BRIEF REPORT

A Tumor Necrosis Factor–α–Inducible Promoter Variant of Interferon-γ Accelerates CD4+ T Cell Depletion in Human Immunodeficiency Virus–1–Infected Individuals

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A polymorphism, −179G/T, in the promoter of the interferon (IFN)–γ gene (*IFNG*) confers differential tumor necrosis factor-α (TNF-α) inducibility to the *IFNG* promoter. The rarer allele, −179T, but not −179G, is inducible by TNF-α. We investigated the effects of *IFNG* −179G/T on AIDS pathogenesis. In 298 African American human immunodeficiency virus (HIV)–1 seroconverters, the *IFNG* −179G/T genotype was associated with accelerated progression to CD4 <200 and AIDS-1993, a finding suggesting that *IFNG* −179T is a risk factor for AIDS progression, as measured by CD4 cell count. It is possible that increased IFN-γ production induced by TNF-α when −179T is present causes CD4 cell depletion by apoptosis.

Host genetic factors have been shown to influence both the risk of human immunodeficiency virus (HIV)–1 transmission and the rate of HIV-1 disease progression. In particular, polymorphisms in the genes for HIV-1 coreceptors and for their natural ligands have been shown to modify both HIV-1 transmission and HIV-1 disease progression and have led to important insights into the pathogenesis of HIV-1 infection [1].

Interferon (IFN)–γ, a cytokine required for the development and propagation of cytotoxic T lymphocytes, is a crucial regulator of cellular immune responses to intracellular pathogens. Both suppressive and inductive effects of IFN-γ on HIV replication have been reported [2]. Infection by HIV and by several other RNA and DNA viruses induces increased sensitivity to cell lysis mediated by tumor necrosis factor (TNF)–α, especially in the presence of IFN-γ [3]. Moreover, TNF-α and IFN-γ together have been shown to act synergistically to potentiate HIV-1 replication and to induce apoptosis both in HIV-1–infected cells and in HIV-1–uninfected cells [3, 4].

Because alterations in IFN-γ levels have been noted in several immune disorders, notably in asthma and multiple sclerosis, there has been an intensive search to identify variants in the IFN-γ gene *(IFNG)*. These results have shown the promoter region of *IFNG* to be highly conserved, a finding suggesting that variations in IFN-γ production are likely due to differential binding to regulatory factors [5]. We recently reported a functional single-nucleotide polymorphism (SNP), −179G/T, in the promoter of *IFNG,* an SNP that is carried by 4% of African Americans (AAs) and by 0.02% of European Americans (EAs) [5]. The *IFNG* promoter–carrying variant allele, −179T, is inducible by TNF-α and constitutively binds nuclear extracts obtained from T cells, whereas the promoter with the more frequent allele, −179G, is nonresponsive to TNF-α [5]. Therefore, we examined the influence of the TNF-α–inducible *IFNG* variant allele, −179T, on HIV-1 infection and progression, in 298 AA HIV-1 seroconverters. Because −179G/T is not polymorphic in EAs (−179T allele, $f \approx 0.01\%$), we could not assess its effects in this population [5].

Patients, materials, and methods. The study group comprised 298 AA HIV-1 seroconverters who were enrolled in either the AIDS Link to the Intravenous Experience (ALIVE) (85%), the Multicenter AIDS Cohort Study (MACS) (10%), or the Multicenter Hemophilia Cohort Study (MHCS) (5%) cohorts and who, respectively, belonged to the following risk groups: injection drug users (IDUs), homosexual men, or hemophiliacs [6–8]. Seroconverters were those individuals who were HIV-1 negative at entry into the study and who subsequently became seropositive. The infection date was estimated as the midpoint between the last seronegative and the first seropositive HIV-1 antibody tests (mean interval, 0.61 years; range, 0.11–1.96 years). Participants were monitored at 6-month intervals. For both the MACS cohort and the MHCS cohort, the censoring

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Table 1. Survival analysis of interferon-γ gene −179G/T, with progression to AIDS endpoints, by the Cox proportional hazard model (dominant model, −179G/T vs. −179G/G).

