MAJOR ARTICLE

Host Immunogenetics and Control of Human Herpesvirus–8 Infection

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(See the editorial commentary by Stebbing, on pages 1051–3.)

Background. Kaposi sarcoma (KS) is primarily caused by human herpesvirus (HHV)–8 infection, and the risk is increased with high HHV-8 lytic or latent antibody titers or the detection of HHV-8 DNA in peripheral blood mononuclear cells (PBMCs). Host genes important for control of HHV-8 infection are not well characterized.

Methods. In 172 HHV-8 latent nuclear antigen (LANA)–seropositive adults in Italy without KS, we examined correlations of common variants in host immune genes with the detection of HHV-8 DNA in PBMCs and with high lytic and latent antibody titers. Twenty-eight single-nucleotide polymorphisms in 14 genes were analyzed. We detected HHV-8 DNA in PBMCs with real-time amplification of the K6 gene, anti-K8.1 (lytic) titers with enzyme-linked immunosorbent assay, and anti-LANA (latent) titers with immunofluorescence.

Results. Detection of HHV-8 DNA in PBMCs was not significantly related to any variant examined. In contrast, a 3-locus haplotype of *IL4*, which contains the −1098G allele (rs2243248), was overrepresented among subjects with high lytic titers (odds ratio [OR], 2.8 [95% confidence interval {CI}, 1.1–6.7]), compared with those with low titers, as was the functional promoter variant of *IL6*, C-236C (rs1800795) (OR, 3.7 [95% CI, 1.1–12.8]). Compared with subjects with low HHV-8 latent antibody titers, analysis of inferred haplotypes for *IL12A* revealed an overrepresentation of −798T/277A in subjects with high HHV-8 latent antibody titers (OR, 2.4 [95% CI, 1.1–5.2]).

Conclusions. Our observations are the first to provide preliminary evidence suggesting that common variants in key host immune genes could influence the control of HHV-8 infection.

Since its discovery in 1994 [1], human herpesvirus (HHV)–8, which is also known as Kaposi sarcoma–associated herpesvirus, has been detected consistently in all clinical variants of Kaposi sarcoma (KS), and it is required for the development of this immune-mediated neoplasm [2, 3]. Among patients with HIV-associated or iatrogenic immunosuppression, the detection of HHV-8 DNA in peripheral blood mononuclear cells (PBMCs) and a high HHV-8 lytic antibody titer are highly predictive of the development of KS [4].

After seroconversion, HHV-8 establishes a life-long infection exhibiting 2 phases, termed "lytic" (active replication of virions) and "latent" (persistence in cells).

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In latency, HHV-8 persists predominantly in B cells [5, 6] and is characterized by a restricted pattern of viral gene expression [7, 8] that facilitates the avoidance of immune surveillance. The virus can reactivate periodically to undergo lytic replication [7, 9], which possibly represents a loss of immunogenic control. Although the factors that regulate this switch have not been well characterized, virions produced during lytic replication can precipitate the spread of virus from the lymphoid reservoir to endothelial cells, which is a critical step for the pathogenesis of KS [5, 10–12].

During the induction of the lytic phase, several viral-encoded cellular homologues [13, 14] with known cytokine or cytokine-receptor signal-transduction potential are up-regulated [10]. These include an interleukin (IL)–6 cytokine gene homologue [15], an IL-8 receptor homologue that functions as a G protein–coupled receptor [16–18], and a viral interferon (IFN) regulatory factor [19]. Viral piracy of these proinflammatory genes and their evolutionary maintenance in the viral genome suggests that they may be important for HHV-8 to evade innate and adaptive immune responses during reactivation and lytic replication. In parallel with human cytokine production, these homologues have been proposed to contribute to the autocrine and paracrine maintenance of KS lesions [20–22]. For example, host IFN-γ, a potent proinflammatory cytokine, has been shown to initiate HHV-8 lytic replication in latently infected endothelial cells [23, 24]. In addition, the results of in vitro studies have suggested that lytic replication is associated with an altered cytokine balance that favors a Th1-dominated lymphocyte response [23]. In turn, this Th1 polarization contributes to enhanced cellular differentiation and the development of spindle cells [25], the tumor cell of KS lesions. Thus, the induction of lytic replication of HHV-8–infected cells and the expression of viral lytic and cellular gene products could play an important role in the persistence of HHV-8 and, ultimately, the pathogenesis of KS.

