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The Interleukin-6^{-174} Promoter Polymorphism is Associated with a Risk of Development of Kaposi's Sarcoma in Renal Transplant Recipients

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Abstract. *Background: Kaposi's sarcoma (KS), an angioproliferative inflammation lesion, is frequently secondary to clinical immunosuppression such as after renal transplantation. KS growth is promoted by the inflammatory cytokine interleukin –6 (IL-6) and is also correlated with human herpesvirus - 8 (HHV-8) infection. Materials and Methods: In a sample of 15 renal transplant patients with KS and 40 patients without KS, we explored the influence of genetic differences in the production of IL-6 by promoter polymorphisms G-174C as well as the correlation with HHV-8 DNA. Results: The G allele homozygotes, which are associated with increased IL-6 production, had increased KS incidence (p=0.008). Therefore increased IL-6 production constitutes a risk factor which should be considered in clinical immunosuppression. Conclusion: In addition to the HHV-8 infection, the interleukin-6 promoter polymorphism G-174C is associated with a risk of development of KS in renal transplant recipients.*

Kaposi's sarcoma (KS) is a vascular proliferative inflammatory condition that is common in elderly Eastern European and Mediterranean males (1). KS is also found to occur in immunosuppressed individuals such as organ transplant recipients or patients infected with the human immunodeficiency virus (HIV) (2, 3). In the early stages of KS, inflammatory cytokines and growth factors promote its development (4, 5) while in its late stages a malignant, apparently monoclonal, phenotype can develop (6).

The exact pathogenesis of KS is still unknown, but DNA sequences from human herpesvirus 8 (HHV-8) are present in different clinical variants of KS (7). Nevertheless, HHV-8 appears necessary but not sufficient for development of KS (8), since many people infected with HHV-8 never develop lesions (9, 10). Immunocompromised individuals, such as people infected with HIV or transplant recipients, may develop KS and epidemiologic evidence suggests that additional environmental, hormonal and genetic cofactors contribute to KS pathogenesis (11, 12).

It is well known that inflammatory cytokines and growth factors promote the development of KS (13). Disturbances in the levels of inflammatory cytokines have been described following transplantation and contribute to the disruption of immune regulation (14, 15). In addition HHV-8 initiates and promotes the development of KS lesions through viral interleukin 6 (vIL-6), a cyclin and the vascular endothelial growth factor (VEGF) that it produces (16). It is possible that the induction of various cytokines due to the transplantation process may synergistically interact with the products of HHV-8 and account for the development and progression of KS (17). A recent study provides evidence that genetically determined differences in the production of human interleukin-6 (IL-6) could contribute to the risk of development of KS in HIV-infected men, independent of HHV-8 infection (13). Specifically, Foster *et al.* (13) observed that the IL-6 promoter polymorphism (G-174C) was associated with altered gene expression and that the homozygotes for the IL-6 G allele, which were associated with increased IL-6 production, were over-represented among patients with KS. Prompted by these observations and since KS is a common malignancy after renal transplantation in Greece (18), we studied whether the IL-6 genotype is associated with the risk of KS development in our renal transplant recipient population.

Materials and Methods

Patients. A total of 1055 renal graft recipients, who received organs between 1983 and 2001, were surveyed for the involvement of KS

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which was encountered in 18 cases (1.7%), 16 men and 2 women. We retrospectively analyzed 15 available samples from those renal transplant recipients who developed KS and 40 patients without KS. All 50 patients had received grafts in the same period and were under a similar immunosuppressive regimen. All the patients with KS had developed tumors over periods of time ranging from 6 to 102 months (mean ± standard deviation=24.7 ± 23.5; median=17; 25% percentile=9.5; 75% percentile=29 months) post transplantation. The frequency of HHV-8 DNA sequences was previously determined in this population; 14 out of 15 KS patients (93%) and 4 out of 40 patients without KS (10%) were positive for HHV8 (19).

IL-6 promoter genotyping. DNA was isolated from peripheral blood samples with the QIAamp Blood Kit (Qiagen, Germany). To confirm the integrity of DNA, a 430-bp sequence in the human GAPDH (glyceraldehyde-3-phosphatate dehydrogenase) gene was amplified (20). The IL-6 polymorphism at position -174, was analyzed by allele-specific polymerase chain reaction (PCR). Specifically we used primers framing a 347 bp region surrounding the G-174C allele to amplify genomic DNA isolated from patients and controls by PCR (sense, 5'-TTGTCAAGACATGCCAAGTGC-3'; antisense, 5'-CAGAATGAGCCTCAGAGACATCTCC-3'). In addition, each PCR reaction contained two additional primers designed to detect the G-174G allele (antisense, 5'-GCAATGACGTCCTTTAGCATCG-3') and another primer designed to detect the G-174C allele (sense, 5'-CCCCCTAGTTGTGTCTTGCCA-3'). We performed multiplex PCR with all four primers in one tube. PCR products were isolated on 2.5% agarose gels and visualized with ethidium bromide. The genotype was confirmed by DNA sequencing.

Individuals with the IL-6 -174 C/C genotype were considered low producers, and those with an IL-6 -174 G/C or G/G genotype were considered high producers (21).

Statistical analysis. The hypothesis of a differences in allele and genotype frequencies between patients with KS and renal transplant recipients without KS was tested with a x2 (3x2 table) for IL-6 – 174. For each genotype the odds ratio (OR), its 95% confidence intervals (CIs) and *p* values were obtained. *P* values are two-sided and significance is at $p<0.05$. For the calculations, GraphPad InStat (version 3.00) was used (GraphPad Software Inc., San Diego, CA, USA).

