MAJOR ARTICLE

Effect of Host Genetics on the Development of Cytomegalovirus Retinitis in Patients with AIDS

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Background. Cytomegalovirus (CMV) retinitis is a common opportunistic infection among patients with AIDS and still causes visual morbidity despite the wide spread usage of highly active antiretroviral therapy (HAART). The ubiquitous CMV pathogen contains a human interleukin-10 (IL-10) homolog in its genome and utilizes it to evade host immune reactions through an IL-10 receptor mediated immune-suppression pathway.

Methods. Effects of *IL-10R1*, *IL*-10 and previously described AIDS restriction gene variants are investigated on the development of CMV retinitis in the Longitudinal Study of the Ocular Complications of AIDS (LSOCA) cohort ($N = 1284$).

Results. In European Americans ($n = 750$), a haplotype carrying an amino acid changing variation in the cytoplasmic domain (S420L) of *IL-10R1* can be protective (OR, 0.14; 95% CI, 0.02–0.94; $P = .04$) against, whereas another haplotype carrying an amino acid changing variation in the extracellular domain (I224V) of *IL-10R1* can be more susceptible (OR, 6.21; 95% CI, 1.22– 31.54; $P = .03$) to CMV retinitis. In African Americans ($n = 534$), potential effects of *IL-10* variants are observed.

Conclusion. Host genetics may have a role in the occurrence of CMV retinitis in patients infected with HIV.

Human cytomegaloviruses (HCMVs) are ubiquitous β-herpesvirus pathogens that infect 80% of the US adults by the age of 40 years, usually producing few or no clinical symptoms. However, in neonates, allogenic transplant recipients, and immunocompromised people, such as patients with AIDS, HCMV is a substantial cause of morbidity and mortality [1]. Even after the introduction of highly active antiretroviral therapy (HAART), HCMV continues to be one of the most frequent opportunistic pathogens in patients with AIDS [2].

CMV retinitis was the main reason (>90%) of vision loss among patients with AIDS in the pre-HAART era [3, 4]. Although immune recovery in response to HAART reduced the incidence of CMV retinitis, the expected decline in the number of new cases of CMV retinitis has leveled off [2]. CMV retinitis related visual morbidity continues to be a problem in the era of HAART due to HAART intolerant and/or unresponsive patients, resistance development to long-term CMV treatment, and improved survival rate increasing the population who remain at risk for CMV retinitis [2].

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Moreover, there are several case reports of patients with elevated $CD4^+$ T cell counts who nevertheless experience a relapse of CMV retinitis [5–8]. Clearly, non-genetic factors such as poor general health, availability of sufficient health care and adherence to therapy are important factors in CMV retinitis development [9–11]. However, the effect of host genetics in CMV activation and progression to CMV retinitis has been largely unexplored.

The genome of HCMV contains several genes that allow it to evade the host immune system [1, 12]. One of these genes codes for a human interleukin 10 (IL-10) homolog called cmvIL-10 [13]. IL-10 is a pleiotropic cytokine that inhibits inflammatory and cell-mediated immune responses through suppression of production of proinflammatory cytokines and the expression of major histocompatibility complex class II and costimulatory molecules [14]. The receptor complex for IL-10 is composed of 2 subunits, IL-10R1 and IL-10R2. The binding of IL-10 to its receptor complex activates the Janus kinase (JAK)–STAT3-STAT1 signal transduction pathway. IL-10R1 (Jak1 associated), is the crucial ligand (IL-10) binding and signal transducing unit of the IL-10 receptor complex [15, 16]. The homolog cmvIL-10 can bind to IL-10R1, initiate signaling through IL-10R complex, and exhibit immunosuppressive properties nearly identical to those of human IL-10 [17, 18]. Genetic polymorphisms of IL-10R1 have been shown to diminish the inhibitory effects of IL-10 on monocytes [19], and to reduce STAT3- and STAT1-activated signaling [20]. Moreover, IL-10R1 polymorphisms can decrease signaling activity of cmvIL-10 through the IL-10 signaling pathway [21]. Potential roles for IL-10 receptor polymorphisms in infectious disease outcomes have also been suggested; specifically, effects of IL-10R1 variants in hepatitis C [22, 23] and progression to AIDS [24]. On the other hand, IL-10 variants that modulate IL-10 transcript production also regulate AIDS progression in HIV-1 infected individuals [24–27].