Findings from preliminary studies have suggested that common variants in host cytokine genes—such as the *IL1* complex, *IL10,* and *TNF*—could be associated with susceptibility to herpesvirus infections, including Epstein-Barr virus, herpes simplex virus–1, and cytomegalovirus [26–29]. The relationship between common genetic variants and the risk of HHV-8 infection was examined in only 1 previously published study. In HIV-seropositive men without AIDS-associated KS, the distribution of common variants in *IL6* at position −236 (also referred to as −174 or rs1800795) did not substantially differ in those who were HHV-8 lytic antibody seropositive and those who were HHV-8 lytic antibody seronegative as determined by only a single HHV-8 lytic antibody assay [30].

In the present investigation, we examined whether common genetic variants from proinflammatory, anti-inflammatory, and chemotactic pathways were associated specifically with markers of infection, including HHV-8 DNA load and high antibody titers against both HHV-8 lytic antigen (K8.1) and latent nuclear antigen (LANA) in HHV-8–seropositive adults without KS or HIV-1 infection.

SUBJECTS, MATERIALS, AND METHODS

Study Population

Using data from HHV-8–seropositive control subjects from the Italian Kaposi Sarcoma Case-Control (KCC) study [31], we examined the relationship between common regulatory polymorphisms in host immune genes and the presence of HHV-8 DNA sequence detected in PBMCs, as well as high HHV-8 lytic and latent antibody titers. We chose to evaluate associations between common variants in cytokine genes and markers of HHV-8 control in subjects who were HHV-8 LANA seropositive but without classic KS, to eliminate the potential effect of disease on gene variants and risk of viral control. We used samples from the 192 population-based control subjects enrolled in the KCC study who had evidence of HHV-8 latent antibodies. DNA adequate for HHV-8 DNA amplification in PBMCs was available from 158 (82.3%) of these subjects. Serum samples and lymphocyte pellets were available from all subjects for antibody quantification and genomic DNA extraction.

All subjects were at least 18 years old and without evidence of KS or HIV-1 infection. As described elsewhere [31], they were identified through the recruitment of control subjects for the KCC study, which included collaborating local primary-care physicians from Sicily, Rome, and Naples between April 1998 and October 2001. Subjects were screened for the presence of HHV-8 LANA antibodies. Those who tested seropositive by immunofluorescence (IFA) were enrolled in the KCC study. Institutional review boards from each study site reviewed and approved the protocol, questionnaire, and related materials before initiation.

Laboratory Methods

HHV-8 LANA seropositivity. As described elsewhere [31, 32], we determined HHV-8 seropositivity by the detection of LANA in a 1:120 dilution of serum with IFA, using the BCBL-1 cell line without induction. Specimens with punctuate nuclear staining were considered to be seropositive.

HHV-8 lytic and latent antibody titers. Antibodies against the lytic antigen K8.1 were determined using ELISA (Viral Epidemiology Section, AIDS Vaccine Program, SAIC-Frederick, National Cancer Institute), as reported elsewhere [33]. Sixteen 2-fold dilutions were made from 1:20 to 1:1,310,720. Absorbance was read at 405 nm on an automated plate reader. The titer was calculated using a standard quadratic titration curve and a cutoff equal to the average of the negative controls plus 0.75. Antibodies against open-reading frame 73 of LANA were determined using IFA [34] performed with latently HHV-8–

Table 1. Common genetic variants in pro- and anti-inflammatory cytokines, chemotactic molecules, and growth factors evaluated in relation to the presence of human herpesvirus (HHV)–8 DNA in peripheral blood mononuclear cells and high HHV-8 lytic and latent antibody titers.

