [60, 62]. Within TNF-α other polymorphisms (*e.g.,* TNF-α −1031, −857) may be useful in representing haplotypic diversity [63]. Another explanation for the racial difference in findings in the present study may be that TNF-α −308A is more tightly linked to another relevant TNF polymorphism in Caucasians than in African Americans, reflecting differences in haplotype structure and diversity [63]. These differences and the inconsistencies in other studies might also be due to linkage disequilibrium with other genes in the MHC (*e.g.,* complement) related to SLE susceptibility [64] or chance variation.

The results of the present study are strengthened by its population-based design and inclusion of only recently diagnosed cases. Most patients were enrolled within 20 months of diagnosis, whereas patients in other studies appear to have been enrolled later in the course of their disease [19, 20]. This could also contribute to differences in findings if TNF or HLA-DR polymorphisms were related to survival. Although Caucasian patients with TNF-α −308A were significantly older, we saw no indication this allele was associated with a marker of severity (*i.e.,* proteinuria). Although other studies often enrolled patients from university clinics, the present study also included community-based rheumatology practices, possibly representing a broader range of disease [44].

Differences in genetic admixture may contribute to inconsistency between studies if the proportion of admixture in controls does not represent the population from which the cases arose. Population-based sampling of controls can help to minimize the effects of selection bias [35] and protect against bias from population-stratification [36]. Controls in the present study were randomly selected from the same geographic region as the cases, reflecting racial frequencies in the study area similar to census estimates and resulting in fewer African-American controls than Caucasian controls or African-American cases. Matching by race can improve the efficiency (cost-wise) of a study, but is not necessary to produce unbiased estimates of association [40]. In a previous study of African Americans, it was unclear whether the controls were derived from the same population base as cases [19]. The percent Caucasian admixture in African Americans varies geographically, and may also be affected by sampling techniques (*i.e.,* population- vs convenience-based). The frequency of TNF-α −308A was considerably lower among African-American controls (8%) in the previous study compared with the present study (15%), a study of healthy African Americans in North Carolina (14%), and a sample of healthy West Africans living in Gambia (19%) [19, 63, 65]. The frequency of the −308A allele in Caucasian controls (17%) was slightly higher than that reported in another sample of Caucasians in North Carolina [65], but similar to controls in other studies of SLE in Caucasians [13, 16, 21]. Admixture within the Caucasian population has also been suggested as a cause of the inconsistent findings on TNF and SLE, a problem avoided in a study of Caucasian families that found no significant transmission of TNF-α −308A or HLA-DR3 with respect to SLE [66]. Interestingly, a TNF-α haplotype defined by variation at −1031, −863, and −857 was associated with some clinical subtypes, suggesting a role of genetic variation in TNF not captured by variation at the −308 locus.

In sum, our findings indicate that the association between the TNF-α −308A allele and SLE in Caucasians is dependent on the presence of the IL-1α −889C/C genotype. This study provides new evidence that epistatic interactions are important to consider in SLE etiology. The complex network of factors involved in the balance of response and regulation of the immune system will certainly be reflected by complex patterns of genetic susceptibility in SLE. Well-designed studies with sufficient sample sizes will be needed to evaluate gene-gene and gene-environment interactions involved in the development of SLE.

ACKNOWLEDGMENT

Special thanks and appreciation are extended to the physicians who participated in the Carolina Lupus Study, and to Ms. Louise Weston who performed most of the laboratory analyses.

REFERENCES

1. Studnicka-Benke A, Steiner G, Petera P, Smolen JS: Tumour necrosis factor alpha and its soluble receptors parallel clinical disease and autoimmune activity in systemic lupus erythematosus. Br J Rheumatol 35:1067, 1996.
2. Gabay C, Cakir N, Moral F, Roux-Lombard P, Meyer O, Dayer JM, et al: Circulating levels of tumor necrosis factor soluble receptors in systemic lupus erythematosus are significantly higher than in other rheumatic diseases and correlate with disease activity. J Rheumatol 24:303, 1997.
3. Aringer M, Feierl E, Steiner G, Stummvoll GH, Hofler E,

Steiner CW, et al: Increased bioactive TNF in human systemic lupus erythematosus: associations with cell death. Lupus 11:102, 2002.

