

5. Summary of Data Reported and Evaluation

5.1 Virus–host interactions

Kaposi's sarcoma herpesvirus/human herpesvirus 8 (KSHV/HHV8) is a gamma-2 herpesvirus (a rhadinovirus) with a 165-kb genome. Its closest relatives are *Herpesvirus saimiri* (HVS), a tumorigenic rhadinovirus of New World primates, and a group of recently identified rhadinoviruses in Old World monkeys. It contains blocks of conserved herpesvirus genes that encode mainly structural proteins. In addition, several genes similar in sequence to other viral and cellular oncogenes and growth controlling factors are present in the KSHV/HHV8 genome. These include homologues of interleukin 6, the antiapoptotic protein bcl-2, a D-type cyclin and a chemokine receptor, some of which are known to be functional.

KSHV/HHV8 has been found in B cells, macrophages and dendritic cells *in vivo*. It establishes a persistent infection in endothelial Kaposi's sarcoma spindle cells and in primary effusion lymphoma cells, which involves a disease-specific pattern of expression, with at least four (in Kaposi's sarcoma) or seven (in primary effusion lymphoma cells) viral genes. Lytic replication occurs in a subpopulation of infected spindle and haematopoietic cells.

KSHV/HHV8 DNA is readily detected in Kaposi's sarcoma lesions, primary effusion lymphoma cells and some lymphoid tissue from patients with multicentric Castleman's disease by Southern blotting or polymerase chain reaction (PCR). In contrast, only small amounts of viral DNA are generally present in non-neoplastic tissue from KSHV/HHV8-infected individuals, in particular in peripheral blood mononuclear cells and semen, requiring the use of sensitive PCR techniques for detection. Serological methods have been developed for the detection of antibodies to a latent nuclear protein and to defined and undefined structural antigens, including immunofluorescence assays, enzyme-linked immunosorbent assays and western blotting. Serological and PCR testing of peripheral blood mononuclear cells and semen shows that infection with KSHV/HHV8 is uncommon among the general populations of northern Europe and the United States, but more common in some Mediterranean countries and frequent in parts of Africa; however,

precise estimates of prevalence rates, especially in non-endemic areas, are still not available. There is some evidence that KSHV/HHV8 is sexually transmitted, but other routes of transmission are likely and probably account for a high prevalence in parts of southern Europe and Africa.

5.2 Human carcinogenicity

DNA analysis has consistently demonstrated the presence of KSHV/HHV8 at high (> 90%) rates in Kaposi's sarcoma lesions and at a generally low rate in neoplastic and non-neoplastic tissues from control patients. The load of viral DNA is higher in tissue from Kaposi's sarcomas than in unaffected tissues from the same patients. When mononuclear cells from Kaposi's sarcoma patients and controls were examined by PCR, KSHV/HHV8 was detected in significantly more cases (up to 50%) than controls. Despite differences in sensitivity, in specificity and in the antigens examined, all of the available serological studies are consistent in showing high rates of antibody-positivity in Kaposi's sarcoma patients and lower rates of seropositivity among various controls. Studies among HIV-1-positive and -negative populations at different risks for Kaposi's sarcoma indicate that seroprevalence is generally in accordance with the risk for developing the disease. The limited number of longitudinal analyses based on either detection of KSHV/HHV8 DNA by PCR or the presence of antibodies to KSHV/HHV8 suggest that KSHV/HHV8 infection precedes the development of Kaposi's sarcoma in the majority of cases.

Thus, the strength of association between infection with this virus and Kaposi's sarcoma is high, as measured by PCR, Southern blotting and serology, with odds ratio greater than 10 being found in most studies involving large numbers of cases and well-defined controls. This association is found in studies with various designs and for all epidemiological types of Kaposi's sarcoma.

Primary effusion lymphoma has been recognized as a new disease entity only since the identification of KSHV/HHV8. It has a characteristic morphology and cell surface phenotype, and all of the cases reported in the literature that showed these characteristics have been found to contain KSHV/HHV8 DNA, sometimes at high copy numbers. The vast majority of cases also contain clonal EBV. Owing to the rarity of this malignancy, no epidemiological studies are yet available.