Results

The association of IL-6 G-174C polymorphism and the risk of development of KS after renal transplantation was determined in the renal transplant population with and without KS (Table I). Among the renal transplant recipients with KS, the distribution of the IL-6 genotypes was: GG in 10 patients (66.7%), GC in 4 patients (26.7%) and CC in 1 patient (6.7%). The distribution of genotypes in the KS patients differed significantly from that in the 40 renal transplant recipients without KS (GG in 11 [27.5%], GC in 22 [55%] and CC in 7 [17.5%]; $p=0.028$, $\chi^2=7.12$). The contribution of individual genotypes to the risk of KS was also determined. The IL-6 GG genotype, which is associated with increased production of IL-6, was over-represented among the renal transplant recipients with KS (66.7% *versus* 27.5%; $p=0.008$, $\chi^2=7.1$; OR=5.3 [95% CI, 1.5 to 18.9]). The CC genotype, associated with decreased IL-6 production, was similarly represented in patients with KS and in renal recipients without KS (6.7% *versus* 17.5%; $p=0.3$, $\chi^2=1.03$; OR=0.34 [95% CI, 0.04 to 3.0]).

The pathogenesis of KS in renal transplant recipients has been closely linked to infection with HHV-8. In an effort to validate the contribution of the IL-6 genotype to HHV-8 positivity, we compared genotype frequencies in our study group with HHV-8 infection (*i.e.*, documented HHV-8 DNA sequences detection by nested PCR - GG in 9, GC in 8, and CC in one), with frequencies in individuals without evidence of HHV-8 infection (*i.e.*, no HHV-8 DNA sequences detection by nested PCR – GG in 12, GC in 18, and CC in 7). The results showed a non-significant relationship between GG genotype and HHV-8 presence. The GG genotype was present in 50% (9 out of 18) of the HHV-8-infected subjects and in 32.4% (12 out of 37) of the HHV-8-negative ones ($p=0.21$). Similarly the CC genotype was observed in 5.5% (1 of 18) of HHV-8-positive subjects and in 18.9% (7 out of 37) of the HHV-8-negative ones ($p=0.18$) with also a non-significant difference.

The risk of development of KS after renal transplantation was then estimated in KS patients with HHV-8 infection (*i.e.*, with documentation plus those with KS). The distribution of IL-6 genotypes in the group with KS (GG in 9, GC in 4 and CC in one) was compared with the distribution in renal transplant recipients without KS who had HHV-8 infection (GG in none, GC in 4 and CC in none). The GG genotype was present in 64.3% (9 out of 14) of the patients with KS and HHV-8 infection but in none of the HHV-8-positive patients without KS ($p=0.023$).

Discussion

A previous study by Foster *et al.* (13) reported that KS is strongly associated with the -174 polymorphism in the promoter region of the human IL-6 gene in men infected with human immunodeficiency virus (HIV). Since disturbances in the levels of inflammatory cytokines have also been described following transplantation (14, 15), in the present study we wished to investigate the possible IL-6 genotype association with the risk of KS development in our renal transplant recipient population. In agreement with the study linked to HIV-related KS, our results showed that homozygotes for the IL-6-174 allele G, which is associated with increased IL-6 production, were found to be over-represented among renal transplant recipients with KS (22).

The IL-6 promoter polymorphism (G-174C) has been suggested to be both biologically and clinically important,

Table I. *Association between IL-6 G-174C polymorphism and Kaposi's sarcoma (KS) in renal transplant recipients.*

Genotype IL-6 -174	No. (%) of renal transplant recipients with KS n=15	No. (%) of renal transplant recipients without KS n=40	Evidence for association (*P* value) (χ^2 – 1d.f.)
GG	10 (66.7)	11 (27.5)	0.008 (7.1)
GC	4 (26.7)	22 (55)	0.061 (3.5)
CC	1 (6.7)	7 (17.5)	0.30 (1.0)

since compared with the G allele, the C allele has been associated with lower plasma levels of IL-6, reduced risk of development of Alzheimer's disease (23) and appears to protect against systemic onset of juvenile chronic arthritis (22). Nonetheless, our findings provide preliminary evidence that the IL-6 promoter polymorphism (G-174C) may contribute to the pathogenesis of KS. This is further suggested by the observation that HHV-8 encodes a viral homologue of the human IL-6 gene (24). It is of interest that the -174C allele of IL-6 is rarer in the African-Caribbean population (5%), where seroprevalence of HHV-8 is high during childhood, than in the North American white population (40.3%), where HHV-8 transmission occurs later in life, through sexual contact (4, 22). It is possible that heritable differences in the IL-6 gene modify the risk of acquiring HHV-8, but our findings cannot support this hypothesis. In our study we found that the GG genotype was present in 64.3% (9 out of 14) of the patients with KS and HHV-8 infection but in none of the HHV-8-positive patients without KS ($p=0.023$). Since there is a proportion of HHV-8-infected renal transplant recipients who have not developed KS, maybe the genetic background and the HHV-8 positivity act synergistically for the development of KS.

The present study had a limitation in that the number of patients was small and further validation with larger numbers of patients will be necessary to confirm our findings. However our small sample population was because, in the Greek population, only 1.7% of the transplantation patients develop KS.

In summary, the present study suggests that the genetic predisposition of patients as well as the presence of HHV-8 is an additional factor leading to KS. The IL-6 promoter polymorphism (G-174C), which determines differences in the production of human IL-6, could be related to the risk of development of KS in renal transplant recipients.

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