Human herpesvirus-8 in hematological diseases

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Abstract

The huge amount of experimental and clinical observations supporting the possible involvement of human herpesvirus 8 (HHV-8) or Kaposi sarcoma herpesvirus (KSHV) in human lymphoproliferative diseases was critically reviewed during a workshop organized by the Italian Society for Experimental Hematology in Florence, Italy, on July 3rd, 1997. The organizers have prepared this report for the readers of Haematologica.

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Key words: human herpesvirus 8, hematological diseases

Human herpesvirus 8 (HHV-8) or Kaposi sarcoma herpesvirus (KSHV) is a new herpesvirus whose sequences were found for the first time in 1994 in Kaposi sarcoma (KS) tissue, but not in normal skin, of an AIDS patient. Two years afterwards, a complete viral particle was isolated, the entire genome was sequenced, the virus was propagated in culture and its infectivity was demonstrated.

HHV-8 is a member of the γ herpesvirus subfamily, which also includes another human lymphotropic virus, namely Epstein-Barr virus (EBV). More precisely, HHV-8 is a member of the genus Radhinovirus, whose prototype is herpesvirus saimiri, a monkey lymphotropic virus that causes lymphomas in primates other than its species of origin. The HHV-8 genome is approximately 165 kb long and has a central segment of low-GC DNA (L DNA, 140 kb), containing about 80 open reading frames and flanked by multiple repetitive high-GC DNA (H DNA). The HHV-8 genome, like other radhinoviruses, contains several genes that seem to be derived from the host cell. These genes code for enzymes involved in nucleotide metabolism, proteins interfering with the immune system (IL-6, IRF, chemokines) and regulators of cell growth (cyclin D2, bcl-2).

Epidemiology of HHV-8 infection

HHV-8 epidemiological studies have been based on two types of methods: first, on molecular methods requiring the use of direct or nested polymerase chain reaction (PCR), aimed at defining the presence of viral DNA sequences or, rarely, of viral transcripts; second, on serological assays, aimed at defining the presence of antibodies against different viral antigens in the population examined.

The search for viral sequences has been mainly performed on KS tissues, the material in which HHV-8 viral sequences were firstly described. In summary, it may be stated that practically all the KS tissue samples examined resulted positive for the presence of the viral sequences, including AIDS-related as well as non-AIDS-related (i.e. classic or endemic) KS. Paraffin embedded samples of KS diagnosed in pre-AIDS era also scored positive for HHV-8 sequences. Moreover, the viral sequences occurred in all histologic variants of KS. These results led to the still frequently used name of KSHV (Kaposi sarcoma herpesvirus). Being the virus lymphotropic, the lymphoid tissues were extensively examined for the presence of viral sequences, but these were found only in the rare body cavity based lymphomas and in some atypical lymphoproliferations, as discussed below.

Concerning the general epidemiology of this new herpesvirus, even more interesting are the results of the research of viral sequences in the peripheral blood mononuclear cells (PBMCs) or in other tissues and fluids of KS patients and normal individuals. These results substantially confirmed the strong relation between KS and HHV-8 infection, as will be discussed later. Regarding the presence of viral sequences in PBMCs of normal subjects, attention must be focused on conflicting results reported in studies screening different populations. Two Italian studies reported the presence of viral sequences in a significant proportion of PBMCs and lymphoid tissues of normal individuals as well as in a very high percentage of semen samples and, to a lesser extent, in urothelial cell specimens. These results were not confirmed for the PBMCs of normal blood donors in UK and USA; moreover, these results were not confirmed in semen, which has obvious interest for the possible relation to sexual transmission of this virus.
It is possible that the observed differences may not be due to technical variations (all the cited studies used nested PCR) but, at least partially, to demographic or geographic differences of the populations studied. At the moment, it is not clear whether the virus is in fact ubiquitous, or whether it is rare in some populations and frequent in others, namely those populations in which classic, non-AIDS-related KS is relatively more frequent.