	Unadjusted (n = 298)			Adjusted[a] (n = 278)		
Condition	No. of events	RH (95% CI)	P	No. of events	RH (95% CI	P
CD4 <200	111	2.47 (1.18–4.82)	.012	100	2.31 (1.04–4.60)	.028
AIDS-1993	127	2.42 (1.28–4.29)	.005	123	2.47 (1.23–4.45)	.006
AIDS-1987	65	1.31 (0.47–3.63)	.485	64	1.26 (0.44–3.58)	.672

NOTE. CI, confidence interval; RH, relative hazard.

[a] Adjusted for genetic covariates *CCR2* 64I, *HLA-B**57, *HLA-B**35Px, and *HLA* class I zygosity; 20 subjects missing ⩾1 HLA genotype covariates were excluded from the adjusted analysis.

date was the earliest of either the date of the last recorded visit or 31 December 1995, to avoid the possibly confounding effects of highly active anti-retroviral therapy (HAART); because administration of HAART therapy was delayed in the ALIVE cohort, 31 July 1997 was used as a censoring date.

High-risk, exposed, uninfected (HREU) AAs (n = 78) were those individuals with high-risk HIV-1 exposure as a result of (1) either sharing needles or any self-reported visit to a "shooting gallery" (ALIVE), (2) being in the top tenth percentile for number of anal-receptive sexual partners (MACS), or (3) having received documented HIV-1–contaminated lots of Factor VIII (MHCS). HIV-1 seronegative AAs (n = 385) were those individuals who, as a result of their inclusion in a risk group (i.e., IDUs, homosexual men, or hemophiliacs), were at risk for HIV-1 infection.

IFNG −179 G/T was genotyped by polymerase chain reaction (PCR)–restriction fragment-length polymorphism (RFLP) assay, as reported elsewhere [5]. In brief, PCR was performed by use of primers 5′-ATCAATGTGCTTTGTGAATGAA-3′ and 5′-CCGAGAGAATTAAGCCAAAGA-3′. The 461-bp PCR product was then digested with *Ava*II (New England Biolabs), which resulted in 3 fragments (242, 159, and 60 bp) and 2 fragments (401 and 60 bp), for the G and T alleles, respectively.

The distributions of allele and genotype frequencies in HIV-1 seroconverters, HIV-1–seronegative individuals, and HREU individuals were compared by Fisher's exact test. Kaplan-Meier survival statistics and the Cox proportional hazards models were used to assess the effects of the *IFNG* −179T allele on the rate of progression to AIDS (SAS Package; SAS). Three endpoints reflecting advancing morbidity were evaluated: (1) CD4 <200 cells/mm^3 (CD4 <200); (2) CD4 <200 cells/mm^3 or AIDS-defining conditions as defined by the Centers for Disease Control (CDC) in 1993 (AIDS-1993) [9]; and (3) AIDS-defining conditions as defined by the CDC in 1987 (AIDS-1987). Cox-model analyses were performed, both unadjusted and adjusted, considering the following AIDS-modifying genetic factors as covariates: *CCR2* 64I, *HLA-B**57, *HLA-B**35Px, and *HLA* class I zygosity [1, 10]. *CCR5* Δ32 was not included as a covariate, because it is rare in AAs. Participants were stratified by sex and by age at seroconversion (0–20, >20–40, and >40 years). All P values are 2-tailed. This study received institutional review-board approval, and informed consent was obtained from each study participant.

