The Journal of Infectious Diseases

MAJOR ARTICLE

Risk of Classic Kaposi Sarcoma With Combinations of Killer Immunoglobulin-Like Receptor and Human Leukocyte Antigen Loci: A Population-Based Case-control Study

James J. Goedert,[1,a] Maureen P. Martin,[2,4,a] Francesco Vitale,[5] Carmela Lauria,[6] Denise Whitby,[3] Ying Qi,[2,4] Xiaojiang Gao,[2,4] and Mary Carrington[2,4]

[1]Division of Cancer Epidemiology and Genetics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, and [2]Cancer and Inflammation Program, Laboratory of Experimental Immunology, and [3]Viral Oncology Section, AIDS and Cancer Virus Program, Leidos Biomedical Research, Frederick National Laboratory for Cancer Research, Maryland; [4]Ragon Institute of MGH, MIT, and Harvard, Cambridge, Massachusetts; [5]Dipartimento di Igiene e Microbiologia Giuseppe D'Alessandro, Universitá degli Studi di Palermo, and [6]Lega Italiana per la Lotta Contro i Tumori-Sez Ragusa, Italy

Background. Kaposi sarcoma (KS) is a complication of KS-associated herpesvirus (KSHV) infection. Other oncogenic viral infections and malignancies are associated with certain *HLA* alleles and their natural killer (NK) cell immunoglobulin-like receptor (*KIR*) ligands. We tested whether *HLA-KIR* influences the risk of KSHV infection or KS.

Methods. In population-based case-control studies, we compared *HLA* class I and *KIR* gene frequencies in 250 classic (non-AIDS) KS cases, 280 KSHV-seropositive controls, and 576 KSHV-seronegative controls composing discovery and validation cohorts. Logistic regression was used to calculate sex- and age-adjusted odds ratios (ORs) and 95% confidence intervals.

Results. In both the discovery and validation cohorts, KS was associated with *HLA-A*11:01* (adjusted OR for the combined cohorts, 0.4; $P = .002$) and *HLA-C*07:01* (adjusted OR, 1.6; $P = .002$). Consistent associations across cohorts were also observed with activating *KIR3DS1* plus *HLA-B Bw4-80I* and homozygosity for *HLA-C* group 1. With *KIR3DS1* plus *HLA-B Bw4-80I*, the KSHV seroprevalence was 40% lower (adjusted OR for the combined cohorts, 0.6; $P = .01$), but the KS risk was 2-fold higher (adjusted OR, 2.1; $P = .002$). Similarly, the KSHV seroprevalence was 40% lower (adjusted OR, 0.6; $P = .01$) but the KS risk 80% higher with *HLA-C* group 1 homozygosity (adjusted OR, 1.8; $P = .005$).

Conclusions. *KIR*-mediated NK cell activation may decrease then risk of KSHV infection but enhance KSHV dissemination and progression to KS if infection occurs.

Keywords. Kaposi sarcoma; Italy; case-control study; human genetics; major histocompatibility complex; human leukocyte antigens; natural killer–cell immunoglobulin-like receptors.

Infection with Kaposi sarcoma (KS)–associated herpesvirus (KSHV; also called "human herpesvirus 8") is required but not sufficient for development of KS [1], and the prevalence of KSHV seropositivity far exceeds the incidence of KS. KSHV infection is generally acquired during childhood, probably via saliva, in KSHV-endemic populations of sub-Saharan Africa and the Mediterranean region [2]. Given KSHV infection, the risk for KS is profoundly increased with human immunodeficiency virus infection (HIV; hereafter, "AIDS-associated KS") and also substantially increased with the use of corticosteroids and other immunosuppressive medications [3–5]. The risk for classic KS, which occurs without HIV infection, is significantly increased in men, individuals, and nonsmokers [3, 4].

The gene encoding human leukocyte antigen (*HLA*) and related genes are centrally involved in the immunological response to infectious diseases and thus would be expected to affect the risk of developing KSHV infection or KS. Only tenuous support for this hypothesis has been reported. In a meta-analysis of 12 publications from 1981–1994, Ioannidis et al concluded that the risk of AIDS-associated KS was significantly decreased with *HLA-DR3* and significantly increased with *HLA-B*35* and *Cw4*, 2 loci that are in strong linkage disequilibrium [6]. They noted, however, that these studies had serious deficiencies, ranging from exceedingly small sample size to uncontrolled confounding by strong *HLA* associations with AIDS, especially for *HLA-B*35*. A 2005 analysis in the Multicenter AIDS Cohort Study, which matched 147 AIDS-associated KS cases to 147 HIV-seropositive KSHV-seropositive controls, only found associations with *HLA-B*27*, which is also associated with slower HIV progression, and with 1 *HLA* class II haplotype (*DRB1*13:02-DQB1*06:04*) [7]. Unfortunately, that latter study included no KSHV-seronegative controls and did not examine *HLA-A* or *HLA-C* alleles. Previous studies of *HLA* associations with classic KS and with non–AIDS-associated KS

Received 5 June 2015; accepted 3 August 2015; published online 12 August 2015.