4. Segal R, Bermas BL, Dayan M, Kalush F, Shearer GM, Mozes E: Kinetics of cytokine production in experimental systemic lupus erythematosus: involvement of T helper cell 1/T helper cell 2-type cytokines in disease. J Immunol 158:3009, 1997.
5. Lemay S, Mao C, Singh AK: Cytokine gene expression in the MRL/lpr model of lupus nephritis. Kidney Int 50:85, 1996.
6. Boswell JM, Yui MA, Burt DW, Kelley VE: Increased tumor necrosis factor and IL-1 beta gene expression in the kidneys of mice with lupus nephritis. J Immunol 141: 3050, 1988.
7. Dayan M, Zinger H, Kalush F, Mor G, Amir-Zaltzman Y, Kohen F, et al: The beneficial effects of treatment with tamoxifen and anti-oestradiol antibody on experimental systemic lupus erythematosus are associated with cytokine modulations. Immunology 90:101, 1997.
8. Segal R, Dayan M, Zinger H, Mozes E: Suppression of experimental systemic lupus erythematosus (SLE) in mice via TNF inhibition by an anti-TNFalpha monoclonal antibody and by pentoxiphylline. Lupus 10:23, 2001.
9. Jacob CO, Lee SK, Strassmann G: Mutational analysis of TNF-alpha gene reveals a regulatory role for the 3′-untranslated region in the genetic predisposition to lupus-like autoimmune disease. J Immunol 156:3043, 1996.
10. Fujimura T, Hirose S, Jiang Y, Kodera S, Ohmuro H, Zhang D, et al: Dissection of the effects of tumor necrosis factor-alpha and class II gene polymorphisms within the MHC on murine systemic lupus erythematosus (SLE). Int Immunol 10:1467, 1998.
11. Kontoyiannis D, Kollias G: Accelerated autoimmunity and lupus nephritis in NZB mice with an engineered heterozygous deficiency in tumor necrosis factor. Eur J Immunol 30:2038, 2000.
12. Goldstein R, Sengar DP: Comparative studies of the major histocompatibility complex in French Canadian and non-French Canadian Caucasians with systemic lupus erythematosus. Arthritis Rheum 36:1121, 1993.
13. Wilson AG, Gordon C, di Giovine FS, de Vries N, van de Putte LB, Emery P, et al: A genetic association between systemic lupus erythematosus and tumor necrosis factor alpha. Eur J Immunol 24:191, 1994.
14. Danis VA, Millington M, Hyland V, Lawford R, Huang Q, Grennan D: Increased frequency of the uncommon allele of a tumour necrosis factor alpha gene polymorphism in rheumatoid arthritis and systemic lupus erythematosus. Dis Markers 12:127, 1995.
15. 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 47:551, 1996.
16. Rudwaleit M, Tikly M, Khamashta M, Gibson K, Klinke J, Hughes G, et al: Interethnic differences in the association of tumor necrosis factor promoter polymorphisms with systemic lupus erythematosus. J Rheumatol 23:1725, 1996.
17. Lu LY, Ding WZ, Fici D, Deulofeut R, Cheng HH, Cheu CC, et al: Molecular analysis of major histocompatibility complex allelic associations with systemic lupus erythematosus in Taiwan. Arthritis Rheum 40:1138, 1997.
18. Chen CJ, Yen JH, Tsai WC, Wu CS, Chiang W, Tsai JJ, et al: The TNF2 allele does not contribute towards susceptibility to systemic lupus erythematosus. Immunol Lett 55:1, 1997.
19. Sullivan KE, Wooten C, Schmeckpeper BJ, Goldman D, Petri MA: A promoter polymorphism of tumor necrosis factor alpha associated with systemic lupus erythematosus in African-Americans. Arthritis Rheum 40:2207, 1997.
20. Rood MJ, van Krugten MV, Zanelli E, van der Linden MW, Keijsers V, Schreuder GM, et al: TNF−308A and HLA-DR3 alleles contribute independently to susceptibility to systemic lupus erythematosus. Arthritis Rheum 43:129–34, 2000.
21. van der Linden MW, van der Slik AR, Zanelli E, Giphart MJ, Pieterman E, Schreuder GM, 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 2:373, 2001.
22. Zuniga J, Vargas-Alarcon 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 (SLE). Genes Immun 2:363, 2001.
23. Bettinotti MP, Hartung K, Deicher H, Messer G, Keller E, Weiss EH, et al: Polymorphism of the tumor necrosis factor beta gene in systemic lupus erythematosus: TNFB-MHC haplotypes. Immunogenetics 37:449, 1993.
24. Kim TG, Kim HY, Lee SH, Cho CS, Park SH, Choi HB, et al: Systemic lupus erythematosus with nephritis is strongly associated with the TNFB*2 homozygote in the Korean population. Hum Immunol 46:10, 1996.
25. Lee SH, Park SH, Min JK, Kim SI, Yoo WH, Hong YS, et al: Decreased tumour necrosis factor-beta production in TNFB*2 homozygote: an important predisposing factor of lupus nephritis in Koreans. Lupus 6:603, 1997.
26. Zhang J, Ai R, Chow F: The polymorphisms of HLA-DR and TNF B loci in northern Chinese Han nationality and susceptibility to systemic lupus erythematosus. Chin Med Sci J 12:107, 1997.
27. Hartung K, Baur MP, Coldewey R, Fricke M, Kalden JR, Lakomek HJ, et al: Major histocompatibility complex haplotypes and complement C4 alleles in systemic lupus erythematosus. Results of a multicenter study. J Clin Invest 90:1346, 1992.
28. Jacob CO, Fronek Z, Lewis GD, Koo M, Hansen JA, McDevitt HO: Heritable major histocompatibility com-

plex class II-associated differences in production of tumor necrosis factor alpha: relevance to genetic predisposition to systemic lupus erythematosus. Proc Natl Acad Sci U S A 87:1233, 1990.

