In summary, a picture of PML glial cell pathobiology is gradually emerging in which the central motif seems to be cellular p53 inactivation caused by binding with JCV large T-antigen. Aneuploidy and overexpression of cyclins A and Bi and of cell proliferation antigens such as PCNA and Ki-67 are other salient features of glial cell reaction to JCV infection. Finally, formal demonstration of p53 T-protein complex formation by means of coimmunoprecipitation studies will lend further support to the above-delineated pathogenetic construct of JCV strategies for glial cell cycle deregulation.

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Human herpesvirus 8 and its diseases

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Based on the epidemiology and the clinical course of disease, Kaposi's sarcoma has long been suspected to be either caused by, or associated with, an infectious agent. A variety of viruses and bacteria have been implicated, including cytomegalovirus, the human immunodeficiency virus (HIV) and mycoplasma. In 1994, Chang and coworkers reported the presence of a herpesvirus-like DNA, detected by representational difference analysis in tissue of Kaposi's sarcoma (1). Subsequent work confirmed these findings and a new human herpesvirus, referred to as Kaposi's sarcoma-associated herpesvirus (KSHV), or human herpesvirus 8 (HHV8), was detected.

The virus

HHV8 belongs to the family of the herpesviruses and, based on the nucleotide sequence, is most closely related to herpesvirus saimiri, an animal virus causing lymphoproliferative disorders in new-world primates, and to the Epstein-Barr virus. HHV8 is the first representative of the subfamily of the ~y2-herpesvirgenus *Rhadinovirus* (2). The linear viral genome is approximately 165 kb and consists of a 140.5 kb coding region flanked by terminal repeats (3). Within the genome, two types of genes have been described: conserved gene regions similar to other herpesviruses that code for structural and replication-associated proteins. In addition, the HHV8 genome reveals regions unique to this virus, coding for a variety of gene homologues to human genes, including viral homologues for interleukin-6, *bcl-2,* a G-protein-coupled receptor, cyclin D, and macrophage inflammatory proteins. *In vitro* analysis revealed that many of these viral analogs show functional activity similar to that seen in the natural counterpart. In addition, virus-derived gene products, as well as the infection of primary human endothelial cells, have been associated with transforming activity. Most of these data, however, have been performed in permanently HHV8 infected lymphoma cell lines and little is known about the primary infection of endothelial cells.

Detection of human heroesvirus 8

In the original description of the herpesviruses-like DNA sequences of HHV8, a polymerase chain reaction (PCR)-based method, the representational difference analysis, has been used to detect the viral sequences. The vast majority of subsequent analyses of clinical material used POR to detect fragments of the viral DNA, either in fresh tissue samples or in formalin-fixed biopsy material (1, 4). In addition, to increase the sensitivity of the test, a nested PCR, i.e., two consecutive PCRs with an inner and an outer primer pair, have been performed. Although the tests can be performed by using appropriate controls, these assays are prone to produce false positive results. However, so far no other test achieves equal sensitivity. Although successful cultivation of the virus in cell cultures, using fresh Kaposis sarcoma tissue, has been reported, this technique is restricted to an experimental design.

PCR *in situ* hybridization has shown that HHV8 DNA is present in the spindle and vascular cells of Kaposi's sarcoma (5, 6). In addition, using the same technique, HHV8 DNA has been reported to be present in the epidermal cells overlying Kaposi's sarcoma tissue as well as in the pneumocytes of patients with the tumor. Using isotopic and nonradioisotopic colorimetric *in situ* hybridization with probes directed against the open reading frame (ORE) K12/T0.7 and the cyclin D viral homolog, a restricted viral gene expression in most tumor cells of Kaposi's sarcoma have been reported (7-9). Conflicting results have further been reported with respect to the presence of HHV8 RNA expression in various epithelial cells.

kann~i'~c ~IL,JIII0 ~1HU WWV$1

Since the original description of Kaposi's sarcoma in 1872, a variety of different clinical forms of this lesion have been described. The classic, or endemic form of Kaposi's sarcoma, originally found in older men of Eastern European or Mediterranean origin, usually shows an indolent clinical course. In contrast, the more recently described forms associated with prolonged immunosuppression, as seen in solid organ transplant recipients and especially in patients infected with HIV, show a more aggressive clinical behavior often disseminating and involving inner organs such as the lung or the gastrointestinal tract. Furthermore, additional variants of Kaposi's sarcoma in Africa have been described.

Histologically, all clinical forms of Kaposi's sarcoma show similar features: in the early or patchy stage, jagged and dilated vascular spaces with interstitial inflammatory cells and extravasated red blood cells can be seen. In the more characteristic plaque and finally nodular stage, the tumor is made up of plump spindle cells with irregular slit-like vascular spaces, aligned by a recognizable endothelium and filled with erythrocytes. Using PCR, usually in the nested form, HHV8 has been detected in 90-100% of Kaposi's sarcoma, including all clinical forms mentioned above. It can therefore be concluded that HHV8 DNA is present in all variants of the tumor and that the detection of HHV8 DNA can be used as a diagnostic test for this disease. Failure to detect HHV8 in a given lesion (and a positive internal control to confirm the presence of appropriate DNA) should shed doubts on the primary diagnosis. The detection of HHV8 can further be used as a diagnostic tool in clinical sped-

mens in which the diagnosis of Kaposi's sarcoma is difficult to achieve due to the small size of the biopsy and crash artifacts, as in biopsies of the lung and the gastrointestinal tract. In addition, it has been shown that the detection of HHV8 in the bronchoalveolar lavage has a high sensitivity and specificity for the diagnosis of Kaposi's sarcoma (10).