[a]J. J. G. and M. P. M contributed equally to this work.

Correspondence: J. J. Goedert, 9609 Medical Center Dr, Rm 6E106, MSC 9767, Bethesda, MD 20892-9704 (goedertj@mail.nih.gov).

The Journal of Infectious Diseases® 2016;213:432–8

DOI: 10.1093/infdis/jiv413

in Africa have been predominantly null [8–14], but they have been seriously underpowered, with only 62 cases in the largest study.

Because the innate immune system plays a key role in the response to viral infections and cancer, our understanding of KSHV disease pathogenesis may be advanced by considering interactions between HLA and killer cell immunoglobulin-like receptor (KIR) ligand pairs. Positioned on the surface of natural killer (NK) cells, KIRs are a polymorphic family of receptors that regulate NK-mediated cytotoxicity when ligated to requisite HLA class I molecules. Three studies have reported significant associations of *KIR3DS1* (activating), *KIR3DL1* (inhibitory), or *HLA-C group* alleles for cervical cancer or precancer, which results from persistent infection with oncogenic types of human papillomavirus (HPV) [15–17]. More recently, Guerini et al studied *KIR* and *HLA* class I ligand pairs in 32 northern Italian persons with classic KS and 51 controls, of whom 18 were KSHV seropositive [18]. They found that activating *KIR/HLA* genotypes, specifically *KIR3DS1*, *KIR2DS1*, and *KIR2DS1+HLA-C group 2*, were significantly more frequent in persons with classic KS [18].

To further test the hypothesis that *HLA* and *KIR* influence the risk of KSHV seropositivity or KS, we compared *HLA* class I and *KIR/HLA* ligand frequencies in a 2-phase study that included a 250 persons with classic KS, 280 KSHV-seropositive controls, and 576 KSHV-seronegative controls in Italy.

METHODS

Subjects

As described previously in depth [3, 4], individuals with histologically confirmed classic KS who were seronegative for HIV and had no history of transplantation were recruited in Italy from the provinces of Lazio (including Rome), Campania (including Naples), and the entire island of Sicily. Contemporaneous controls with a similar age and sex distribution were recruited from the rosters of primary care physicians in the same geographic areas. KSHV seropositivity was determined by an immunofluorescence assay (IFA), performed at a 1:120 dilution with uninduced BCBL-1 cells, plus an enzyme immunoassay with recombinant K8.1 structural glycoprotein at a 1:20 plasma dilution. Subjects were considered KSHV seropositive if they had uninduced IFA positivity or a K8.1 optical density of >1.2. KSHV-seronegative individuals had uninduced IFA negativity plus a K8.1 optical density of ≤1.2. Twenty-four controls with missing or ambiguous KSHV serologic findings were included in the current study for comparisons of persons with classic KS to all controls. The current analysis was composed of discovery (phase 1) and validation (phase 2) cohorts (Table 1).

HLA Class I Genotyping

High-resolution genotyping for *HLA* class I loci was performed by polymerase chain reaction–sequence-based typing, as recommended by the 13th International Histocompatibility Workshop (available at: http://www.ihwg.org/tmanual/TMcon tents.htm). *HLA* sequences were analyzed using the ASSIGN software (Conexio Genomics).

KIR Genotyping

KIR genotyping for the presence or absence of each *KIR* gene was conducted by polymerase chain reaction with sequence-specific priming as described previously [19], with some modifications. PCR was conducted using SYBR Green Master Mix with Platinum Taq (Life Technologies). The presence and absence of specific PCR products was detected by melting curve analysis on the 7900 Real-Time PCR System (Applied Biosystems).

Statistical Analyses

First, to assess associations with KSHV seroprevalence, KSHV-seropositive controls and KSHV-seronegative controls were compared on their *HLA* allele, *KIR* gene, and *KIR* ligand frequencies. Second, to assess associations with disease, frequencies of these genotypes among individuals with classic KS were compared to those among controls. Patients with classic KS were compared to controls, with and without stratification on KSHV serostatus. The frequency of the inhibitory *KIR2DL1* and *KIR2DL2/3* alleles (*KIR2DL2* and *KIR2DL3* are alleles of the same locus, and the gene that encodes these receptors is present on virtually all haplotypes), which interact with HLA-*C* group 2 and -*C* group 1 alleles, respectively, is nearly 100%. Thus, analysis was restricted to assessment of variation in their ligand frequency.