Gene	Chromosome	Common variant (alias)	dbSNP identifier
Proinflammatory cytokines			
IL1A	2q14	G+114T (Ex5 +21)	rs17561
		IVS4 C-96T	rs2856841
		IVS4 A-109C	rs2071374
		Ex1 C+12T (−889)	rs1800587
IL1B	2q14	Ex5 C+14T (+3954)	rs1143634
		C-580T (−31)	rs1143627
		T-1060C (−511)	rs16944
IL2	4q26	IVS1 G-100T (−385)	rs2069762
IL6	7p21	C-236G (−174)	rs1800795
IL12A	3p12-q13.2	IVS2 A-798T	rs582054
		Ex7 A+277G (+8685)	rs568408
TNFA	6p21.3	C-1036T (−857)	rs1799724
		A-487G (−308)	rs1800629
IFNG	12q14	IVS3 G+284A	rs1861494
Anti-inflammatory cytokines			
IL4	5q31.1	G-1098T	rs2243248
		T-588C (−524)	rs2243250
		Ex1 C-168T (−33)	rs2070874
IL5	5q31.1	T-745C	rs2069812
IL10	1q31-q32	C-853T (−819)	s1800871
		A-1116G (−1082)	rs1800896
IL13	5q31	C-1069T (−1112)	rs1800925
		Ex4 A+98G (+130, +144)	rs20541
Chemotactic and growth factors			
IL8	4q13-q21	A-351T (−251)	rs4073
		IVS1 G+230T (+396)	rs2227307
		IVS1 C-204T (+781)	rs2227306
IL8RB	2q35	Ex3 T+1235C	rs1126579
		Ex3 G-1010A (+1440)	rs1126580
VEGF	6p12	236 bp 3′ of STP C→T	rs3025039

NOTE. Common genetic variant nomenclature adapted from Antonarakis SE and the Nomenclature Working Group [36]. dbSNP, SNP500Cancer single-nucleotide polymorphism database.

infected primary effusion lymphoma cell line BCP-1. Twofold dilutions were made from 1:100 to 1:204,800. The titer was determined to be the reciprocal of the last dilution at which punctate nuclear fluorescence was observed. All serum samples were tested by researchers blinded to infection status.

HHV-8 real-time PCR. HHV-8 DNA was detected and quantitated in previously stored frozen (−70°C) PBMCs using real-time TaqMan amplification (ABI), as described elsewhere [33]. Briefly, viral DNA was extracted from PBMCs using the QIAmp DNA blood kit (Qiagen). HHV-8 DNA was amplified using primers to the K6 region and quantitated with a plasmid-based standard curve and ABI Prism 7700 (ABI) [12, 33]. Cell quantitation was performed using a real-time polymerase chain reaction (PCR) assay for human ERV3, as described elsewhere [35]. The viral load was calculated as the number of viral copies per 10^6 cells. The lower limit of detection was determined to be 3 copies/10^6 PBMCs.

Common genetic variants. Genomic DNA was extracted from cryopreserved lymphocyte pellets using a modified salt precipitation–extraction method (Gentra Systems). As shown in table 1, 28 common single-nucleotide polymorphisms (SNPs) were selected from 14 genes *(IL1A, IL1B, IL2, IL4, IL5, IL6, IL8, IL8RB, IL10, IL12A, IL13, TNF, IFNG,* and *VEGF)*. Common genetic variants in candidate genes were selected a priori on the basis of the following criteria: (1) a minor allele frequency of >5% in the SNP500Cancer data set (Caucasians only); (2) demonstrated functional data; (3) previous associations with autoimmune, infectious, or cancer outcomes; and (4) a plausible contribution to HHV-8 infection status or the pathogenesis of KS. All assays are publicly available (including primers, probes, and conditions) on the SNP500Cancer Web site (available at: http://snp500cancer.nci.nih.gov) [37]. Genotype analysis was performed using optimized TaqMan assays and analyzed on the ABI 7900HT platform (ABI). A blinded

duplicate analysis of 5% of study samples demonstrated >99% concordance. All common variants are referred to in accordance with the recommendations set forth by the Nomenclature Working Group [36].