Primary effusion lymohoma and multicentric Cateleman's disease

Two additional diseases have been associated with HHV8. The body cavity-based lymphoma, later referred to as primary effusion lymphoma, is a rare malignant non-Hodgkin's lymphoma, usually emerging as serous effusions without a detectable mass or lymph node involvement (11), although organ involvement has been described. The tumor is mainly, but not exclusively, associated with HIV-infection and morphologically bridges large-cell immunoblastic lymphoma and anaplastic large-cell lymphoma. The tumor cells express CD45, but other B-cell-associated antigens are frequently absent. Clonal immunoglobulin gene rearrangement is present, but *c-myc* gene rearrangement is missing. In addition, the majority of primary effusion lymphomas, but not all, also harbor the Epstein-Barr virus. Human herpesvirus 8 is today thought to be invariably associated with this lymphoma and the detection of HHV8 can therefore be used for the diagnosis of this disease. Various cell lines derived from primary effusion lymphoma have been established and can maintain the virus in *vitro.* These cell lines have been important in the study of viral replication and have served as as a basis for serological analysis. In contrast, HHV8 DNA has rarely been detected in other forms of malignant non-Hodgkin's lymphoma associated with immunosuppression.

HHV8 DNA has further been detected in most patients with multicentric Castleman's disease (or multicentric angiofollicular lymphoid hyperplasia) associated with HIV-infection (12). In HIV-negative patients, HHV8 was detected in only 41% of the multicentric variant of this disease. On the other hand, in the localized torm of this disease, HHV8 has only rarely been detected.

Epidemiology of HHV8

Based on either immunofluorescent analysis of HHV8 containing lymphoma cell lines or recombinant viral proteins, a variety of serological tests and epidemiological analyses have been performed (13-16). It should, however, to be kept in mind that this first generation of serological assays gives varying results of the prevalence of HHV8. Nevertheless, most studies gave concordant results, demonstrating that HHV8 is, in contrast to other herpesviruses, not a ubiquitous human infection. Antibodies to HHV8 can be detected in about 90% of individuals with Kaposi's sarcoma. In the general population of the Western world, a seroprevalence of 0-25% has been reported, although it is usually estimated that the seroprevalence in the general population is about 5%. In high endemic areas, such as Central Africa, a much higher seroprevalence has been reported, up to 100% of the general population. A higher rate of seroprevalence has further been reported in patients attending clinics for sexually transmitted diseases and in patients at increased risk of developing Kaposis sarcoma, especially HIV-positive homosexual men. On the other hand, in hemophiliacs no increased seroprevalence compared with the general population has been observed. Taken together, serological data confirm the strong correlation between Kaposi's sarcoma and HHV8 infection and suggest that the virus is primarily sexually transmitted in countries with low a low prevalence of Kaposi's sarcoma. Other ways of transmission, however, must be present, as suggested by the fact that Kaposi's sarcoma has been found in young children in areas where HHV8 is endemic. In addition, it has recently been shown that HHV8 can be transmitted by solid organ transplantation and that transplant patients have an increased risk of developing Kaposi's sarcoma (17).

HHV8 in other diseases

A bewildering number of papers report the presence of HHV8 DNA in various diseases and lesions. The most intriguing is the presence of HHV8 in multiple myeloma and in a proportion of patients with monoclonal gammopathy of unknown significance (18). Some groups have confirmed these data whereas others have been unable to detect evidence of HHV8 in this disease, either by POR or by serological tests (19). The reason for these conflicting results is currently unknown and further data are urgently needed to define the role of HHV8 in this disease. A variety of other reports describe the detection of HHV8 in squamous cell carcinomas, pemphigus vulgaris, sarcoidosis and angiosarcomas. However, most of these findings have not been confirmed by other groups and it remains to be seen whether these controversial reports, usually based on detection of HHV8 DNA by PCR, have originated false-positive results generated by nested PCR or whether these findings are due to marked regional differences in the prevalence of HHV8. Nevertheless, because of these discrepancies, it is unlikely that HHV8 is involved in the basic pathogenesis of these diseases.

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Prions and the immune system

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A wealth of evidence points to the identity of PrPsc with the prion, the transmissible agent causing spongiform encephalopathies (1, 2). To address the question of central nervous system (CNS) pathogenesis, we grafted neuroectoderm from mice, which overexpress PrPc, into the brain of scrapie-resistant PrP-deficient mice and inoculated it with scrapie prions. Infected grafts developed scrapie and contained high amounts of PrPSc and infectivity, while neighboring cells remained unaffected. The host life span was not reduced. Therefore, availability of endogenous PrPcto the infectious agent, rather than deposition of PrPSc, correlates with scrapie neurotoxicity *in vivo* (3). We then addressed the spread of prions from peripheral sites to the CNS by transplanting neuroectoderm from overexpressing PrP to the brain of Prn-pSc recipients. Scrapie was not detected in grafts after intraocular, intraperitoneal or subcutaneous inoculation. Immunity to PrP developed in several animals soon after grafting but anti-PrP titers did not influence the course of the disease after subcutaneous inoculation and no transport of intraocular infectivity was detected in animals tolerant to PrP (4). Adoptive transfer of PrP-expressing bone marrow cells restored prion replication in the spleen but did not reconstitute neuroinvasion via the intraperitoneal route (5). These results indicate that PrPc supports infectious spread from the periphery to the CNS and imply that neuroinvasion depends on the neuroimmune interface (6). We also showed that B-lymphocytes are crucial for neuroinvasion (7), independently of whether they express PrPc or not (8). This may indicate a target for postexposure prophylaxis (9-10).

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