Table 1. Characteristics of the Study Population

Characteristic	Discovery Phase 1				Validation Phase 2		
	Classic KS Cases, No. (%)	Controls, No. (%)			Classic KS Cases, No. (%)	Controls, No. (%)	
		KSHV Positive	KSHV Negative	Other[a]		KSHV Positive	KSHV Negative
Sex							
Male	90 (70)	134 (66)	242 (73)	18 (75)	75 (62)	54 (70)	180 (74)
Female	39 (30)	69 (34)	91 (27)	6 (25)	46 (38)	23 (30)	63 (26)
Age, y, mean (range)	73 (29–93)	74 (46–91)	71 (32–92)	71 (35–90)	75 (28–94)	74 (39–90)	71 (32–91)
Total	129	203	333	24	121	77	243

Abbreviations: KS, Kaposi sarcoma; KSHV, Kaposi sarcoma–associated herpesvirus.

[a] Data are for controls with missing or ambiguous KSHV serologic findings.

Unlike several of the inhibitory *KIRs*, activating *KIRs* are present on only a fraction of *KIR* haplotypes, and therefore activating *KIR* with known or putative HLA ligands were interrogated together. For all analyses, logistic regression was used to estimate odds ratios (ORs), adjusted for age and sex, and corresponding 95% confidence intervals (CIs). The significant associations identified in phase 1 were tested in phase 2, followed by analysis of both phases combined.

RESULTS

HLA alleles and *KIR* genes were successfully determined in 1130 participants, including 250 persons with classic KS, 280 KSHV-seropositive controls, 576 KSHV-seronegative controls, and 24 KSHV-seroindeterminant controls (Table 1). There were 793 men and 337 women, with a mean age of 72 years (range, 28–94 years). Case and control groups did not differ by phase of the study, although phase 1 was larger than phase 2 (689 vs 441).

KSHV Seroprevalence

Among the controls, we compared KSHV-seropositive individuals to KSHV-seronegative individuals for possible associations of KSHV seroprevalence with each *HLA-A, HLA-B,* and *HLA-C* allele and *KIR* gene in phase 1. Four (*HLA-A*30:02*, *HLA-B*15:01*, and *HLA-B*35:02* and *KIR2DL3*) were nominally associated with seroprevalence, but none of these findings were replicated in phase 2 (Supplementary Table 1). Based on this, the KSHV-seropositive and KSHV-seronegative control groups were pooled for comparison to persons with classic KS with regard to single *HLA* alleles and *KIR* genes.

Risk of Classic KS

Two *HLA* alleles were reproducibly associated with classic KS, compared with all controls in phase 1 and 2 (Table 2). Persons with classic KS had a reduced frequency of *A*11:01* in phase 1 (adjusted OR, 0.4; 95% CI, .2–.9), which was replicated in phase 2 (adjusted OR, 0.4; 95% CI, .2–.8). Conversely, there was a significantly increased risk of classic KS for participants carrying *C*07:01* in phase 1 (adjusted OR, 1.5; 95% CI, 1.0–2.2), which was replicated in phase 2 (adjusted OR, 1.7; 95% CI, 1.1–2.6). None of the other *HLA* alleles and none of the *KIR* genes was reproducibly associated with classic KS (see Supplementary Tables 2–6 for results of analyses performed in phase 1).

KIR Ligands With KSHV Seroprevalence

In contrast to single *HLA* alleles and *KIR* genes, joint classification of participants according to their *HLA-B* and *-C KIR* ligand motifs revealed associations with KSHV seroprevalence and classic KS (Table 3 and Figure 1). With the compound genotype *HLA-B Bw4-80I* plus *KIR3DS1*, KSHV seroprevalence was significantly reduced in phase 1 (adjusted OR, 0.6; 95% CI, .3–.9), with an identical point estimate but nonsignificant 95% CI in phase 2 (adjusted OR, 0.6; 95% CI, .3–1.2). With both phases

Table 2. Association Between *HLA* Genotype and Classic Kaposi Sarcoma (KS)

HLA Genotype	Discovery Phase 1				Validation Phase 2				Combined			
	Classic KS Cases, No. (%)	Controls,[a] No. (%)	aOR (95% CI)	*P* Value	Classic KS Cases, No. (%)	Controls,[a] No. (%)	aOR (95% CI)	*P* Value	Classic KS Cases, No. (%)	Controls,[a] No. (%)	aOR (95% CI)	*P* Value
Comparison 1												
*A*11:01*	6 (4.7)	63 (11.6)	0.4 (.2–.9)	.02	7 (5.9)	39 (12.5)	0.4 (.2–.8)	.02	13 (5.2)	102 (11.9)	0.4 (.2–.7)	.002
Others	123 (95.3)	479 (88.4)	. . .		112 (94.1)	274 (87.5)	. . .		235 (94.8)	753 (88.1)	. . .	
Comparison 2												
*C*07:01*	55 (42.6)	175 (32.9)	1.5 (1.0–2.2)	.04	55 (45.5)	107 (34.1)	1.7 (1.1–2.6)	.02	110 (44)	282 (33.3)	1.6 (1.2–2.1)	.002
Others	74 (57.4)	357 (67.1)	. . .		66 (54.5)	207 (65.9)	. . .		140 (56.0)	564 (66.7)	. . .	