For this, study we took a candidate-gene approach to investigate potential effects of host genetics on the development of CMV retinitis. We focused primarily on polymorphisms in the *IL-10R1* gene, but also explored polymorphisms in the *IL-10* gene. The study participants were HIV-infected European American and African American patients enrolled in the Longitudinal Study of the Ocular Complications of AIDS (LSOCA) cohort. As HCMV-related organ diseases are seen typically in the later years of HIV infection, correlate with AIDS progression (such as $CD4^+$ T cell counts), and HAART treatment, we also examined variants in genes [28, 29] that have been shown to influence HIV-1 infection, AIDS progression, therapy response, and antiviral drug metabolism to account for potential genetic confounding factors.

PATIENTS AND METHODS

Study Population and Clinical Assessment of CMV retinitis

Study patients included 750 European American and 534 African American individuals enrolled in the Longitudinal Studies of the Ocular Complications of AIDS (LSOCA) cohort. LSOCA is a prospective multicenter observational study of patients with incident or prevalent cases of AIDS diagnosed according to the 1993 surveillance case definition of the Centers for Disease Control and Prevention. Eligible patients included those with either incident and prevalent cases of CMV retinitis, as well as those without CMV retinitis. The first patient was enrolled into the study in September 1998 and enrollment was still continuing through 30 September 2009. Each patient provided a written consent for study participation. Further information on the study design and implementation and ophthalmologic examinations have been described in previous publications [4, 10, 11].

Genotyping and Haplotype Construction

The *IL-10R1* region was genotyped for high frequency (≥5%) functional and haplotype tagging variants (single-nucleotide polymorphisms, or SNPs) of rs3135932 (Exon-4, replacement, also reported as SNP3 in the literature [19]), rs2228055 (Exon-5, replacement, also reported as SNP6 in the literature [19]), rs4252279, rs4252314, rs4252286 (Intron-6), rs2229113 (Exon-7, replacement, also reported as SNP4 in the literature [19]), and rs2229114 (Exon-7, replacement, also reported as SNP8 in the literature [19]). Additionally, 11 haplotype tagging SNPs covering the *IL-10* region were selected (promoter region: rs17351243, rs4072227, rs6667202, rs1800890, rs1800896, and rs1800894; intronic: rs3021094 and rs3024508; 3′ UTR: rs3024496, rs3024498, and rs3024500). Figure 1 shows individually genotyped SNPs and inferred haplotypes for *IL-10R1* in the European American and African American groups. Previously identified [28–31] functional polymorphisms for AIDS restriction and therapy response genes were genotyped for *CCR5Δ32* (rs333), *CCR2–64I* (rs1799864), *CCR5 P1* (rs1799988),

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Figure 1. Inferred haplotypes, their frequencies and LD structure across the *IL-10R1* region in samples from European American patients (*A*) and in African American patients (*B*). Black filled rectangles show the exons. Lines indicate the actual physical position of SNPs with respect to each other. The brightness of red color represents the pairwise D′ values: high D′ values are shown with bright red, low D′ values are shown in light red and blue squares. The single-nucleotide polymorphisms rs3135932, rs2228055, rs2229113, and rs2229114 are also known as SNP3, SNP6, SNP4, and SNP8, respectively, in the literature [19]. Do et al. [24] suggest that rs2229113 and rs2229114 may be involved in progression to AIDS.