Statistical analysis. For the purposes of the analysis, markers of HHV-8 infection were defined as the presence of HHV-8 DNA in PBMCs or high lytic or latent antibody titers. The HHV-8 DNA load in PBMCs was dichotomized into 2 groups—present or absent—where DNA levels $\geq$10 copies/10^6 cells were considered to be present, and DNA levels <10 copies/10^6 cells were considered to be absent. Lytic and latent antibody titers were categorized into tertiles. High lytic and latent antibody titers were defined by the highest tertile (lytic, $\geq$1:520; latent, $\geq$1:51,200), whereas low titers were defined by the combined lowest 2 tertiles.

Differences in genotype frequencies in subjects with evidence of HHV-8 DNA in PBMCs and high lytic or latent antibody titers were determined using the Pearson χ^2 test. Deviation from Hardy-Weinberg equilibrium was examined for each locus. Linkage disequilibrium (LD) between multiple loci was measured using the standardized disequilibrium coefficient (Lewotin's D′) and the correlation coefficient (r^2). For single loci, the relative risk of a high HHV-8 burden was estimated by the prevalence odds ratios (ORs) and 95% confidence intervals (CIs), which were calculated using logistic regression. The most frequent homozygous genotype occurring among participants with a low HHV-8 burden served as the referent. For genotypes occurring in the population at a rate of <5%, rare allele-containing genotypes were combined. Statistical significance, based on multivariate logistic models, was calculated using the likelihood-ratio test for logistic regression. A 2-tailed $P \leq .05$ was considered to be statistically significant. Analyses are presented without adjustment for multiple comparisons. Genotypic distributions and linkage were examined using Stata software (version 7.0; StataCorp).

Haplotype frequencies occurring in the population with a frequency of >1% were estimated for 9 genes *(IL1A, IL1B, IL4, IL8, IL8RB, IL10, IL12A, IL13,* and *TNF)* using the expectation-maximization progressive insertion algorithm as implemented in HaploStats (version 1.1.0; available at: http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm). Differences in the overall haplotype profile among case and control subjects were evaluated using the global score test based on empirical distributions of a minimum of 10,000 and a maximum of 50,000 permutations and adjusted for age and sex [38]. Associations of HHV-8 control and haplotypes were estimated using generalized linear models based on iterative, weighted regression substitution [38, 39]. For internal validity, associations of HHV-8 control and haplotypes were confirmed using an alternative approach of first assigning individual posterior probabilities of phase to each person, using the coalescent approach implemented in PHASE (version 2.0.2; Mathematical Genetics Group) [40, 41], and then performing logistic regression to estimate haplotype effects, weighted by these posterior probabilities. Data are presented on the basis of additive models generated using HaploStats.

RESULTS

PBMC HHV-8 PCR. Viral DNA was detected in PBMCs in 26 (16.5% [95% CI 10.6%–22.3%]) of 158 subjects. The geometric mean level of HHV-8 DNA in PBMCs was 58 copies/10^6 cells (range, 13–2128 copies/10^6 cells). The detection of HHV-8 DNA did not differ significantly by sex or age (table 2).

The distributions of genotypes are presented in table 3 for all common variants, stratified by HHV-8 DNA in PBMCs and high lytic and latent antibody titers, respectively. The detection of HHV-8 DNA in PBMCs was not significantly associated with any of the single-locus markers examined ($\geq$0.11). Rare homozygotes of *IL13* at positions −1069C and +98A were overrepresented among subjects with, compared with those without, detectable HHV-8 DNA in PBMCs (*IL13* −1069T: OR, 18.9 [95% CI 1.4–257] and *IL13* +98A: OR, 17.3 [95% CI 1.4–221]), although statistical significance was not observed when rare homozygotes were combined with heterozygotes at each *IL13* locus.

Haplotypes were estimated separately for subjects with HHV-8 DNA in PBMCs and those without, using unphased data for 9 of 14 cytokine genes with genotypes at multiple loci (table 4). For each of the 9 genes, the simulated global score test did not suggest that the overall haplotype profile significantly differed by the presence or absence of HHV-8 DNA in PBMCs ($P \geq .11$). Haplotype frequency estimates did not differ substantially by haplotype estimation method or after adjustment for sex and age (data not shown).