Absolutely relevant to these kinds of questions are the results obtained by screening for the presence of anti-HHV-8 antibodies by serologic assays. The first attempt to approach this problem was done by screening for antibodies against a polypeptide (p40) expressed by BC-1 cells (HHV-8 infected lymphoma cell line) induced by n-butirate. These antibodies are likely to be directed against lytic antigens and were detected by an IFA procedure in sera of AIDS patients with KS in a substantially higher percentage than in HIV infected KS negative patients. A second group used an IFA assay examining the presence of antibodies against latent nuclear antigens (LNA), using initially the same BC-1 cell line and subsequently the BCP-1 cell line as starting material. The authors showed that seroconversion to positivity occurs before the clinical appearance of KS in AIDS patients. Moreover, the same authors observed the absence of these antibodies in US blood donors, while in Italian blood donors the percentage of positivity was 4%. On the other hand, IFA positivity reached 51% in Uganda HIV negative patients without KS. A third group used a similar approach by screening for antibodies against LNA in an additional HHV-8 positive lymphoma cell line, i.e. BCBL-1. The results confirmed the strict relation with the risk for KS development, revealed a significant increase of antibody prevalence in a STD patient population and a positivity of 1% in HIV negative US blood donors. These results were compared to those obtained using an ELISA test for antibodies against LNA in an additional HHV-8 positive lymphoma cell line, i.e. BCBL-1. The results confirmed the strict relation with the risk for KS development, revealed a significant increase of antibody prevalence in a STD patient population and a positivity of 1% in HIV negative US blood donors. These results were compared to those obtained using an ELISA test for antibodies against LNA in an additional HHV-8 positive lymphoma cell line, i.e. BCBL-1.

Data presented at the Workshop of the Italian Society for Experimental Hematology (SIES; held in Florence, July 3rd, 1997) by Luppi et al., working with Weiss’s group in London, showed that the presence of anti-LNA antibodies in Italian blood donors was definitely higher than in US or UK donors and that the frequency of positivity was higher in the regions with higher incidence of classic endemic KS, namely the South portion of Italy. Moreover, these authors showed data suggesting that there are in fact no differences in the prevalence of anti-HHV-8 antibodies in Italian lymphoma patients as compared with the normal population in Italy. These data seem to confirm the idea that there is a sort of North-South gradient in the diffusion of HHV-8 infection, and perfectly fit with the previous epidemiologic expectations about the prevalence of the undefined etiologic agent of KS.

At the same SIES workshop, Parravicini et al. reported a similar serologic assay performed on blood donors, homosexual men (HIV positive and negative), and intravenous drug users (IVDUs) from the area of Milan, Italy. The results confirmed the relation between the infection and the sexual behavior, while no differences were reported between blood donors and IVDUs. It has to be noted that the percentage of positive blood donors in the Milan area (2.5%; Parravicini et al.) is definitely lower than that reported in the nearby area of Piacenza (8%; Luppi et al.) in spite of the fact that the serologic methods used are strictly similar. Others (Schulz et al., personal communication) have also been observed striking differences between very near communities in Northeastern Italy. These results, curiously reminiscent of those observed in HTLV-I epidemiology, are still waiting for a convincing explanation.

In summary, it seems reasonable to conclude that HHV-8 serology needs to be refined by a methodological point of view and that, in near future, it is likely to offer more important insights in the epidemiology and possible pathogenetic mechanisms of HHV-8 infection.

**Association of HHV-8 with hematologic diseases**

In the early phases of the characterization of HHV-8, it was realized that the virus belongs to a family of lymphotropic viruses. This feature of HHV-8 led several investigators to postulate and experimentally investigate the involvement of HHV-8 in hematopoietic neoplasia. Knowledge regarding the involvement of HHV-8 in lymphoid tumors has been accumulating rapidly, both in the context of immunocompetent hosts as well as HIV-infected patients. Today, the association of HHV-8 infection is well established in the instance of body cavity based lymphoma (BCBL) and AIDS-related multicentric Castleman’s disease (MCD).