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; KSHV, Kaposi sarcoma–associated herpesvirus.

[a] Data are for controls positive for KSHV, negative for KSHV, or missing/ambiguous data on KSHV status combined.

Table 3. Opposing Effects of HLA-C Group and KIR3DS1+HLA-B Bw4 80I on the Risk of Kaposi Sarcoma (KS)–Associated Herpesvirus (KSHV) Seroprevalence and Classic KS

Genotype	KSHV-Positive Controls, No. (%)	KSHV-Negative Controls, No. (%)	aOR (95% CI)	*P* Value	Classic KS Cases, No. (%)	KSHV-Positive Controls, No. (%)	aOR (95% CI)	*P* Value
Discovery phase 1								
Comparison 1								
3DS1+HLA-B Bw4 80I	24 (12.0)	64 (19.7)	0.6 (.3–.9)	.03	29 (22.5)	24 (12.0)	2.1 (1.2–3.8)	.02
All others	176 (88.0)	261 (80.3)	. . .		100 (77.5)	176 (88.0)		
Comparison 2								
C1/C1	45 (22.8)	83 (26.5)	0.7 (.4–1.1)	NS	46 (35.9)	45 (22.8)	1.8 (1.1–3.1)	.03[a]
C2+	152 (77.2)	230 (73.5)	. . .		82 (64.1)	152 (77.2)		
Comparison 3								
C1/C1 (–C*07:01)	15 (11.9)	34 (15.7)	0.7 (.4–1.4)	NS	22 (29.7)	15 (11.9)	3.1 (1.5–6.5)	.002
C2+ (–C*07:01)	111 (88.1)	183 (84.3)	. . .		52 (70.3)	111 (88.1)		
Validation phase 2								
Comparison 1								
3DS1+HLA-B Bw4 80I	9 (11.7)	48 (20.2)	0.6 (.3–1.2)	NS	26 (21.9)	9 (11.7)	2.1 (.9–4.8)	NS
All others	68 (88.3)	189 (79.8)	. . .		93 (78.1)	68 (88.3)		
Comparison 2								
C1/C1	15 (20.0)	80 (33.9)	0.5 (.3–1.0)	0.04	42 (35.3)	15 (20.0)	1.9 (1.0–4.0)	NS[a]
C2+	60 (80.0)	156 (66.1)	. . .		77 (64.7)	60 (80.0)		
Comparison 3								
C1/C1 (–C*07:01)	6 (11.5)	38 (24.5)	0.4 (.2–1.0)	.05	15 (22.7)	6 (11.5)	2.3 (.8–6.3)	NS
C2+ (–C*07:01)	46 (88.5)	117 (75.5)	. . .		51 (77.3)	46 (88.5)		
Combined								
Comparison 1								
3DS1+HLA-B Bw4 80I	33 (11.9)	112 (19.9)	0.6 (.4–.9)	.01	55 (22.2)	33 (11.9)	2.1 (1.3–3.4)	.002
All others	244 (88.1)	450 (80.1)	. . .		193 (77.8)	244 (88.1)		
Comparison 2								
C1/C1 vs	60 (22.1)	163 (29.7)	0.6 (.4–.9)	.01	88 (35.6)	60 (22.1)	1.8 (1.2–2.7)	.005[a]
C2+	212 (77.9)	386 (70.3)	. . .		159 (64.4)	212 (77.9)		
Comparison 3								
C1/C1 (–C*07:01)	21 (11.8)	72 (19.3)	0.6 (.3–.9)	.03	37 (26.4)	21 (11.8)	2.7 (1.5–4.9)	.001
C2+ (–C*07:01)	157 (88.2)	300 (80.6)	. . .		103 (73.6)	157 (88.2)		

C1 denotes HLA-C*01, HLA-C*03, HLA-C*07, and HLA-C*08, and C2 denotes HLA-C*02, HLA-C*04, HLA-C*05, and HLA-C*06 [20, 21]. HLA-B Bw4 80I denotes HLA-B*27:02, HLA-B*38:01, HLA-B*49:01, HLA-B*51, HLA-B*57, and HLA-B*58 [22, 23].

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; NS, not significant.