High HHV-8 lytic antibody titer. By definition, 57 (33.1%) of 172 subjects had high lytic antibody titers (geometric mean, 1197; range, 520–3533). As shown in table 2, men were 2.9-fold more likely than women to have high HHV-8 lytic antibody titers ($P = .006$). However, the geometric mean HHV-8 lytic antibody titer was not substantially different by sex (men, 383 [95% CI, 297–493] and women, 232 [95% CI, 154–348]; $P = .09$).

The presence of a high HHV-8 lytic antibody titer was significantly associated with genotype frequencies for G-containing variants of *IL4* at position −1098, a promoter variant of *IL6*, C-236C (table 2), as well as *IL1B* heterozygotes at positions −580 and −1060 (table 3). Allelic distributions of *IL1B* at these linked loci ($D' = 1.0$) did not differ substantially by level of viral lytic titer (−580C: OR, 0.9 [95% CI, 0.6–1.3] and −1060T: OR, 0.9 [95% CI, 0.7–1.3]). Haplotype frequency estimates confirmed single-locus marker associations of high lytic antibody titers for *IL4* (table 5). Overall, the global score test for *IL4* was significant ($P = .05$). At the individual level, compared

Table 2. Genotypes in cytokines, chemotactic molecules, and growth factors and risk of human herpesvirus (HHV)–8 DNA peripheral blood mononuclear cells and high HHV-8 lytic and latent antibody titers

		HHV-8 DNA			Lytic antibody titer			Latent antibody titer		
Variable/gene and locus (alias)	Genotype	Positive	Negative	OR (95% CI)	High	Low	OR (95% CI)	High	Low	OR (95% CI)
Male sex		51	142	1.0 (0.56–1.82)	47	71	**2.9(1.3–6.3)**	36	81	1.2 (0.6–2.4)
Age ⩾ 75 years (median)		41	92	1.6 (0.91–2.67)	32	56	1.4 (0.7–2.6)	27	60	1.1 (0.6–2.2)
IL4										
G-1098T										
	TT	23	114	1.0	44	102	1.0	46	99	1.0
	GT	3	16	1.0 (0.3–3.7)	12	12	**2.8 (1.1–7.0)**	4	20	0.4 (0.1–1.4)
	GG	0	1	NA	1	0	NA	1	0	NA
	GT + GG	3	17	0.9 (0.2–2.3)	13	12	**3.0 (1.2–7.4)**	5	20	0.6 (0.2–1.6)
T-588C (−524)										
	CC	19	100	1.0	45	85	1.0	37	92	1.0
	TC	6	26	1.4 (0.5–3.8)	11	24	0.9 (0.4–2.0)	11	24	1.2 (0.5–2.6)
	TT	1	2	3.9 (0.3–50.4)	1	2	1.7 (0.1–21.4)	1	2	1.4 (0.1–16.4)
	TC + TT	7	28	1.5 (0.6–4.0)	12	26	0.9 (0.4–2.1)	12	26	1.2 (0.5–2.5)
Ex1 C-168T (−33)										
	CC	19	103	1.0	45	88	1.0	38	94	1.0
	TC	6	26	1.4 (0.5–4.0)	11	24	0.9 (0.4–2.1)	11	24	1.2 (0.5–2.6)
	TT	1	1	7.7 (0.4–147)	1	1	5.3 (0.3–96.5)	1	1	3.0 (0.2–52.9)
	TC + TT	7	27	1.6 (0.6–4.3)	12	25	1.0 (0.5–2.3)	12	25	1.2 (0.6–2.7)
IL6										
C-236G (−174)										
	GG	17	76	1.0	29	70	1.0	29	69	1.0
	CG	9	42	0.9 (0.4–2.2)	21	36	1.5 (0.7–3.1)	15	42	0.8 (0.4–1.8)
	CC	0	11	NA	7	6	**3.7 (1.1–12.8)**	6	7	2.0 (0.6–6.7)
	CG + CC	9	53	0.7 (0.3–1.7)	NA	NA	NA	NA	NA	NA
IL12A										
IVS2 A-798T										
	TT	5	39	1.0	14	34	1.0	16	31	1.0
	AT	16	64	2.1 (0.7–6.3)	28	54	1.1 (0.5–2.5)	24	58	0.8 (0.4–1.7)
	AA	4	19	1.8 (0.4–7.9)	11	18	1.2 (0.4–2.3)	7	22	0.6 (0.2–1.7)
Ex7 A+277G (+8685)										
	GG	23	94	1.0	40	89	1.0	32	96	1.0
	AG	3	29	0.3 (0.1–1.3)	14	20	1.2 (0.5–2.7)	15	19	**2.4 (1.1–5.4)**
	AA	0	3	NA	1	2	1.1 (0.1–14.1)	2	1	6.0 (0.5–68.6)
	AG + AA	3	32	0.3 (0.1–1.2)	15	22	1.2 (0.5–2.6)	17	20	**2.6 (1.2–5.7)**