Table 1. Demographic and Clinical Characteristics of Study Patients, Who Were Enrolled from the Longitudinal Study of the Ocular Complications of AIDS (LSOCA) Cohort

Characteristic	European Americans		African Americans	
	Case patients (n = 200)	Control patients (n = 550)	Case patients (n = 110)	Control patients (n = 424)
Male sex, % of patients	92	91	64	68
Age at study entry, years[a]				
Mean ± SD	42.2 ± 7.2	44.0 ± 8.6	39.0 ± 8.2	42.1 ± 8.4
Median (25th, 75th percentile)	41.0 (37, 47)	43.0 (38, 49)	38.0 (34, 44)	42.0 (37, 47)
Nadir CD4$^+$ T cell count, cells/mL[a]				
Mean ± SD	32.0 ± 75.1	79.3 ± 92.5	28.4 ± 66.9	61.6 ± 70.2
Median (25th, 75th percentile)	10.0 (5, 25)	50.0 (18, 120)	9.0 (3, 22)	32.0 (10, 95)
Baseline HIV viral load, $\log_{10}$ copies/mL[a]				
Mean ± SD	5.2 ± 1.0	5.0 ± 0.9	5.5 ± 0.5	5.1 ± 0.9
Median (25th, 75th percentile)	5.5 (4.9, 5.9)	5.3 (4.6, 5.7)	5.6 (5.2, 5.9)	5.3 (4.7, 5.7)
Time since AIDS diagnosis, years[b]				
Mean ± SD	5.5 ± 3.3	5.1 ± 3.7	3.8 ± 2.9	4.0 ± 3.6
Median (25th, 75th percentile)	5.2 (3.3, 7.3)	4.7 (2.1, 7.2)	3.5 (1.5, 5.7)	3.1 (1.0, 6.2)
HAART use, % of patients[c]	81	84	67	82

[a] Significantly different between the CMV retinitis case group and the control group for both European Americans and African Americans.
[b] Years before study entry
[c] Significantly different between the CMV retinitis case group and the control group for African Americans (P = .001)

SDF-3′A (rs1801157), *RANTES -403A* (rs2107538), *RANTES -28G* (rs2280788), *RANTES-In1.1C* (rs2280789), *CX3CR1-V249I* (rs3732379), *CX3CR1-T280M* (rs3732378), *IFNG-179T* (rs2069709), *MDR1-C3435T* (rs1045642), and *MCP-1364G* (rs2857657, intronic *767G*, representative of haplotype 7). All SNPs were genotyped using the ABI-TaqMan method (Applied Biosystems).

All haplotypes were inferred by the expectation maximization algorithm with SAS Genetics (SAS Institute) and the HaploView software [32]. The presence of *CCR5_59353C* (rs1799988) in the absence of *CCR2–64I* and *CCR5Δ32* defines the *CCR5 P1* promoter haplotype +.*P1*.+. [31] The *RANTES -403A*, *RANTES -28G, RANTES-In1.1C* genotypes define the *RANTES* haplotypes. *RANTES* -H1 = G-C-T; *RANTES*-H2 = A-C-T; and *RANTES*-H3 = A-C-C (low producer haplotype) [30, 33].

Statistical Analyses

Each SNP and haplotype found at ⩾1% frequency in the study population was evaluated as a risk factor for CMV retinitis by 3 different models of inheritance: allelic, dominant, and codominant (additive). Allelic analyses examined individual allele effects. Genotypes were coded as 0, 1, or 2 copies of the rare allele for the codominant (additive) model. The dominant model analyzed genotypes as absence or presence of the rare alleles. Odds ratios (ORs) for the codominant and dominant models were calculated by logistic regression. Nominal P values were calculated. *IL-10R1* and *IL-10* haplotypes were further analyzed with a more powerful haplotype trend regression approach [34], in which an estimated haplotype matrix of posterior probabilities for each individual was used in the regression models. The whole effect of haplotypes was assessed by the global tests followed by individual tests for each haplotype. Further, a stepwise regression approach was used to obtain the most parsimonious model. All regression models were adjusted for the square root of the nadir CD4$^+$ T cell count, the $\log_{10}$HIV-1 load, age, sex, and HAART use. All analyses were performed with SAS software, version 9.1 (SAS Institute). Case patients were defined as study patients who received a diagnosis of CMV retinitis, and control patients were defined as study patients who did not develop CMV retinitis.