[a] Adjusted for HLA-C*07:01.

combined, the KSHV seroprevalence was reduced with *HLA-B Bw4-80I* plus *KIR3DS1* (adjusted OR, 0.6; 95% CI, .4–.9).

Similarly, homozygosity for *HLA-C group 1* (the ligand for *KIR2DL2/3*) was associated with reduced seroprevalence, which was nonsignificant in phase 1 (adjusted OR, 0.7; 95% CI, .4–1.1), but reached significance in phase 2 (adjusted OR, 0.5; 95% CI, .3–1.0). With both phases combined, *HLA-C* group 1 homozygosity was associated with a lower KSHV seroprevalence (adjusted OR, 0.6; 95% CI, .4–.9). Excluding *C*07:01* (a constituent of *C group 1*) had little effect (Table 3).

KIR Ligands With Classic KS

Because the risk of KSHV seroprevalence was associated with *KIR* ligands, persons with classic KS were compared only to the KSHV-seropositive controls, thereby evaluating the risk of classic KS conditional on KSHV infection. In this analysis, classic KS was significantly increased with the compound genotype *HLA-B Bw4-80I* plus *KIR3DS1* in phase 1 (adjusted OR, 2.1; 95% CI, 1.2–3.8), with an identical point estimate but nonsignificant CI in phase 2 (adjusted OR, 2.1; 95% CI, .9–4.8). With both phases combined, classic KS was increased with *HLA-B Bw4-80I* plus *KIR3DS1* (adjusted OR, 2.1; 95% CI, 1.3–3.4). These associations suggest that this compound genotype confers protection against KSHV infection but that, among those who do become infected, there is an increased risk of developing classic KS.

A similar pattern was seen with *HLA-C group 1* homozygosity, for which the risk of classic KS was significantly increased in phase 1 (adjusted OR, 1.8; 95% CI, 1.1–3.1), phase 2 (adjusted OR, 1.9; 95% CI, 1.0–4.0), and both phases combined (adjusted OR, 1.8; 95% CI, 1.2–2.7). The risk of classic KS with *C group 1* homozygosity was higher with exclusion of *C*07:01* (adjusted OR, 2.7; 95% CI, 1.5–4.9; Table 3), an allele that is a constituent of *C group 1* and that itself was associated with classic KS

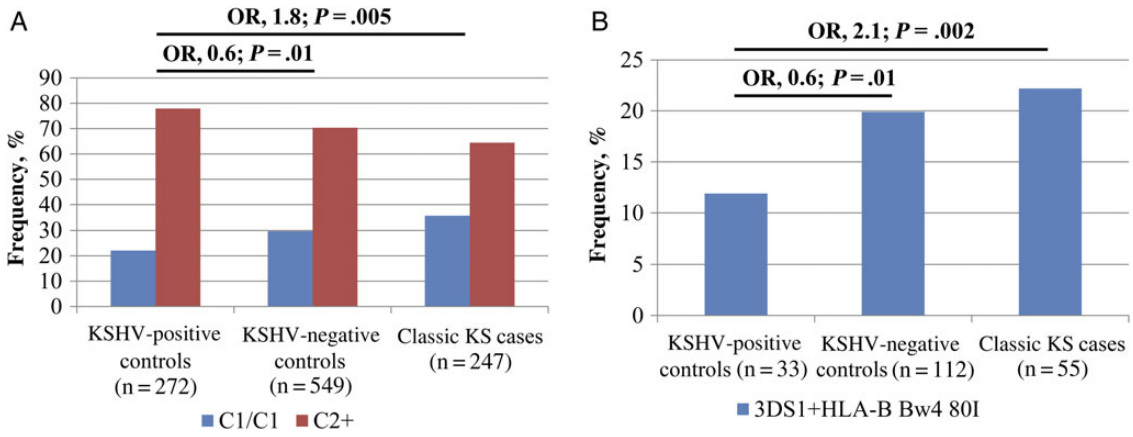

Figure 1. Opposing effects of *HLA-C* group 1 (*A*) and *KIR3DS1/Bw4-80I* (*B*) on the risk of Kaposi sarcoma (KS)–associated herpesvirus (KSHV) infection and classic KS after KSHV infection. *A*, HLA-C group 1 alleles, which serve as ligands for the inhibitory KIR2DL2/3, were significantly associated with protection against KSHV infection. This KIR/HLA combination has a relatively weak natural killer (NK) cell inhibitory potential relative to KIR2DL1 in the presence of its HLA-C group 2 ligand, which is strongly inhibitory. On the other hand, C group 1 alleles were associated with an increased risk of classic KS among KSHV infected subjects. *B*, The combination of the activating KIR3DS1 with HLA-B Bw4-80I was protective against infection but associated with an increased risk of classic KS. Abbreviation: OR, odds ratio.