Results. We first examined the effects of *IFNG* −179G/T on HIV-1 susceptibility, by comparing allele and genotype frequencies, between AA HIV-1 seroconverters (n = 298) and either AA HIV-1–seronegative individuals (n = 385) or HREU individuals (n = 78). The allele frequency of *IFNG* −179T was 0.029 in seroconverters (17 G/T heterozygotes), 0.023 in seronegative individuals (16 G/T heterozygotes and 1 T/T homozygote), and 0.026 in HREU individuals (4 G/T heterozygotes). There were no significant differences, in either allele or genotype frequencies of *IFNG* −179, between the AA HIV-1 seroconverters and either AA HIV-1–seronegative individuals (odds ratio [OR], 1.31; 95% confidence interval [CI], 0.62–2.78; P = .48) or AA HREU individuals (OR, 1.12; 95% CI, 0.35–4.71; P = 1.0). We also observed no differences, in Hardy-Weinberg equilibrium, in any of the 3 groups. These results suggest that *IFNG* −179 SNP had no apparent role in HIV-1 infection in our study group, in which exposure to HIV-1 had been predominantly via blood-borne routes.

The *IFNG* −179G/T genotype was significantly associated with both accelerated CD4 cell depletion (table 1) (relative hazard [RH], 2.47 [Cox model]; 95% CI, 1.18–4.82; P = .012) and accelerated AIDS-1993 (RH, 2.42; 95% CI, 1.28–4.29; P = .005), in the unadjusted analysis, and, after adjustment for the effects of the genetic covariates, these accelerating effects remained significant (for CD4 <200, adjusted RH, 2.31; 95% CI, 1.04–4.60; P = .03; and, for AIDS-1993, adjusted RH, 2.47; 95% CI, 1.23–4.45; P = .006). The CD4 <200–free and AIDS-1993–free survival curves illustrate that the −179G/T genotype was associated with a significantly accelerated progression to both CD4 <200 and AIDS-1993 (P = .006 and P = .002 [log-rank test], respectively) (figure 1). However, this effect was not significant for AIDS-1987, although a trend in the same direction was observed (table 1). Similar significant associations with accelerated progression for −179T carriers were obtained when either only participants with a seroconversion interval of <12

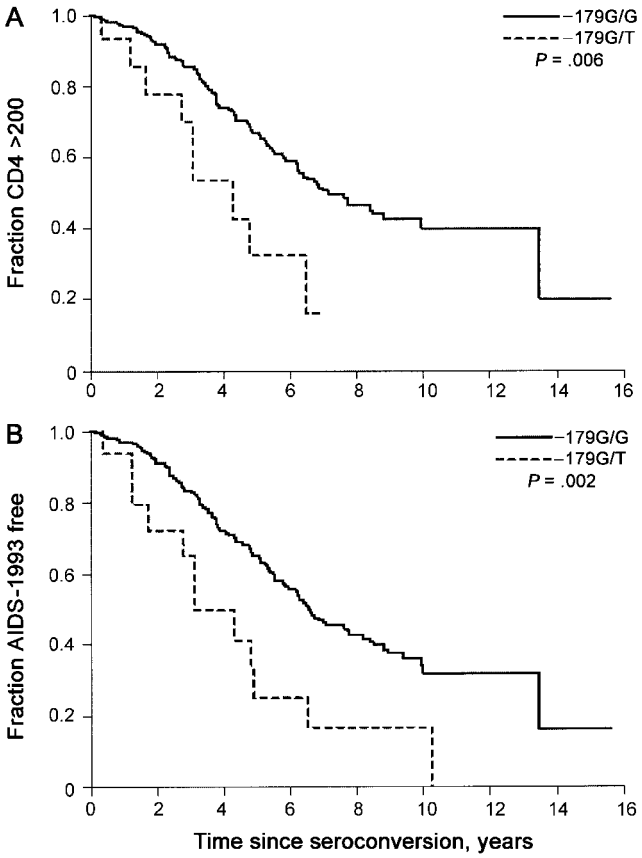

Figure 1. Kaplan–Meier survival curves of human immunodeficiency virus–1 progression to CD4 <200 *(A)* and AIDS-1993 *(B)*, after seroconversion, on the basis of the presence *(broken line)* or absence *(solid line)* of the *IFNG* −179G/T genotype. The *IFNG* −179T/T genotype was absent in this study group. *P* values are from a 2-sided log-rank test.

months (data not shown) or from participants only from the ALIVE cohort were included in the analyses (data not shown).