Structural analysis of *IL-10R1* amino acid changes was carried on the x-ray structure of human IL-10/IL-10R1 complex, pdb code 1Y6K [35].

RESULTS

Within the group of European American patients (n = 750), case patients with CMV retinitis were slightly younger than control patients (42.2 vs 44.0 years; P = .01), had a higher initial $\log_{10}$HIV viral load (mean, 5.21 vs 5.03 $\log_{10}$ copies/mL; P = .03), and had a lower nadir CD4$^+$ T cell count (mean, 32 vs 79 cells/mL; P < .001) (Table 1). Similar trends were observed for the group of African American patients (n = 534): case patients with CMV retinitis were younger than control patients (39.0 vs 42.1 years; P = .004), had a higher $\log_{10}$HIV viral load (mean, 5.48 vs 5.06 $\log_{10}$ copies/mL; P < .001), and a lower nadir CD4$^+$ T cell count (mean, 28 vs 67 cells/mL; P < .001).

Table 2. Allelic Distribution And Association Tests Of *IL-10R1* Single-Nucleotide Polymorphisms (SNPs) in Case Patients with Cytomegalovirus Retinitis and in Control Patients

	European Americans								African Americans							
	Allele frequency, %		Allelic model		Codominant model[a]		Dominant model[a]		Allele frequency, %		Allelic model		Codominant model[a]		Dominant model[a]	
SNP	Case patients (n = 200)	Control patients (n = 550)	OR	P	OR	P	OR	P	Case patients (n = 110)	Control patients (n = 424)	OR	P	OR	P	OR	P
rs3135932 (A/G)	17	14	1.21	0.23	1.27	0.18	1.39	0.11	2	3	0.72	0.49	0.72	0.54	0.72	0.54
rs2228055 (A/G)	7	5	1.49	0.11	1.55	0.14	1.55	0.16	0.5	2	0.24	0.13	0.24	0.20	0.24	0.20
rs4252279 (C/T)	9	11	0.81	0.31	0.79	0.26	0.87	0.60	11	11	1.00	0.99	0.99	0.97	0.98	0.94
rs4252314 (A/G)	3	4	0.68	0.26	0.66	0.27	0.59	0.21	0.5	1	0.48	0.48	0.56	0.61	0.56	0.61
rs4252286 (G/A)	4	2	1.61	0.15	1.95	0.08	1.95	0.08	0.9	0.4	2.50	0.28	1.22	0.84	1.22	0.84
rs2229113 (G/A)	30	29	1.03	0.81	1.08	0.61	1.19	0.35	19	20	1.07	0.74	1.02	0.92	1.07	0.75
rs2229114 (C/T)	2	5	**0.44**	**0.03**	**0.42**	**0.03**	**0.42**	**0.04**	2	0.6	3.25	0.06	1.61	0.60	1.61	0.60

NOTE. Values with a statistically significant difference are in boldface. The single-nucleotide polymorphisms rs3135932, rs2228055, rs2229113, and rs2229114 are also known as SNP3, SNP6, SNP4, and SNP8, respectively, in the literature [19]. Do et al. [24] suggest that rs2229113 and rs2229114 may be involved in progression to AIDS.

[a] Logistic regression model is adjusted for the square root of the nadir CD4$^+$ T cell count, the $\log_{10}$ HIV-1 load, age, sex, and HAART use.