**Body cavity based lymphomas (BCBL)**

The term BCBL designates lymphomas presenting exclusively or predominantly as effusions in the serous cavities of the body (pleura, pericardium and peritoneum) in the absence of solid tumor masses. Lymphomas consistent with BCBL have been reported in AIDS patients since 1989. Yet the concept that BCBL is a distinct disease entity has emerged only during the last two years with the report of its consistent and selective association with HHV-8 infection. It is now widely accepted that any possible definition of BCBL requires the consideration of HHV-8 infection.
of multidisciplinary criteria, including epidemiologic, pathologic, phenotypic, genotypic and clinical features as well as patterns of viral infection. Most cases of BCBL reported until now have developed in association with AIDS. The disease may also arise in immunocompetent hosts, although preliminary epidemiologic data suggest that the frequency of BCBL in immunocompetent hosts is significantly lower than that of HIV infected individuals.

Clinically, the overwhelming majority of BCBL are characterized by growth in liquid phase in the absence of solid tumor masses. However, recent autopic studies, the results of which have been presented by Carbone et al. at the SIES Workshop, defined that the basic pathologic feature of BCBL is a net predilection for diffuse spreading along the serous membranes without infiltrative or destructive growth patterns. In this context, the serous membranes are involved by multiple small tumor foci that appear slightly thickened. That BCBL is capable of solid growth is also demonstrated by the fact that, very rarely, some cases of BCBL extend into tissues underlying the serous cavities, including the omentum and the outer part of the gastrointestinal tract wall, as well as other solid organs, namely lymph nodes, mediastinum and lung.

Although the precise diagnosis of BCBL requires confirmation of HHV-8 infection within the tumor clone, morphologic and phenotypic features may be highly suggestive of this lymphoma. Morphologically, BCBL cells display peculiar features which are relatively specific among lymphomatous effusions. Cells of BCBL bridge the features of large cell immunoblastic and anaplastic large cell lymphomas. Cells are usually large and irregularly shaped, with abundant cytoplasm, and variably chromatic and pleomorphic nuclei. One or more prominent nucleoli are generally present. Phenotypically, BCBL also display several peculiarities when compared to the majority of non-Hodgkin lymphomas. First, BCBL tend to express indeterminate (null) phenotypes, lacking expression of any lineage associated B or T lymphocyte antigens. Immunogenotypic studies, though, demonstrate the consistent derivation of BCBL from B cells. Second, BCBL cells frequently express activation markers, such as CD30, CD38, CD71 and epithelial membrane antigen (EMA). The expression of activation antigens by BCBL cells has been assumed to correlate with the viral infection of the tumor clone.

BCBL is characterized by a peculiar pattern of viral infection, involving both HHV-8 and, in AIDS-related cases, also EBV. Infection by HHV-8 occurs in all cases of AIDS-related and AIDS-unrelated BCBL and is a sine qua non for BCBL diagnosis. In situ hybridization studies of BCBL have unequivocally defined that the HHV-8 genome is harbored by the tumor cells. Studies by semi-quantitative PCR and Southern hybridization have defined that the number of HHV-8 DNA copies (20-60 copies per cell) detected in BCBL cells is relatively high, consistent with a pathogenetic role of the virus in BCBL development. The consistency of BCBL infection by HHV-8, as well as its selectivity among non-Hodgkin’s lymphomas, further adds to the hypothesis that HHV-8 is indeed pathogenic for BCBL. In addition to HHV-8, EBV is also commonly detected in AIDS-related BCBL, though not in AIDS-unrelated cases. Overall, it remains questionable whether HHV-8 and EBV play a synergistic action in the development of AIDS-related BCBL, or whether their effect is simply additive. The detection of HHV-8 positive, EBV negative cases of BCBL suggests that EBV is not an absolute requirement for the clinicopathologic manifestation of BCBL.

At the genetic level, BCBL consistently lack the molecular lesions commonly detected in mature B-cell neoplasia, including activation of the proto-oncogenes c-MYC, BCL-2, BCL-6, N-RAS and K-RAS, as well as mutations of the p53 tumor suppressor gene. Available BCBL karyotypes do not point to any recurrent cytogenetic abnormality specific for BCBL. However, alterations of the chromosomal region 1q21-q25, which frequently associate with other types of EBV positive AIDS-related NHL, have been detected also in AIDS-related BCBL, suggesting that this chromosomal abnormality might be a common feature of EBV positive, AIDS-related lymphomas. Advanced karyotyping techniques, such as comparative genomic hybridization and SKY, might reveal recurrent cytogenetic abnormalities specific for BCBL which are undetectable by conventional cytogenetics.