(Table 2). This pattern of a given genotype protecting against infection but increasing the risk of disease among those who do become infected was thus consistent for *HLA-C group 1* homozygosity and *HLA-B Bw4-80I* plus *KIR3DS1*.

DISCUSSION

This is the first comprehensive study to assess the role of *HLA* and *KIR* on the risk of KSHV seroprevalence and classic KS. There were several major findings. First, we observed significant opposing effects of activating *KIR/HLA* combinations on seroprevalence and the risk of classic KS. Second, seroprevalence had no consistently significant association with any individual *HLA* allele or *KIR* gene. Third, in contrast to seroprevalence, the risk of classic KS was significantly and reproducibly associated with 2 *HLA* alleles when compared to combined control groups.

In both phases of our study, the risk of classic KS was significantly reduced in people with *A*11:01*, and it was increased for those with *C*07:01*. Whether or not *A*11:01* and *C*07:01* have similar associations in African KS remains to be determined. African KS differs from the Mediterranean form in terms of epidemiologic characteristics and clinical outcomes, and there are significant differences in *HLA* allele frequencies in these populations. Thus, it is unlikely that this will be the case. Intriguingly, *A*11:01* is well known to be associated with decreased risk for nasopharyngeal carcinoma [24–26]. Nasopharyngeal carcinoma is strongly associated with Epstein-Barr virus infection, which, like KSHV, is a γ-herpes virus. This implies that *A*11:01* may present herpesvirus-related antigenic epitopes to cytotoxic T lymphocytes, resulting in effective control of the virus in the lytic phase. Functional cytotoxic T-lymphocyte assays to confirm this will need to be performed. There is clinical evidence to suggest that lytic KSHV replication is important for progression to KS. In a clinical trial [27], patients with AIDS and cytomegalovirus retinitis were randomly assigned to one of 3 treatment arms: intravenous ganciclovir alone, oral plus ocular-implant ganciclovir, or placebo plus ocular-implant ganciclovir. Compared with oral placebo, the risk of AIDS-associated KS was reduced 75% with oral ganciclovir and 93% with intravenous ganciclovir [27], highly significant effects that the authors attribute to the blockage of lytic KSHV replication by ganciclovir.

The significant opposing effects of activating *KIR/HLA* combinations on seroprevalence versus classic KS merit special attention. *HLA-C group 1* alleles, which serve as ligands for the inhibitory *KIR2DL2/3*, were significantly associated with protection against KSHV seroprevalence. This *KIR/HLA* combination has a relatively weak NK cell inhibitory potential relative to that of *KIR2DL1* in the presence of its *HLA-C group 2* ligand [28], which is strongly inhibitory. On the other hand, *C group 1* alleles were associated with an increased risk of classic KS among KSHV-infected subjects. Likewise, the combination of the activating *KIR3DS1* with *HLA-B Bw4-80I* was protective against seroprevalence but associated with an increased risk of classic KS (Table 3 and Figure 1). Our findings corroborate the classic KS association with activating *KIR* reported by Guerini et al [18] and add the novel association with KSHV seroprevalence. We hypothesize that strong NK cell activation protects against seropositivity but is a risk factor for classic KS after KSHV infection (Figure 2), perhaps because of the known association of KS with inflammation [29]. These findings expand previously reported and remarkably similar *HLA-KIR* associations with HPV-induced cervical neoplasia [16, 17] and with Epstein-Barr virus–related nasopharyngeal carcinoma [30], in which the risk of the neoplasm was increased in the presence of activating *KIR/HLA* combinations.

Figure 2. Model of the hypothetical effects of natural killer (NK) cell activation on Kaposi sarcoma (KS)–associated herpesvirus (KSHV) infection and the risk of KS. NK cell–activating genotypes (left) protect against KSHV infection, but once infected these same genotypes enhance inflammation and development of KS (right).

The modulation of NK cell–mediated inflammation by *KIR* may be beneficial against infectious agents but detrimental in controlling neoplasia. More specifically, emerging data suggest that *KIR* may help to control certain viruses, including HIV and hepatitis C virus, while also increasing the risk for some inflammation-mediated conditions, including cancers [31–33]. A key aspect of KS is its association with inflammation. Indeed, it is well known that there is a propensity for the development of KS lesions at local sites of inflammation, such as herpes zoster skin lesions [34] and foci of trauma (Koebner phenomenon) [35]. Furthermore, several viral genes encode proteins that contribute to a proinflammatory environment, including viral interleukin 6, viral CC chemokine homologs, and viral interferon regulatory factor [36]. Matthews et al have recently demonstrated that NK cells activated by cytokines successfully kill KSHV-infected fibroblasts [37]. However, multiple perturbations of systemic immunity are a hallmark of KS, and KS is exacerbated by administration of exogenous interferon γ [38, 39]. The role of NK cells and *KIR* in the development of KS has not been studied in depth. Further functional studies are warranted to clarify the findings described herein and to understand their role in the development of KS.