Also, the percentage of African American patients receiving HAART was lower in the case group with CMV retinitis than in the control group (67% vs 82%; $P = .001$) (Table 1). In addition, European American and African American patient groups showed statistically significant differences ($P \leqslant .01$) with respect to the percentage who were male (91% vs 67%), age (43.5 vs 41.5 years), nadir CD4$^+$ T cell count (mean, 67.6 vs 55.1 cells/mL), and time since AIDS diagnosis before study enrollment (5.2 vs 4.0 years). Because there were significant differences in these clinical covariates both between and within the group, the dominant and codominant association models were adjusted for the nadir CD4$^+$ T cell count, the $\log_{10}$HIV viral load, age, sex, and HAART use.

Analyses for the European American Group

In SNP-based analyses, we found that the S (serine) to L (leucine) amino acid changing (replacement) mutation (S420L), rs2229114, that is present in the cytoplasmic domain of *IL-10R1* was overrepresented in the control patients (ie, those who did not develop CMV retinitis) in all 3 models of association tests ($P = .03$–$.04$; Table 2). Moreover, none of the homozygote carriers of this mutation developed CMV retinitis. In contrast, another replacement (isoleucine to valine change) mutation, rs2228055, and an intronic variant, rs4252286, showed increased frequency in the case group with CMV retinitis, but the association test results were not significant (Table 2). Previously reported strong linkage disequilibrium between rs3135932 (SNP3) and rs2229113 (SNP4) also was observed in our data ($D' = 0.90$, $P < .0001$). However, rs2229114, rs2228055, and rs4252286 did not show significant linkage disequilibrium with each other or with any other SNP (Figure 1).

We were concerned that the rs2229114-T CMVR protective effect could also be associated with AIDS progression. So, we compared the allelic distribution of rs2229114-T in the patients with the highest and lowest 25% percentile CD4$^+$ T cell counts and HIV viral load among the CMV-negative patients. The rs2229114-T frequency was not significantly different ($P = .44$) between the highest and lowest 25% percentile CD4$^+$ T cell groups (5.4% vs 4.3%), and was not significantly different ($P = .78$) between the highest and lowest 25% percentile HIV viral load groups (4.2% vs 4.4%). Similar analyses with the low and high 50% percentiles also did not show a significant difference in rs2229114-T frequency distribution among these groups (nadir CD4$^+$ T cell count, 5.0% vs 3.0%; $P = .23$; HIV viral load, 5.3% vs 3.2%; $P = .61$).

None of the gene variants associated with AIDS progression and HAART response that we examined had a significant association with CMV retinitis (Table 3). Although an allelic association test indicated CMV retinitis susceptibility for the *CCR5* promoter haplotype, *+.P1.+*, the effect was conflicting (codominant vs dominant model) after correcting for the clinical covariates (Table 3).

Analyses of haplotypes also indicated that certain *IL-10R1*

Table 3. Allelic Distribution and Association Tests for Gene Variants Related to AIDS Progression and Response to Highly Active Antiretroviral Therapy in Case Patients with Cytomegalovirus Retinitis and in Control Patients

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haplotypes were associated significantly with CMV retinitis development. In the haplotype trend regression analysis, 8 parsimonious haplotypes were identified, of which the haplotype AACAGGT protected against CMV retinitis (OR, 0.14; $P = .04$), whereas the haplotype AGCAGGC conferred susceptibility (OR, 6.21; $P = .03$) (Table 4). Compared with the most common and wild-type haplotype, AACAGGC, the protective effect of AACAGGT was still evident in the dominant model (OR, 0.38; $P = .03$) and in the additive model (OR, 0.38, $P = .03$). Also, patients with the AGCAGGC haplotype had higher odds of developing CMV retinitis than did patients with the wild-type haplotype in the dominant model (OR, 1.72, $P = .05$) and in the additive model (OR, 1.92; $P = .05$). All haplotype associations were statistically significant, irrespective of the clinical covariate corrections. Moreover, the haplotype analyses results agreed with the SNP-based analyses. The protective haplotype AACAGGT had only a single variant difference, compared with the wild-type haplotype AACAGGC, which was the protective SNP rs2229114. Similarly, the susceptibility haplotype AGCAGGC had only a single variant difference, compared with the wild-type haplotype—that is, the rs2228055 SNP that displayed a nonsignificant trend towards CMV retinitis susceptibility.