As soon as BCBL was recognized as an individual nosologic entity, several investigators were concerned about the precise B-cell subset giving rise to the lymphoma. Several data presented at the SIES Workshop may help answering this question. First, Fais et al. reported that BCBL frequently associates with somatic hypermutation of hypervariable regions of immunoglobulin (Ig) genes. The presence of somatic Ig hypermutation is a well codified feature of B-cells which reside in the germinal center (GC) or, alternatively, have transited through the GC. Conversely, somatic Ig hypermutation is absent in virgin B-cells. In this respect, the association of BCBL with somatic Ig hypermutation indicates that BCBL derives from B-cells that have transited through the GC. The relationship of BCBL with GC B-cells is further supported by additional data presented at the SIES Workshop, indicating that BCBL frequently harbor mutations of the S’ noncoding regions of the BCL-6 proto-oncogene. Similar to Ig mutations, also BCL-6 mutations are regarded as a marker of GC or post-GC B-cells.

Further refinement of the maturation stage of BCBL cells may be derived from analysis of the CD138/syndecan-1 antigen in these lymphomas. The CD138/syndecan-1 molecule is involved in cell-to-cell and cell-to-extracellular matrix adhesion and its expression among mature B-cells selectively associates with late stages of B-cell differentiation, namely plasmacells.
A recent study, the results of which have been presented at the SIES Workshop, has demonstrated that expression of CD138/syndecan-1 selectively clusters with BCBL among B-cell non-Hodgkin’s lymphomas. Several studies have investigated the occurrence of HHV-8 infection in other types of lymphoproliferative disorders. By using conventional strategies, i.e. Southern blot and/or single step PCR, virtually all cases of systemic non-Hodgkin’s lymphomas have been scored negative for infection both in immunocompetent and immunodeficient patients. Confirmation of these data has been provided by Capello et al. at the SIES Workshop. By the same experimental strategies, infection by HHV-8 also scores negative in virtually all primary central nervous system lymphomas (PCNSL), although one case of PCNSL arising post-iatrogenic immunosuppression was found to harbor HHV-8 infection by single-step PCR. Conversely, Luppi et al. has reported positivity for HHV-8 in a subset of cases of angioimmunoblastic lymphadenopathy with dysproteinemia and in a fraction of reactive lymphadenopathies. Infection by HHV-8 in reactive lymphadenopathies has also been confirmed by other authors. Remarkably, HHV-8 infection in these settings occurred both in HIV-positive and in HIV-negative individuals. Most commonly, the HHV-8 positive reactive lymphadenopathies showed an almost identical histology, characterized by a predominant follicular lesion, with giant germinal center hyperplasia and increased vascularity. Infection by EBV was also relatively frequent in these lesions.

**Multicentric Castleman’s disease**

Multicentric Castleman’s disease (MCD), also called multicentric angiofollicular lymphoid hyperplasia, is an atypical, polyclonal lymphoproliferative disorder frequently associated with severe systemic symptoms. Whereas AIDS-related MCD is closely linked with KS (75% of cases), non-AIDS-related MCD associates with KS in only 13% of cases. In patients affected by both KS and MCD, KS may be already present at diagnosis or develop during the course of the disease. HHV-8 infection has been reported in 100% AIDS-related MCD patients both with and without KS. Among MCD of the immunocompetent host, HHV-8 infection is restricted to approximately 40% of the cases. The biologic significance of HHV-8 infection in MCD, and its relationship with KS development, is presently unclear. It is suggestive that MCD and KS share several features, including the fact that both proliferations display vascular hyperplasia, associate with immune dysregulation, and are sustained by the growth factor activity of IL-6 which is present at high levels in the involved tissues.