29. Rudwaleit M, Tikly M, Gibson K, Pile K, Wordsworth P: HLA class II antigens associated with systemic lupus erythematosus in black South Africans. Ann Rheum Dis 54:678, 1995.
30. Santamaria P, Gehrz RC, Bryan MK, Barbosa JJ: Involvement of class II MHC molecules in the LPS-induction of IL-1/TNF secretions by human monocytes. Quantitative differences at the polymorphic level. J Immunol 143:913, 1989.
31. Freedman BI, Spray BJ, Heise ER, Espeland MA, Canzanello VJ: A race-controlled human leukocyte antigen frequency analysis in lupus nephritis. The South-Eastern Organ Procurement Foundation. Am J Kidney Dis 21: 378, 1993.
32. Vermeire S, Noman M, Van Assche G, Baert F, Van Steen K, Esters N, et al: Autoimmunity associated with anti-tumor necrosis factor alpha treatment in Crohn's disease: a prospective cohort study. Gastroenterology 125:32, 2003.
33. Shakoor N, Michalska M, Harris CA, Block JA: Drug-induced systemic lupus erythematosus associated with etanercept therapy. Lancet 359:579, 2002.
34. Aringer M, Smolen JS: SLE—Complex cytokine effects in a complex autoimmune disease: tumor necrosis factor in systemic lupus erythematosus. Arthritis Res Ther 5:172, 2003.
35. Little J, Bradley L, Bray MS, Clyne M, Dorman J, Ellsworth DL, et al: Reporting, appraising, and integrating data on genotype prevalence and gene-disease associations. Am J Epidemiol 156:300, 2002.
36. Wacholder S, Rothman N, Caporaso N: Counterpoint: bias from population stratification is not a major threat to the validity of conclusions from epidemiological studies of common polymorphisms and cancer. Cancer Epidemiol Biomarkers Prev 11:513, 2002.
37. Zhao B, Stavchansky SA, Bowden RA, Bowman PD: Effect of interleukin-1beta and tumor necrosis factor-alpha on gene expression in human endothelial cells. Am J Physiol Cell Physiol 284:C1577, 2003.
38. Parks CG, Cooper GS, Dooley MA, Treadwell EL, St Clair EW, Gilkeson GS, et al. Systemic lupus erythematosus and genetic variation in the interleukin-1 gene cluster: a population-based study in the Southeastern United States. Ann Rheum Dis 63:91, 2004.
39. Cooper GS, Dooley MA, Treadwell EL, St Clair EW, Gilkeson GS: Hormonal and reproductive risk factors for development of systemic lupus erythematosus: results of a population-based, case-control study. Arthritis Rheum 46:1830, 2002.
40. Rothman KJ, Greenland S: Modern epidemiology. Philadelphia: Lippincott-Raven, 1998.
41. Galbraith GM, Steed RB, Sanders JJ, Pandey JP: Tumor necrosis factor alpha production by oral leukocytes: influence of tumor necrosis factor genotype. J Periodontol 69:428, 1998.
42. Ota M, Seki T, Fukushima H, Tsuji K, Inoko H: HLA-DRB1 genotyping by modified PCR-RFLP method combined with group-specific primers. Tissue Antigens 39: 187, 1992.
43. Moore JH, Williams SM: New strategies for identifying gene-gene interactions in hypertension. Ann Med 34:88, 2002.
44. Cooper GS, Parks CG, Treadwell EL, St Clair EW, Gilkeson GS, Cohen PL, et al: Differences by race, sex and age in the clinical and immunologic features of recently diagnosed systemic lupus erythematosus patients in the southeastern United States. Lupus 11:161, 2002.
45. Kameda H, Pandey JP, Kaburaki J, Inoko H, Kuwana M: Immunoglobulin allotype gene polymorphisms in systemic sclerosis: interactive effect of MHC class II and KM genes on anticentromere antibody production. Ann Rheum Dis 57:366, 1998.
46. Danis VA, Kulesz AJ, Nelson DS, Brooks PM: Cytokine regulation of human monocyte interleukin-1 (IL-1) production in vitro. Enhancement of IL-1 production by interferon (IFN) gamma, tumour necrosis factor-alpha, IL-2 and IL-1, and inhibition by IFN- alpha. Clin Exp Immunol 80:435, 1990.
47. Barrera P, Joosten LA, den Broeder AA, van de Putte LB, van Riel PL, van den Berg WB: Effects of treatment with a fully human anti-tumour necrosis factor alpha monoclonal antibody on the local and systemic homeostasis of interleukin 1 and TNFalpha in patients with rheumatoid arthritis. Ann Rheum Dis 60:660, 2001.
48. Lorenz HM, Antoni C, Valerius T, Repp R, Grunke M, Schwerdtner N, et al: In vivo blockade of TNF-alpha by intravenous infusion of a chimeric monoclonal TNF-alpha antibody in patients with rheumatoid arthritis. Short term cellular and molecular effects. J Immunol 156:1646, 1996.
49. Dominici R, Cattaneo M, Malferrari G, Archi D, Mariani C, Grimaldi LM, et al: Cloning and functional analysis of the allelic polymorphism in the transcription regulatory region of interleukin-1 alpha. Immunogenetics 54:82, 2002.
50. Shirodaria S, Smith J, McKay IJ, Kennett CN, Hughes FJ: Polymorphisms in the IL-1A gene are correlated with levels of interleukin-1alpha protein in gingival crevicular fluid of teeth with severe periodontal disease. J Dent Res 79:1864, 2000.
51. Hulkkonen J, Laippala P, Hurme M: A rare allele combination of the interleukin-1 gene complex is associated with high interleukin-1 beta plasma levels in healthy individuals. Eur Cytokine Netw 11:251, 2000.
52. Brennan DC, Yui MA, Wuthrich RP, Kelley VE: Tumor necrosis factor and IL-1 in New Zealand Black/White