We also evaluated potential structural implications that may be associated with the replacement (isoleucine [Ile] to valine [Val] change) SNP rs2228055. Ile224 is located in the proximity of the membrane and included in the large internal hydrophobic core in its bottom part formed by Leu132, Ile134, Ile139, Val203, Phe201, and Leu226. The replacement mutation Ile to Val (I224V, rs2228055) is a change of one hydrophobic to another hydrophobic residue. However, the volume of Val residue is much less than that of Ile (Figure 2).

Omnibus CMV association analyses of *IL-10* haplotypes were not statistically significant (results not shown). Previously reported *IL-10* proximal promoter haplotypes [26, 36], formed by SNPs rs1800872, rs1800871, and rs1800896 (such as ATA, ACC, and GCC) and associated with differential IL-10 production, were inferred with proxy SNPs rs1800872 and rs1800896 because of the high linkage disequilibrium between rs1800871 and rs1800872. Individual *IL-10* haplotypes or grouping of these proximal promoter haplotypes did not have a statistically significant effect on the development of CMV retinitis (results not shown).

Analyses for the African American Group

None of the *IL-10R1* SNPs that we examined showed any statistically significant association with the development of CMV retinitis (Table 2). Two *IL-10* SNPs, rs3024500 and rs3024496, also showed a trend toward increased susceptibility for CMV retinitis in models adjusted for the clinical covariates ($P = .02$–$.05$; Table 5). There was a strong linkage disequilibrium between these 2 SNPs ($D' = 0.9$, $P < .0001$), which implies that the susceptibility signal may be driven by just 1 of these SNPs or by an untyped SNP in this haplotype group. Moreover, the *IL-10* rs4072227 variant showed a statistically significant increase in CMV retinitis risk in an allelic model, but that effect was not as significant after correcting for clinical covariates (Table 5). As nadir $CD4^+$ T cell count, HIV viral load, and HAART use have significant effects on development of CMV retinitis, we tested whether the aforementioned *IL-10* SNPs had any effect on these clinical covariates but we did not observe any significant effects. Finally, SNP and haplotype analyses of the examined gene variants related to AIDS progression and

Table 4. Haplotype Trend Regression Analyses of Single-Nucleotide Polymorphisms in *IL-10R1*

	European Americans[a]				African Americans			
	Haplotype frequency, %				Haplotype frequency, %			
Haplotype	Case patients ($n = 200$)	Control patients ($n = 550$)	P	OR (95% CI)	Case patients ($n = 110$)	Control patients ($n = 424$)	P	OR(95% CI)
AACAGGC	49.3	51.2	.94	0.98 (0.58–1.65)	60.4	68.9	.05	0.51 (0.25–1.00)
AACAAGC	3.6	1.9	.12	4.19 (0.69–25.49)	0.9	0.4	.85	1.45 (0.03–63.85)
AACAGAC	13.3	13.9	.87	1.07 (0.49–2.32)	18.9	15.7	.20	1.77 (0.74–4.25)
AACAGGT	**1.7**	**4.1**	**.04**	**0.14 (0.02–0.94)**	2.2	0.4	.25	7.82 (0.24–260)
AATAGGC	8.6	10.1	.35	0.67 (0.28–1.56)	12.1	10.2	.55	1.35 (0.50–3.63)
AGCAGGC	**5.7**	**3.5**	**.03**	**6.21 (1.22–31.54)**	0.9	1.6	.36	0.22 (0.01–5.78)
GACAGAC	12.1	9.2	.12	1.96 (0.84–4.56)	2.2	2.0	.70	1.65 (0.13–20.42)
GACGGAC	2.7	3.6	.40	0.51 (0.11–2.45)	1.8	0.6	.27	6.72 (0.23–193)