Discussion. Our results have demonstrated the genetic association, in HIV-1 seroconverters, between a functional promoter variant (*IFNG* −179T) and accelerated rates of progression to both CD4 <200 and AIDS-1993. The association between the TNF-α–inducible *IFNG* −179T allele and rapid loss of $CD4^+$ T cells is consistent with studies showing that costimulation by TNF-α and IFN-γ induces a state of increased sensitivity to apoptosis of HIV-1–infected and HIV-1–uninfected promonocytic and T cell lines [3, 4]. Results of in vitro reporter assays suggest that the *IFNG* −179T allele is inducible by TNF-α and that it increases IFN-γ transcription activity several fold [5]. Therefore, it is possible that this allele may render both HIV-1–infected cells and HIV-1–uninfected bystander cells more susceptible to apoptosis in the presence of TNF-α. Alternatively, elevated IFN-γ transcription activity afforded by *IFNG* −179T on TNF-α induction may accelerate disease progression by predisposing macrophages to infection with CXCR4-utilizing HIV-1 strains [11].

It has been shown that elevated TNF-α levels are found in the majority of patients in the early stage of HIV-1 infection and are correlated with IFN-γ levels, a finding likely representing activation of the cytotoxic T cell compartment [12]. In the late stage of HIV-1 infection, this correlation disappears, and TNF-α is instead correlated with interleukin-10 [13]. Although it is likely that the smaller number of AIDS-1987 events accounts for the lack of association between *IFNG* −179T and AIDS-associated conditions, it is also likely that the lack of association could be due to the decline of IFN-γ and TNF-α synergism in later stages of infection. It is also possible that, in the absence of HIV-1 infection, *IFNG* −179T may be associated with lower CD4 cell counts. In one report using data from the Los Angeles MACS, a strong correlation between the $CD4^+$ T cell number at the time of progression to AIDS and the $CD4^+$ T cell number before HIV infection was observed [14]. However, in a preliminary study, we were unable to detect differences, in $CD4^+$ T cell counts, between HIV-1–uninfected *IFNG* −179T carriers and HIV-1–uninfected *IFNG* −179T noncarriers (data not shown).

It should be noted that the low allele frequency of *IFNG* −179T imposes several unavoidable limitations on this study. The association found in AAs was obtained from only 17 seroconverters who carried the *IFNG* −179G/T genotype; thus, the possibility of statistical fluctuation cannot formally be excluded. The lack of significant association between −179T and progression to AIDS-1987 (AIDS-defining conditions) may be due to the smaller number of events for the later outcome. Validation of this association in additional, African or AA cohorts is necessary. However, considering the strength of the association, the importance of IFN-γ in the cellular immune response to intracellular pathogens, the reported role of IFN-γ in HIV-1 pathogenesis, and the functional importance of *IFNG* −179G/T, the association seems both reasonable and biologically plausible.

The results of this genetic study, if confirmed, call for caution in the use of IFN-γ in the treatment of AIDS. In fact, clinical trials of IFN-γ have provided no clear evidence of clinical benefits but have provided evidence of both acceleration of AIDS progression and decline of CD4 cell counts [2, 15]. Further elucidation of the mechanism responsible for the association between *IFNG* −179T and AIDS progression may provide insight into immunological changes in HIV-1 pathogenesis. Up to 4% of AAs carry the *IFNG* −179T variant. It remains to be determined whether this variant allele is associated with susceptibility to other intracellular pathogens or immune disorders, particularly those disproportionately affecting people of African descent.

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