NOTE. Odds ratios (ORs) were adjusted for sex and age. The most frequent genotype among control subjects (low HHV-8 burden) served as the referent. Rare genotypes for which the frequency was <5% are combined. Bold type indicates statistical significance. CI, confidence interval; NA, not applicable.

with subjects with low HHV-8 lytic antibody titers, the 3-locus haplotype of *IL4* (−1098G/−588C/−168C) was associated with increased likelihood of high HHV-8 lytic antibody titers (OR, 2.8 [95% CI, 1.1–6.7]). The coalescent approach yielded a similar estimate (OR, 2.5 [95% CI, 1.4–4.7]), and there was no notable difference by sex and age (data not shown).

High HHV-8 latent antibody titer. By definition, 51 (29.7%) of 172 subjects had high latent antibody titers (geometric mean titer, 1:88,180; range, 1:51,200–1:204,800). The distribution of high anti–latent viral antigen did not significantly differ by sex or age (table 2). As shown in table 2, the minor allele at *IL12A*, +277A, was overrepresented in subjects with high HHV-8 latent antibody titers, compared with those with low latent titers (OR, 2.6 [95% CI, 1.2–5.7]).

The difference in the global score test was significant overall for a 2-locus haplotype of *IL12A* (simulated global score test, $P = .02$) (table 5), which suggests that common variations in this gene could influence latent viral antibody titers. Compared with subjects with low titers, the frequency estimate of the *IL12A* (−798T/+277A) haplotype was higher in subjects with high HHV-8 latent antibody titers ($P = .006$), and the relative frequency of this haplotype significantly increased in subjects with high viral titers (OR, 2.4 [95% CI, 1.1–5.2]). This finding was confirmed using the coalescent method (OR, 2.3 [95% CI,

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

1.4–4.0]) and was not substantially altered when adjusted for sex and age.

DISCUSSION

In HHV-8–infected adults from Italy without HIV-1 infection or KS, our results suggest that host immunogenetics could contribute to the control of HHV-8 infection. In an evaluation of common variants drawn from key cytokine, chemotactic, and growth-factor genes, the presence of HHV-8 DNA in PBMCs was not significantly related to any single variant. In persons without KS, the level of detection of HHV-8 DNA in the peripheral blood is low; thus, our lack of significant findings were not unexpected. However, we observed that a high HHV-8 lytic antibody titer, which is possibly a marker of lytic reactivation, was associated with a common promoter variant in *IL6* (−236C), as well as a haplotype of *IL4* (−1098G/−588C/−168C), and that a high latent HHV-8 antibody titer was associated with a haplotype of *IL12A* (−798T/277A). To our knowledge, this is the first report to identify host genetic factors that influence susceptibility to high antibody titers in HHV-8–infected individuals without HIV-1 infection or KS.