NOTE....Values with a statistically significant difference are in boldface. Haplotypes were estimated using the ordered SNPs: rs3135932, rs2228055, rs4252279, rs4252314, rs4252286, rs2229113, and rs2229114. Logistic regression models are adjusted for the square root of the nadir $CD4^+$ T cell count, the $\log_{10}$ HIV-1 load, age, sex, and HAART use.

[a] Global *P:* for European Americans, $P = 02$; for African Americans, $P = .08$.

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Figure 2. Hydrophobic core of the C-terminal domain of *IL-10R1* in the proximity of cell membrane are shown in brown (at the bottom); β-strands are shown in green, and coils in light brown. Hydrophobic side chains are shown as stick-and-ball models; the atom color code is as follows: C, green; O, red; N, blue. Mutation Ile224Val and the C-terminus are marked; atom CD1 of the Ile224 removed on mutation is shown in violet and highlighted with a bigger radius.

therapy response did not show any significant effect on the risk of development of CMV retinitis or on any clinical covariates tested (Table 3).

Global CMV retinitis association tests for *IL-10R1*, and *IL-10* haplotypes were not significant in the African American patient group (Table 4; results not shown). However, when haplotypes were examined individually, the *IL-10R1* AACAGGC haplotype trended towards conferring protection against CMV retinitis.

DISCUSSION

In this study we used a candidate gene approach to examine the potential effects of *IL-10R1* mutations on CMV retinitis development. Upon observation of significant associations of the examined *IL-10R1* variants with CMV outcome, we extended our analyses to *IL-10* variants. Moreover, we examined variants in genes that have been shown to influence HIV-1 infection, AIDS progression, therapy response and antiviral drug metabolism to account for potential genetic confounding factors. Our results suggest that a haplotype carrying an amino acid changing variation in the cytoplasmic domain of *IL-10R1* can be protective against, whereas another haplotype carrying an amino acid changing variation in the extracellular domain of *IL-10R1* can be more susceptible to CMV retinitis. Overall, our results indicate that *IL-10R1* amino acid changing mutations show a significant association with onset of CMV retinitis even after adjustment for the other genes and clinical covariates examined in European Americans. However, we note that none of our nominally significant *P* values will pass conservative Bonferroni multiple test correction.

The observed effects of *IL-10R1* variants on development of CMV retinitis were specific to European Americans. In African American patients, similar effects of *IL-10R1* variants were not observed, possibly because the derived allele and the associated haplotype frequencies were too low for any statistical power. A potential increased CMV retinitis susceptibility, specific to African Americans, was suggested by 2 *IL-10* variants (that are in high linkage disequilibrium) after adjusting for the effects of AIDS related clinical covariates. The *IL-10* rs3024500-C variant, which was overrepresented among African American patients with CMV retinitis, had previously been shown to increase the risk for severe complications of human ocular infection with *Chlamydia trachomatis* in a Gambian population [37]. Moreover, rs3024500-C allele was part of a higher IL-10 transcript producing haplotype that has been associated with susceptibility to sequelae of human ocular chlamydial infection [38]. Individual *IL-10* polymorphism effects on the development of CMV retinitis in LSOCA African American patients may result from complex interactions between human IL-10 production, HIV infection progression, and HCMV activity. Statistically powerful modeling of potential effects of African American *IL-10* variants (and their haplotypes) on the development of CMV retinitis requires more patients than were available for this study.