**Other lymphoproliferative disorders**

Several studies have investigated the occurrence of HHV-8 infection in other types of lymphoproliferative disorders. By using conventional strategies, i.e. Southern blot and/or single step PCR, virtually all cases of systemic non-Hodgkin’s lymphomas have been scored negative for infection both in immunocompetent and immunodeficient patients. Confirmation of these data has been provided by Capello et al. at the SIES Workshop. By the same experimental strategies, infection by HHV-8 also scores negative in virtually all primary central nervous system lymphomas (PCNSL), although one case of PCNSL arising post-iatrogenic immunosuppression was found to harbor HHV-8 infection by single-step PCR. Conversely, Luppi et al. has reported positivity for HHV-8 in a subset of cases of angioimmunoblastic lymphadenopathy with dysproteinemia and in a fraction of reactive lymphadenopathies. Infection by HHV-8 in reactive lymphadenopathies has also been confirmed by other authors. Remarkably, HHV-8 infection in these settings occurred both in HIV-positive and in HIV-negative individuals. Most commonly, the HHV-8 positive reactive lymphadenopathies showed an almost identical histology, characterized by a predominant follicular lesion, with giant germinal center hyperplasia and increased vascularity. Infection by EBV was also relatively frequent in these lesions.

**HHV-8 infection of hematopoietic cells and Kaposi’s sarcoma**

The precise nature of Kaposi’s sarcoma (KS) is elusive. Although KS is generally regarded as a tumor of endothelial origin, and thus not strictly a hematopoietic neoplasm, it has been long since recognized that hematopoietic cells may contribute to KS development and pathogenesis. In particular, inflammatory cytokines produced by CD8+ T lymphocytes and monocytes-macrophages infiltrating KS biopsies induce the formation of KS spindle cells of endothelial origin and lead to the production of angiogenic factors (bFGF, VEGF) that mediate lesion formation.34

Several studies have demonstrated that sustained levels of HHV-8 infection are indeed present in the PBMCs of KS patients as well as of individuals at high risk for KS development. The pathogenetic link between HHV-8 infection of PBMCs and KS development is suggested by the indirect observation that infection may be detected several years before the occurrence of KS and that HHV-8 detection in the peripheral blood of individuals without KS is predictive of subsequent development of the disease. Data presented at the SIES Workshop have reinforced the notion that hematopoietic cells contribute to KS pathogenesis. By focusing on the role of monocytes and macrophages, Ensoi et al. have reported that the inflammatory cytokines typically associated with KS are able to maintain and rescue HHV-8 infection in cultured PBMCs, including B-cells, monocytes and circulating spindle cells. In parallel, inflammatory cytokines induce the growth and differentiation of monocytes into macrophages and spindle cells with endothelial cell markers characteristic of KS biopsies. HHV-8 positive B cells are rare in KS lesions and their pathogenetic role is obscure. Conversely, since macrophages of KS lesions are frequently infected productively by HHV-8, it is conceivable that cells of the monocye/macrophage lineage vehicle HHV-8 to tissues and transmit the infection to endothelial cells which subsequently give rise to the typical cells of KS lesions.

**Conclusions and perspectives**

The finding of the involvement of HHV-8 in some lymphoproliferative disorders has catalyzed the rapid accumulation of data, leading to an improved knowledge not only of HHV-8 per se, but also of the hematologic disorders bearing HHV-8 infection. It is likely that, if the association between BCBL and HHV-8 had not been discovered, the clinico-pathologic features of this lymphoma would have remained rather undefined, and the notion of BCBL as a unique lymphoma category would still be unfamiliar to most hematologists.

Several challenges stand now in front of experimental and clinical hematologists. First, the actual pathogenetic role of HHV-8 in BCBL needs to be formally demonstrated. Toward this aim, initial steps
may be represented by the definition of the clonality of viral infection and the elucidation of which HHV-8 genes are expressed in BCBL cells. A final answer to the question of pathogenicity, however, will require the establishment of animal models and/or in vitro assays recapitulating the molecular pathology of BCBL. In this respect, the availability of BCBL cell lines may be of substantial help.