Cytokines play a pivotal role in host defenses against infectious agents and surveillance of tumor cells. In particular, cytokines can modulate the Th1/Th2 balance of T lymphocytes: Th1 cells favor cell-mediated immunity, whereas Th2 cells favor humoral immunity [42, 43]. The response to a chronic infection is often dominated by either Th1 or Th2 cytokines, with 1 type suppressing the other type via tight regulatory autocrine and paracrine effects, which results in a polarization of the immune response. Recent evidence has suggested that a Th1 or a predominantly proinflammatory response is associated with the pathogenesis of KS. For example, early-stage KS lesions produce a variety of proinflammatory cytokines (IFN-γ, tumor necrosis factor–α, IL-1, and IL-6), chemotactic molecules (IL-8 and IL-8 receptor–β), and growth factors (basic fibroblast growth factor and vascular endothelial growth factor) [22]. In addition, these inflammatory molecules can be detected in KS lesions [44] and have been shown to support the growth of KS-derived cells in vitro [44–46].

Proinflammatory cytokines may be involved in reactivating endothelial cells latently infected with HHV-8, which is a critical step in triggering viral replication and cellular differentiation into the spindle tumor cells that are characteristic of KS lesions. Evidence suggests that the HHV-8 regulator of transcription activation gene controls the induction and completion of lytic replication from viral latency [47, 48] and is capable of not only inducing high-level expression of viral IL-6 but also reactivating cellular IL-6 expression [49]. The relationship between viral and cellular cytokine expression is not well defined, but it is possible that interactions between host and viral genes that affect cytokine expression could contribute to HHV-8 control and ultimately, the pathogenesis of KS.

Findings from a previously published report suggested that, in HIV-1–infected individuals, cellular *IL6* C-236G genotypes were not significantly related to HHV-8 seropositivity, compared with HHV-8–uninfected G-236G homozygotes (calculated risk estimates, OR_{CG}, 0.9 and OR_{CC}, 0.8) [30]. However, HHV-8 serostatus was evaluated using only HHV-8 lytic antibodies. In our investigation of study subjects without HIV-1 infection or KS, we note that the same promoter variant, C-236C, was associated with a nearly 4-fold increased likelihood of high HHV-8 lytic antibody titers. This finding is particularly intriguing, because this variant has been reported to be associated with a decrease in *IL6* expression, as well as lower plasma levels of circulating IL-6 [50]. Given the potentially important role of IL-6 for the control of HHV-8 infection and the pathogenesis of KS, our finding supports the hypothesis that complex relationships of viral and cellular genes and subsequent gene products may influence the immunogenic control of infection.

Compared with subjects with low HHV-8 lytic antibody titers, a rare haplotype of *IL4* (−1098G/−588C/−168C) was associated with an increased likelihood of high lytic antibody titers. Further analysis of the association of genotype and haplotype frequencies of *IL4* suggested that the G allele at position −1098 could be an important putative susceptibility factor, but additional correlative studies are needed to establish a causal relationship.

IL-4 is a pleiotropic Th2 cytokine that promotes the proliferation and differentiation of B cells, as well as the expression of antigen and isotype switching [51]. Laboratory evidence has suggested that the promoter region variants −588T and −168T are associated with increased transcriptional activity of *IL4,* as well as with increased circulating levels of IgE [52, 53]. Findings from genetic association studies have suggested that carriers of

Table 4. Overall haplotype profile, individual haplotype frequency estimates, and risk of peripheral blood mononuclear cell human herpesvirus (HHV)–8 DNA, and high HHV-8 lytic and latent antibody titers.

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

Table 5. Overall haplotype profile, individual haplotype frequency estimates, and risk of high human herpesvirus (HHV)–8 lytic and latent antibody titers.

Test, gene	Haplotype			Haplotype frequency estimates			*P*	Relative haplotype frequency, OR (95% CI)
				Overall	High HHV-8	Low HHV-8		
High HHV-8 lytic antibody titer								
IL4	−1098	−588	−168					
	T	C	C	0.803	0.763	0.823	.16	1.0
	T	T	T	0.115	0.114	0.115	.93	1.2 (0.6–2.7)
	G	C	C	0.076	0.123	0.053	**.02**	**2.8 (1.1–6.7)**
Simulated global score test							*.05*	
High HHV-8 latent antibody titer								
IL12A	−798	277						
	A	G		0.447	0.407	0.463	.32	1.0
	T	G		0.432	0.397	0.447	.43	1.1 (0.6–1.9)
	T	A		0.121	0.196	0.090	**.006**	**2.4 (1.1–5.2)**
Simulated global score test							*.02*	