The IL-10/IL-10R signaling path is a key pathway for viral persistence. Animal models lacking IL-10 exhibit enhanced pathogen clearance [39–41] and blockage of the IL-10/IL-10R signaling pathway leads to resolution of chronic viral infection [42, 43]. Thus, it is not surprising that human CMV uses its IL-10 homolog, cmvIL-10, to bind to IL-10R1 [13], which leads to immunosuppression [18]. The cmvIL-10 shows only 27% amino acid similarity to human IL-10, yet it has IL-10R1 affinity similar to human IL-10. However, cmvIL-10 has a different interdomain angle than does human IL-10, which changes the orientation of IL-10R1 in the putative cell surface complex [17]. Because of this structural difference, naturally occurring *IL-10R1* amino acid polymorphisms may destabilize the cmvIL-10/IL-10R1 complex. Indeed, the common *IL-10R1* variants rs3135932 (SNP3) and rs2229113 (SNP4) differentially reduce the signaling activity of cmvIL-10 [21]. In this study, we did not observe statistically significant effects of these 2 SNPs on the development of CMV retinitis. Although rs3135932 and rs2229113 variants have been shown to reduce cmvIL-10–induced (and human IL-10–induced) signaling activity, they do not totally diminish the signaling through the IL-10 receptor complex [20, 21]. These 2 SNPs may specifically affect the time to development of CMV-related disease. However, as our analyses concentrated on CMV retinitis as the end point, we might not have captured the potential effects of rs3135932 and rs2229113. The SNPs rs2229114 and rs2228055 and the associated haplotypes that showed an effect on the development of CMV retinitis in this study have not been investigated in any infectious disease outcome so far. Although the mutation Ile to Val (I224V, rs2228055) is a change from hydrophobic to another hydrophobic residue, the Ile is a much larger amino

Table 5. Allelic Distribution and Association Tests of *IL-10* Single-Nucleotide Polymorphisms (SNPs) in Case Patients with Cytomegalovirus Retinitis and in Control Patients

This table is available in its entirety in the online version of the *Journal of Infectious Diseases*

acid than the Val. For this reason the I224V change may affect the conformation of IL-10R1 structure in the proximity of the membrane, which in turn may affect the transduction of downstream signaling of the IL-10R complex. It is much harder to model the potential effects of the S420L (rs2229114) substitution, because this change involves a residue that is in the cytoplasmic domain of IL-10R1, for which no published crystal structure is available. However, 420S residue is a highly conserved residue in chimpanzee, mouse, rat, dog, and cow IL-10R1. Moreover, the cytoplasmic residues are thought to be involved in STAT3 binding and signal transduction. Therefore, S420L change may lead to a reduction in the IL-10 signaling pathway. Antibody blockade of the IL-10/IL-10R pathway in vitro has been shown to enhance $CD4^+$ T cell responses in samples from patients chronically infected with HIV [44]. Our findings suggest that a similar IL-10/IL-10R blockade may be useful in controlling chronic HCMV infections.

No world population, whether industrialized or not, is immune to HCMV infection. Recent surveys show that the prevalence of HCMV reaches 90%–100% in some populations (i.e., in Africa and southeast Asia), in which the incidence of HIV infections is also high [45]. Although usually associated with mild or no clinical symptoms, HCMV infections can lead to drastic end-stage organ diseases which may be fatal when activated in immunocompromised patients. Our findings suggest that host genetics may play a crucial role in the development of one of these end-stage organ diseases, CMV retinitis, in patients with AIDS. Previously, autopsy studies showed a high incidence of other organ CMV infections in patients with CMV retinitis [46]. Indeed, 13% of the LSOCA patients who developed CMV retinitis in this study also received a diagnosis of other CMV-related diseases, including pneumonitis, pancreatitis, gastritis, and colitis, which indicates system-wide disease in patients that have CMV activation. Moreover, patients coinfected with HIV and CMV progress more rapidly to AIDS and death than do HIV-infected patients who are not infected with CMV [11, 47–49]. Our results should stimulate further research, especially with longitudinal follow-up studies, to elucidate the role of IL-10 signalling and other host genetics in initiation and progression of CMV-related diseases.

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