When considering the involvement of HHV-8 in lymphoproliferative disorders, it emerges that HHV-8 selectively associates with two types of B-cell lymphoproliferations, BCBL and MCD, which preferentially develop in immunodeficient patients, and are rare, though not totally absent, in the immunocompetent host. Curiously, however, BCBL and MCD have little in common apart from the association with HHV-8 infection and the predilection for immunocompromised hosts. Recently, HHV-8 infection of bone marrow dendritic cells has been proposed as a pathogenetic mechanism for multiple myeloma. Whereas in BCBL the viral genome is harbored by the tumor clone, in the case of multiple myeloma HHV-8 would act through the production of cytokines by the tumor clone, in the case of multiple myeloma HHV-8 selectively associates with two types of B-cell lymphoproliferations, BCBL and MCD, which preferentially develop in immunodeficient patients, and are rare, though not totally absent, in the immunocompetent host. Curiously, however, BCBL and MCD have little in common apart from the association with HHV-8 infection and the predilection for immunocompromised hosts. Recently, HHV-8 infection of bone marrow dendritic cells has been proposed as a pathogenetic mechanism for multiple myeloma.37

Whereas in BCBL the viral genome is harbored by the tumor clone, in the case of multiple myeloma HHV-8 would act through the production of cytokines by accessory cells which reside in the microenvironment surrounding the neoplastic population. Results of serologic studies, however, have questioned the association between HHV-8 infection and multiple myeloma.38,39 The precise role, if any, of HHV-8 in multiple myeloma is presently awaiting confirmation from studies performed in multiple institutions.

Two years after a previous review article40 this report offers an overview of the huge amount of experimental and clinical observations supporting the possible involvement of lymphotropic viruses in human lymphoproliferative diseases.

Contributions and Acknowledgments
The two authors equally contributed to this report.

Funding
The work by the authors described in this review has been supported by grants from IX AIDS Project, Rome, Italy; AIRC, Milan, Italy; and Fondazione "Piera Pietro e Giovanni Ferrero", Alba, Italy.

Disclosures
Conflict of interest: none. Redundant publications: no substantial overlapping with previous papers.

Manuscript processing
Manuscript received November 4, 1997; accepted February 18, 1998.

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Appendix

Papers presented at the workshop of the Italian Society for Experimental Hematology (SIES) held in Florence, Italy on July 3rd, 1997

Role of HHV-8 in the pathogenesis of Kaposi’s sarcoma

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Recent evidence indicates that, at least in early stage, KS is a cytokine-mediated disease and that a novel herpesvirus termed HHV-8 is associated with KS development. Inflammatory cytokines (IC) produced by CD8+ T cells and monocytes-macrophages infiltrating the tissue induce the formation of KS spindle cells of endothelial origin and the production of angiogenic factors (bFGF, VEGF) that mediate lesion formation. On the other hand, HHV-8 is found in most of the KS lesions and in PBMC from patients with KS or at high risk of KS such as homosexual men. In these individuals viral load appears to be higher than in other groups at low risk of KS or in normal individuals that, at a lower prevalence, are also infected by HHV-8. In addition, viral load increases with lesion progression. This suggests a reactivation and maintenance of HHV-8 infection in KS or in high risk patients. These same individuals present signs of immunoadherence/dysregulation and increased levels of IC, suggesting a role for IC on HHV-8 infection. In fact, IC maintain and rescue HHV-8 infection in cultured PBMC and allow viral PCR detection on previously negative individuals. In addition, IC induce the growth and differentiation of monocytes into macrophages and spindle cells with endothelial cell markers as those found in lesional tissues. The virus is present in B cells, monocytes and spindle cells from the blood of these individuals and express both latent and lytic viral genes. These results indicate that IC increased in KS lesions and in individuals at risk of KS can maintain and promote HHV-8 infection increasing viral load and, at the same time, can induce the histological changes of KS. In addition, since B cells are few or absent in KS and lemosal macrophages are produc-
Clinically, BCBL are characterized by growth in liquid phase in the absence of detectable tumor masses. Pathologically, however, the basic feature of these lymphomas is a net predilection for diffuse spreading along the serous membranes without infiltrative or destructive growth patterns. As seen at autopsy or revealed by computed tomography scan, BCBL presents as multiple small tumor foci involving the serous membranes, which appear slightly thickened. In some cases, the tumor spread may be so extensive that, based on the sole autopsy, it is difficult to define whether the neoplasm has originated in the pleura, the pericardium, or the peritoneum. Although BCBL usually remains localized to the serous body cavities, extension into tissues underlying the serous membranes, including the omentum and the outer parts of the gastrointestinal tract wall, may occasionally occur. Also, BCBL involvement of mediastinal lymph nodes and visceral lymphatics, without parenchymal infiltration, has been observed in some cases. Notably, BCBL cells derived from lymphomatous effusion and those derived from solid tissue localization possess indistinguishable morphologic, immunophenotypic and molecular features. Finally, several reports have defined that BCBL may rarely involve superficial and deep lymph nodes, as well as soft tissues and organs other than serous body cavities. Overall, the clinical and gross pathological features suggest that BCBL is a lymphoma of the serous membranes typically giving rise to lymphomatous effusions without mass formation.