Multicentric Castelman's disease is a rare and usually polyclonal lymphoproliferative disorder. In studies based on very few cases, KSHV/HHV8 has been found in a substantial proportion of HIV-positive patients with this disorder, and a high proportion of these patients also had Kaposi's sarcoma; a much smaller proportion of HIV-negative cases of multicentric Castleman's disease showed KSHV/HHV8 DNA.

KSHV/HHV8 has occasionally been reported to be present in other tumours, but the results are inconsistent. Whereas some of these discrepant results probably reflect the marked geographical differences in KSHV/HHV8 prevalence and the fact that the virus can be detected at several body sites and in samples from some KSHV/HHV8-infected but healthy individuals, other reports are more difficult to explain and remain controversial.

5.3 Animal models

KSHV/HHV8 has not yet been tested for tumorigenicity in experimental animals; however, studies of related viruses have proved informative.

Herpesvirus saimiri (HVS) and *Herpesvirus ateles* (HVA) do not induce disease in their natural hosts, the two New World monkeys, squirrel monkeys and spider monkeys, but they induce tumours and/or lymphoproliferation in a variety of heterologous non-human primates. The natural host populations become infected early in life, perhaps through horizontal transmission, and maintain the virus in latency throughout their lives. Both HVS and HVA are T-lymphotropic viruses and readily transform and immortalize human and simian T cells. As they are gamma-2 herpesviruses (rhadinoviruses), HVS and HVA are more closely related to KSHV/HHV8 than to EBV. Lymphoid cell lines derived either by transformation *in vitro* or from tumour tissues contain viral DNA, express viral proteins and release variable amounts of virus and viral genome copies per cell. HVS induces lymphoid tumours in New Zealand white rabbits.

There is evidence of gamma-2 herpesviruses in Old World monkeys, which may play a role in retroperitoneal fibromatosis, a condition with some similarities to Kaposi's sarcoma.

Bovine herpesvirus type 4 is another gamma-2 herpesvirus which includes a large number of antigenically related isolates distinct from other bovine herpesviruses. This virus has not been established as the etiological agent of a distinct disease entity, but its role in the etiology of some diseases of the genital tract has been suggested. It has never been identified as a potential cause of tumours.

Murid herpesvirus 4 is a B-lymphotropic gamma-2 herpesvirus that causes B-cell proliferation in mice. As it is a gamma-2 herpesvirus, it could serve as a model for KSHV/HHV8 infection.

5.4 Molecular mechanisms of carcinogenesis

The role of KSHV/HHV8 in the pathogenesis of Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman's disease is still poorly understood. The virus is present in the endothelial tumour (spindle) cells of Kaposi's sarcoma lesions and in primary effusion lymphoma cells. The latter are of monoclonal origin, and there is evidence to suggest that Kaposi's sarcoma lesions are also monoclonal. The viral homologue of D-type cyclins, which can disrupt cell cycle control, is expressed in both these tumour types, as are some other proteins of as yet unknown function. In the case of primary effusion lymphoma, several growth factors, a growth factor regulatory protein and a growth factor receptor are also expressed. Some KSHV/HHV8-infected Kaposi's sarcoma spindle cells undergo lytic replication. It is therefore at present unclear whether, as in EBV, a latent programme of gene expression is required for cellular transformation, with lytic infection of spindle cells representing an abortive pathway. Some viral genes whose expression can be upregulated during lytic infection (e.g. several growth factors and a growth factor regulatory protein) may contribute to virus-mediated expansion of Kaposi's sarcoma spindle cells. There is a striking correspondence between genes

encoded by KSHV/HHV8 and human genes involved in the control of cell growth, which are induced after EBV infection. This suggests that the two viruses may use different strategies to modify the same cellular regulatory and signalling pathways. Similar considerations apply to the role of KSHV/HHV8 in the pathogenesis of primary effusion lymphoma, the cells of which can also undergo lytic infection *in vitro*.

KSHV/HHV8 is not always found in multicentric Castleman's disease, especially in HIV-negative cases. There are no published data on which cell type in these lesions harbours KSHV/HHV8; however, KSHV/HHV8 probably plays an indirect role in this disorder, conceivably involving cytokines such as viral interleukin-6, since cellular interleukin-6 has been implicated in its pathogenesis.

5.5 Evaluation

There is compelling but as yet limited evidence for a role of KSHV/HHV8 in the causation of Kaposi's sarcoma.

KSHV/HHV8 is probably carcinogenic to humans (Group 2A).