mice. Enhanced gene expression and acceleration of renal injury. J Immunol 143:3470, 1989.

53. McHale JF, Harari OA, Marshall D, Haskard DO: TNF-alpha and IL-1 sequentially induce endothelial ICAM-1 and VCAM-1 expression in MRL/lpr lupus-prone mice. J Immunol 163:3993, 1999.
54. Rus V, Atamas SP, Shustova V, Luzina IG, Selaru F, Magder LS, et al: Expression of cytokine- and chemokine-related genes in peripheral blood mononuclear cells from lupus patients by cDNA array. Clin Immunol 102:283, 2002.
55. Hanada T, Yoshimura A: Regulation of cytokine signaling and inflammation. Cytokine Growth Factor Rev 13: 413, 2002.
56. D'Alfonso S, Giordano M, Mellai M, Lanceni M, Barizzone N, Marchini M, et al: 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 3:454, 2002.
57. D'Alfonso S, Rampi M, Bocchio D, Colombo G, Scorza-Smeraldi R, Momigliano-Richardi P: Systemic lupus erythematosus candidate genes in the Italian population: evidence for a significant association with interleukin-10. Arthritis Rheum 43:120, 2000.
58. Mehrian R, Quismorio Jr FP, Strassmann G, Stimmler MM, Horwitz DA, Kitridou RC, et al: Synergistic effect between IL-10 and bcl-2 genotypes in determining susceptibility to systemic lupus erythematosus. Arthritis Rheum 41:596, 1998.
59. Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP: Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. J Immunol 166:3915, 2001.
60. Abraham LJ, Kroeger KM: Impact of the −308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. J Leukoc Biol 66:562, 1999.
61. Ozaki K, Ohnishi Y, Iida A, Sekine A, Yamada R, Tsunoda T, et al: Functional SNPs in the lymphotoxin-alpha gene that are associated with susceptibility to myocardial infarction. Nat Genet 32:650, 2002.
62. Bayley JP, de Rooij H, van den Elsen PJ, Huizinga TW, Verweij CL: Functional analysis of linker-scan mutants spanning the −376, −308, −244, and −238 polymorphic sites of the TNF-alpha promoter. Cytokine 14:316, 2001.
63. Ackerman H, Usen S, Mott R, Richardson A, Sisay-Joof F, Katundu P, et al: Haplotypic analysis of the TNF locus by association efficiency and entropy. Gen Biol 4:1–13, 2003.
64. Milner CM, Campbell RD: Genetic organization of the human MHC class III region. Front Biosci 6:D914, 2001.
65. Hassan MI, Aschner Y, Manning CH, Xu J, Aschner JL: Racial differences in selected cytokine allelic and genotypic frequencies among healthy, pregnant women in North Carolina. Cytokine 21:10, 2003.
66. Tsuchiya N, Kawasaki A, Tsao BP, Komata T, Grossman JM, Tokunaga K: Analysis of the association of HLA-DRB1, TNFalpha promoter and TNFR2 (TNFRSF1B) polymorphisms with SLE using transmission disequilibrium test. Genes Immunol 2:317, 2001.