Anatomical pathology of HHV-8+ body cavity-based lymphoma (BCBL)

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HHV-8 infection in immunocompetent subjects.

Pathologic features that may represent the possible clinicopathologic subtypes. However, in a geographic area with a high prevalence of HHV-8 infection, it is possible to identify HHV-8 DNA in the primary cerebral lymphoma of a HIV negative patient after long term steroids, in the lymph node biopsies of 1 HIV negative case of multicentric Castleman’s disease of plasma cell type, of 3 HIV negative cases of angioimmunoblastic lymphadenopathy with dysproteinemia and of 4 non AIDS-related lymphadenopathies with giant germinal center hyperplasia and increased vascularity. In conclusion, differences in the HHV-8 seroprevalence among various Italian regions mirror the different distribution of incidence rates of KS. Prevalence of antibodies to HHV-8 latent antigens in lymphoma patients from Italy is comparable to that found in the healthy population. Serologic findings are consistent with the infrequent occurrence of HHV-8 sequences not only in non-Hodgkin’s lymphomas, AIDS-related or not, but also in a large series of Hodgkin’s disease cases, representative of all histologic subtypes. However, in a geographic area with a high prevalence of HHV-8 infection, it is possible to document the association between HHV-8 infection and reactive lymphadenopathies with peculiar histologic features that may represent the possible clinicopathologic entity which may occur in the course of HHV-8 infection in immunocompetent subjects.

Seroepidemiology of Kaposi’s sarcoma-associated herpesvirus (KSHV) infection in Milan, Italy


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To assess in Milan the seroprevalence of KSHV infection among the general population versus HIV-infected and non-infected groups at risk for Kaposi’s sarcoma (KS). Indirect immunoperoxidase assay to detect IgG antibodies specific for KSHV-associated nuclear antigens present on chronically infected, primary effusion lymphoma-derived cell lines.

We screened sera from general blood donors (n=42), homosexual men with and without HIV infection (n=64 and n=46, respectively) and HIV seropositive intravenous drug abusers (IVDU) (n=94).

Results. Results are given in the Table below.

Seroprevalence of KSHV was found to be significantly higher in homosexual men, irrespective of concomitant infection by HIV, as compared to general blood donors and/or IVDUs (p<0.00001), while the difference between blood donors and IVDUs was not statistically significant. In all groups, KSHV seroprevalence was higher among persons originating from southern Italy, without significant age or sex-related differences. Of note, in HIV seropositive homosexuals or IVDUs, KSHV seroprevalence did not correlate with clinical CDC stage and absolute CD4 cell counts.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Cases (n)</th>
<th>KSHV-pos (n)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>General-(blood donors)</td>
<td>42</td>
<td>1</td>
<td>2.5%</td>
</tr>
<tr>
<td>Homosexual men, HIV-neg.</td>
<td>46</td>
<td>21</td>
<td>46%</td>
</tr>
<tr>
<td>Homosexual men, HIV-pos.</td>
<td>64</td>
<td>25</td>
<td>39%</td>
</tr>
<tr>
<td>IVDUs</td>
<td>94</td>
<td>7</td>
<td>7.5%</td>
</tr>
</tbody>
</table>

These results extend a previous survey conducted on general blood donors from Milan (Nature Medicine 1996; 2:925-28) and indicate that KSHV infection is not ubiquitous in Northern Italy. By contrast, the high seroprevalence rates observed among HIV positive and negative homosexual men (but not among HIV positive IVDUs) is consistent with previous epidemiologic evidences suggesting that KS is due to a sexually transmissible agent other than HIV.

