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 Carrington2,4

1Division of Cancer Epidemiology and Genetics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, and 2Cancer and Inflammation Program, Laboratory of Experimental Immunology, and 3Viral Oncology Section, AIDS and Cancer Virus Program, Leidos Biomedical Research, Frederick National Laboratory for Cancer Research, Maryland; 4Ragon Institute of MGH, MIT, and Harvard, Cambridge, Massachusetts; 5Dipartimento di Igiene e Microbiologia Giuseppe D’Alessandro, Università degli Studi di Palermo, and 6Lega 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. KIR-mediated NK cell activation may decrease then risk of KSHV infection but enhance KSHV dissemination and progression to KS if infection occurs.

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
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 pre-cancer, 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/TMcontents.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**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Discovery Phase 1</th>
<th>Validation Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Classic KS Cases, No. (%)</td>
<td>KSHV Positive</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>90 (70)</td>
<td>134 (66)</td>
</tr>
<tr>
<td>Female</td>
<td>39 (30)</td>
<td>69 (34)</td>
</tr>
<tr>
<td>Age, y, mean (range)</td>
<td>73 (29–93)</td>
<td>74 (46–91)</td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
<td>203</td>
</tr>
</tbody>
</table>

Abbreviations: KS, Kaposi sarcoma; KSHV, Kaposi sarcoma-associated herpesvirus.
* 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, 0.2–0.9), which was replicated in phase 2 (adjusted OR, 0.4; 95% CI, 0.2–0.8). 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, 0.3–0.9), with an identical point estimate but nonsignificant 95% CI in phase 2 (adjusted OR, 0.6; 95% CI, 0.3–1.2). With both phases
combined, the KSHV seroprevalence was reduced with HLA-B Bw4-80I plus KIR3DS1 (adjusted OR, 0.6; 95% CI, 0.4–0.9).

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, 0.4–1.1), but reached significance in phase 2 (adjusted OR, 0.5; 95% CI, 0.3–0.9). With both phases combined, HLA-C group 1 homozygosity was associated with a lower KSHV seroprevalence (adjusted OR, 0.6; 95% CI, 0.4–0.9). 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, 0.9–4.8). 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.

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Discovery phase 1</th>
<th>Validation phase 2</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KSHV-Positive Controls, No. (%)</td>
<td>KSHV-Negative Controls, No. (%)</td>
<td>aOR (95% CI)</td>
</tr>
<tr>
<td>Comparison 1</td>
<td>3DS1+HLA-B Bw4 80I</td>
<td>24 (12.0)</td>
<td>64 (19.7)</td>
</tr>
<tr>
<td>All others</td>
<td>176 (88.0)</td>
<td>261 (80.3)</td>
<td>100 (77.5)</td>
</tr>
<tr>
<td>Comparison 2</td>
<td>C1/C1</td>
<td>45 (22.8)</td>
<td>83 (26.5)</td>
</tr>
<tr>
<td>C2+</td>
<td>152 (77.2)</td>
<td>230 (73.5)</td>
<td>82 (64.1)</td>
</tr>
<tr>
<td>Comparison 3</td>
<td>C1/C1 (−C*07:01)</td>
<td>15 (11.9)</td>
<td>34 (15.7)</td>
</tr>
<tr>
<td>C2+ (−C*07:01)</td>
<td>111 (88.1)</td>
<td>183 (84.3)</td>
<td>52 (70.3)</td>
</tr>
<tr>
<td>Combined</td>
<td>3DS1+HLA-B Bw4 80I</td>
<td>9 (11.7)</td>
<td>48 (20.2)</td>
</tr>
<tr>
<td>All others</td>
<td>68 (88.3)</td>
<td>189 (79.8)</td>
<td>93 (81.1)</td>
</tr>
<tr>
<td>Comparison 2</td>
<td>C1/C1</td>
<td>15 (20.0)</td>
<td>80 (33.9)</td>
</tr>
<tr>
<td>C2+</td>
<td>60 (80.0)</td>
<td>156 (66.1)</td>
<td>77 (64.7)</td>
</tr>
<tr>
<td>Comparison 3</td>
<td>C1/C1 (−C*07:01)</td>
<td>6 (11.5)</td>
<td>38 (21.5)</td>
</tr>
<tr>
<td>C2+ (−C*07:01)</td>
<td>46 (88.5)</td>
<td>117 (75.5)</td>
<td>51 (77.3)</td>
</tr>
</tbody>
</table>

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.

* Adjusted for HLA-C*07:01.
to suggest that lytic KSHV replication is important for
lytic phase. Functional cytotoxic T-lymphocyte assays to con-
lymphocytes, resulting in effective control of the virus in the
present herpesvirus-related antigenic epitopes to cytotoxic T
lymphocyte, is a
is strongly associated with Epstein-Barr virus infection, which,
deroung epidemiologic characteristics and clinical outcomes, and there are
signiﬁcantly reduced in people with
nasopharyngeal carcinoma [24]. Nasopharyngeal carcinoma
A*11:01 is well known to be associated with decreased risk for
nasopharyngeal carcinoma [25–26]. 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 con-
firm 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
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 attrib-
ute to the blockage of lytic KSHV replication by ganciclovir.

**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 sero-
prevalence 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 signiﬁ-
cantly 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 ep-
ideimiologic characteristics and clinical outcomes, and there are
signiﬁcant differences in HLA allele frequencies in these popu-
lations. 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. 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 con-
firm this will need to be performed. There is clinical evidence
to suggest that lytic KSHV replication is important for

**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.
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.

**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 HHSN26120080001E).

**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.

**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 copylefted and are the sole responsibility of the author, so questions or comments should be addressed to the author.

**References**