The strengths of this study include analysis of a well-defined population [4, 40], a 2-phase design to validate associations, stratified assessment of KSHV serostatus and classic KS disease, state-of-the-art *HLA* and *KIR* genotyping, avoidance of confounding by HIV, and substantial size (13-fold larger than the only prior study of *HLA-KIR* and classic KS [18]). In summary, this study uncovered reproducible associations of *HLA-A*11:01* and *HLA-C*07:01* with the risk of classic KS. It also uncovered countervailing *HLA-KIR* ligand associations with KSHV serostatus and classic KS. These findings suggest that KIR-mediated NK cytotoxicity may retard initial KSHV infection. After infection, however, it may, along with other factors, enhance KSHV dissemination to lymphatic endothelial cells and progression to KS.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

Acknowledgments. We thank the many colleagues who contributed to this project and, especially, the study participants.

Disclaimer. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

Financial support. This work was supported by the National Cancer Intramural Research Program (ZIA CP 010214) and the Frederick National Laboratory for Cancer Research (contract HHSN261200800001E).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science **1994**; 266:1865–9.
2. Minhas V, Wood C. Epidemiology and transmission of Kaposi's sarcoma-associated herpesvirus. Viruses **2014**; 6:4178–94.
3. Anderson LA, Lauria C, Romano N, et al. Risk factors for classical Kaposi sarcoma in a population-based case-control study in Sicily. Cancer Epidemiol Biomarkers Prev **2008**; 17:3435–43.
4. Goedert JJ, Vitale F, Lauria C, et al. Risk factors for classical Kaposi's sarcoma. J. Natl.Cancer Inst **2002**; 94:1712–8.
5. Uldrick TS, Whitby D. Update on KSHV epidemiology, Kaposi Sarcoma pathogenesis, and treatment of Kaposi Sarcoma. Cancer Lett **2011**; 305:150–62.
6. Ioannidis JP, Skolnik PR, Chalmers TC, Lau J. Human leukocyte antigen associations of epidemic Kaposi's sarcoma. AIDS **1995**; 9:649–51.
7. Dorak MT, Yee LJ, Tang J, et al. HLA-B, -DRB1/3/4/5, and -DQB1 gene polymorphisms in human immunodeficiency virus-related Kaposi's sarcoma. J Med Virol **2005**; 76:302–10.
8. Masala MV, Carcassi C, Cottoni F, Mulargia M, Contu L, Cerimele D. Classic Kaposi's sarcoma in Sardinia: HLA positive and negative associations. Int J Dermatol **2005**; 44:743–5.
9. Guttman-Yassky E, Cohen A, Kra-Oz Z, et al. Familial clustering of classic Kaposi sarcoma. J Infect Dis **2004**; 189:2023–6.
10. Strichman-Almashanu L, Weltfriend S, Gideoni O, Friedman-Birnbaum R, Pollack S. No significant association between HLA antigens and classic Kaposi sarcoma: molecular analysis of 49 Jewish patients. J Clin Immunol **1995**; 15:205–9.
11. Kaloterakis A, Papasteriades C, Filiotou A, Economidou J, Hadjiyannis S, Stratigos J. HLA in familial and nonfamilial Mediterranean Kaposi's sarcoma in Greece. Tissue Antigens **1995**; 45:117–9.
12. Papasteriades C, Kaloterakis A, Filiotou A, et al. Histocompatibility antigens HLA-A, -B, -DR in Greek patients with Kaposi's sarcoma. Tissue Antigens **1984**; 24:313–5.
13. Lemarchand-Venencie F, Lepace V, Belaich S, et al. [HLA typing in classical and African Kaposi's disease]. Pathol Biol (Paris) **1986**; 34:761–4.
14. Melbye M, Kestens L, Biggar RJ, Schreuder GM, Gigase PL. HLA studies of endemic African Kaposi's sarcoma patients and matched controls: no association with HLA-DR5. Int J Cancer **1987**; 39:182–4.
15. Arnheim L, Dillner J, Sanjeevi CB. A population-based cohort study of KIR genes and genotypes in relation to cervical intraepithelial neoplasia. Tissue Antigens **2005**; 65:252–9.
16. Carrington M, Wang S, Martin MP, et al. Hierarchy of resistance to cervical neoplasia mediated by combinations of killer immunoglobulin-like receptor and human leukocyte antigen loci. J Exp Med **2005**; 201:1069–75.
17. Martin MP, Borecki IB, Zhang Z, et al. HLA-Cw group 1 ligands for KIR increase susceptibility to invasive cervical cancer. Immunogenetics **2010**; 62:761–5.
18. Guerini FR, Mancuso R, Agostini S, et al. Activating KIR/HLA complexes in classic Kaposi's Sarcoma. Infect Agent Cancer **2012**; 7:9.
19. Martin MP, Carrington M. KIR locus polymorphisms: genotyping and disease association analysis. Methods Mol Biol **2008**; 415:49–64.
20. Biassoni R, Falco M, Cambiaggi A, et al. Amino acid substitutions can influence the natural killer (NK)-mediated recognition of HLA-C molecules. Role of serine-77 and lysine-80 in the target cell protection from lysis mediated by "group 2" or "group 1" NK clones. J Exp Med **1995**; 182:605–9.
21. Colonna M, Borsellino G, Falco M, Ferrara GB, Strominger JL. HLA-C is the inhibitory ligand that determines dominant resistance to lysis by NK1- and NK2-specific natural killer cells. Proc Natl Acad Sci U S A **1993**; 90:12000–4.
22. Cella M, Longo A, Ferrara GB, Strominger JL, Colonna M. NK3-specific natural killer cells are selectively inhibited by Bw4- positive HLA alleles with isoleucine 80. J Exp Med **1994**; 180:1235–42.