NOTE. Relative haplotype frequencies adjusted for sex and age. Rare haplotypes with frequencies <1% were combined (data not shown). Empirical *P* values were based on a minimum of 10,000 and maximum of 50,000 permutations. Bold type indicates statistical significance. Frequencies add to 100%. CI, confidence interval; NA, haplotype does not occur with any frequency; OR, odds ratio.

the high-functioning −588T allele are at an increased risk of Th2-mediated diseases, including asthma, atopy, and severe infection with respiratory syncytial virus [54–56]. In our investigation, loci at positions −1098 and −588 were in strong linkage ($D' = 1.0$). Although it is possible that the −588C-containing haplotype (−1098G/−588C/−168C) results in nominal or decreased IL-4 expression, the referent haplotype differed only at position −1098, which suggests that this variant could be informative or, perhaps, be a marker in LD with another variant that has not yet been evaluated.

A 2-loci haplotype of *IL12A* (−798T/277A) was overrepresented in subjects with evidence of high latent antibody titers. IL-12 is a heterodimer that is composed of 2 covalently linked chains, p40 and p35, that are independently encoded by genes on different chromosomes. The p40 chain is encoded by *IL12B* (5q31.1–q33.1), whereas the p70 chain is encoded by *IL12A* (3p12–q13.2). IL12p70 is a Th1-polarizing cytokine that is capable of stimulating naive T cells and inducing IFN-γ production and cell-mediated immunity [57, 58]. In vitro evidence has suggested that these gene products may interact, with IL12p40 antagonizing the effect of IL12p70 in humans [59]. Findings from genetic association studies have suggested that the *IL12B* allele, which is characterized by increased IL12p40 expression, was associated with type 1 diabetes [60], as well as mortality from cerebral malaria with a reduction in nitric oxide production among children [61], whereas the low-functioning allele was associated with an increased risk of asthma [62]. Although the functional effects of *IL12B* variants may be insightful, the exact function of *IL12A* haplotypes has not yet been determined.

It is tempting to speculate that complex relationships among immunoglobulin classes, viral and cellular Th1 and Th2 cytokine genes, and gene products are important for HHV-8 antibody presentation and the control of infection. However, caution in the interpretation of these findings is warranted. Our findings are limited by the cross-sectional interpretation of viral markers in peripheral blood. Accordingly, an increase in antibody titers may reflect unchecked chronic HHV-8 replication resulting in immune stimulation; alternatively, it may represent immune control of a recent HHV-8 reactivation. In addition, our observations are based on an HHV-8 latent seropositive study population from Italy, an area where HHV-8 infection and KS are endemic. Although the modes of HHV-8 transmission remain uncertain, infection typically begins in childhood and plateaus in the sixth decade of life. The mean age of our study population was 75 years, which suggests that our participants may have been infected with HHV-8 for many years. However, most subjects will likely never develop KS, which may be attributed to environmental or genetic factors not evaluated. In this capacity, it is not clear whether our findings related to HHV-8 control are applicable to younger study populations. Despite stringent criteria for selecting candidate genes a priori, our findings could represent false-positive associations due to LD with additional variants not evaluated or to small sample size. However, by using 2 statistical methods based on different algorithms to estimate frequencies of inferred haplotypes, we decreased the likelihood of false positives resulting from imputation.

In our study, we observed a possible influence of common genetic variants in key host defense genes on markers of poorly controlled HHV-8 infection, which is not completely unexpected, given the role of proinflammatory cytokines in HHV-8 reactivation, as well as KS pathogenesis. Overall, among HHV-

8–seropositive Italians without KS, markers of poorly controlled HHV-8 infection were associated with haplotypes of *IL4* and *IL12A* and the promoter region variant, *IL6* −236C. Our data provide preliminary evidence for genetic variations in cytokines that could influence the overall control of HHV-8 in HIV-negative individuals without KS. Additional studies are required to confirm these preliminary findings and to investigate a biological basis for alternations in cytokine pathways involved in the immunogenic control of HHV-8 infection.

CLASSIC KAPOSI SARCOMA WORKING GROUP

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