23. Gumperz JE, Litwin V, Phillips JH, Lanier LL, Parham P. The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor. J Exp Med **1995**; 181:1133–44.
24. Burt RD, Vaughan TL, McKnight B, et al. Associations between human leukocyte antigen type and nasopharyngeal carcinoma in Caucasians in the United States. Cancer Epidemiol Biomarkers Prev **1996**; 5:879–87.
25. Hildesheim A, Apple RJ, Chen CJ, et al. Association of HLA class I and II alleles and extended haplotypes with nasopharyngeal carcinoma in Taiwan. J Natl Cancer Inst **2002**; 94:1780–9.
26. Goldsmith DB, West TM, Morton R. HLA associations with nasopharyngeal carcinoma in Southern Chinese: a meta-analysis. Clin Otolaryngol Allied Sci **2002**; 27:61–7.
27. Martin DF, Kuppermann BD, Wolitz RA, Palestine AG, Li H, Robinson CA. Oral ganciclovir for patients with cytomegalovirus retinitis treated with a ganciclovir implant. Roche Ganciclovir Study Group. N Engl J Med **1999**; 340: 1063–70.
28. Winter CC, Gumperz JE, Parham P, Long EO, Wagtmann N. Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. J Immunol **1998**; 161:571–7.
29. Ganem D. KSHV and the pathogenesis of Kaposi sarcoma: listening to human biology and medicine. J Clin Invest **2010**; 120:939–49.
30. Butsch Kovacic M, Martin M, Gao X, et al. Variation of the killer cell immunoglobulin-like receptors and HLA-C genes in nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev **2005**; 14(11 Pt 1):2673–7.
31. Seich Al Basatena NK, Macnamara A, Vine AM, et al. KIR2DL2 enhances protective and detrimental HLA class I-mediated immunity in chronic viral infection. PLoS Pathog **2011**; 7:e1002270.
32. Kulkarni S, Martin MP, Carrington M. The Yin and Yang of HLA and KIR in human disease. Semin Immunol **2008**; 20:343–52.
33. Martin MP, Carrington M. Immunogenetics of HIV disease. Immunol Rev **2013**; 254:245–64.
34. Niedt GW, Prioleau PG. Kaposi's sarcoma occurring in a dermatome previously involved by herpes zoster. J Am Acad Dermatol **1988**; 18(2 Pt 2):448–51.
35. French PD, Harris JR, Mercey DE. The Koebner phenomenon and AIDS-related Kaposi's sarcoma. Br J Dermatol **1994**; 131:746–7.
36. Moore PS, Boshoff C, Weiss RA, Chang Y. Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. Science **1996**; 274:1739–44.
37. Matthews NC, Goodier MR, Robey RC, Bower M, Gotch FM. Killing of Kaposi's sarcoma-associated herpesvirus-infected fibroblasts during latent infection by activated natural killer cells. Eur J Immunol **2011**; 41:1958–68.
38. Ganser A, Brucher W, Brodt HR, et al. Treatment of AIDS-related Kaposi's sarcoma with recombinant gamma-interferon. Onkologie **1986**; 9:163–6.
39. Krigel RL, Odajnyk CM, Laubenstein LJ, et al. Therapeutic trial of interferon-gamma in patients with epidemic Kaposi's sarcoma. J Biol Response Mod **1985**; 4:358–64.
40. Anderson LA, Lauria C, Romano N, et al. Risk factors for classical Kaposi sarcoma in a population-based case-control study in Sicily. Cancer Epidemiol Biomarkers Prev **2008**; 17:3435–43.