Supported by IX AIDS Project (N 9403-04), Istituto Superiore di Sanità, Rome, Italy.

Molecular analyses of VH and VL genes used by 5 cases of primary effusion lymphomas (PEL)

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The VH and VL segments encoding the B cell antigen receptor (Ig) can accumulate point mutations following adequate stimulation of B cells by antigen. This mechanism is usually operating in the germinal centers where both isotypic switch and affinity maturation occur. The mutation events are thought to occur randomly, whereas the subsequent selection of the cell with a given receptor is regulated by affinity of the receptor for the stimulating antigen. The cells that have been positively selected present an accumulation of replacement (R) mutations at the level of the gene segments encoding the antibody CDR regions.

It is generally accepted that a ratio of ≥ 3 between the R and silent (S) mutations in the CDRs vs. those
Human herpesvirus-8 in hematological diseases

detected in the framework region is consistent with antigen selection. Thus, the molecular analysis of VH and VL genes can be employed to determine the history of a certain B cell clone. We have sequenced the VH and VL genes used by 4 cell lines (HBL6, BC1, BC2, BC3) derived from primary effusion lymphomas (PEL) and from 2 biotic samples. HBL6 and BC1 were derived from the same patient at different times.

VH gene usage among the samples was the following: 2 cases used a VH3 family gene (VH3-73 and VH3-23 respectively), 2 cases employed a VH4 gene (both VH4-39) and 1 case used a VH5 gene (VH5-51). The use of JH segments in our samples displayed an apparent bias since the otherwise most frequently used JH (JH4) was not found among PEL. The malignant cells expressed a JH5 (2 cases) or JH6 (3 cases) segments. The D segments were not clearly assignable in three cases whereas the other two cases used DN1 and D21-9 segments respectively. A significant number of point mutations were found in the samples that employed VH3 and VH5 genes.

The R:S ratio was indicative of antigen selection in the VH3 genes (R:S ratio=6 in the VH3-73 gene and 4 in the VH3-23 gene). The VH5 gene in the cell lines HBL-6 and BC1 (derived from the same patient) had an R:S ratio < 3, whereas a ratio > 3 was detected in the light chain gene (DPK24, R:S ratio=6) from the same cells. The VH4 family gene was found in germ-line configuration. We also determined the heavy chain class of the cell lines studied. The cell lines BC2 and BC3 expressed a $\mu$ heavy chain, whereas BC1 and HBL6 cell lines were both $\gamma_2$, a finding that may suggest a further process of intraclonal maturation.

The presence and the pattern of the point mutations at VH and VL level in the cases reported suggest that these cells passed through germinal centers at some stages of their development and that were subjected to a process of antigen selection.

Overall, these data suggest that (a) PEL frequently derives from GC- or post-GC B cells and (b) antigen-induced stimulation and selection are involved in the pathogenesis of this peculiar type of lymphoma.

Comparative analysis of HHV-8 infection in human tumors

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Human herpesvirus 8 (HHV-8) is a $\gamma_2$ herpesvirus recently identified in Kaposi’s sarcoma (KS). Subsequent research showed that detection of HHV-8 DNA is a consistent feature of all clinical variant of KS, of body cavity-based lymphoma (BCBL) and of multicentric Castelman’s disease. Controversial results have been obtained in molecular studies with regard to the presence of HHV-8 sequences in other proliferative lesions. To verify the value of the different technical approaches in the evaluation of HHV-8 infection, we have analyzed a panel of B-cell non Hodgkin lymphomas (B-NHL) by different strategies, the sensitivity of which had been defined in reconstruction experiments. HHV-8 infection was investigated in 30 systemic B-NHL and in 31 primary central nervous system lymphomas (PCNSL). All PCNSL and systemic B-NHL scored negative by Southern blot and single-step PCR, suggesting a viral load ≤ 100 viral copies/200,000 human haploid genome equivalent (HHGE). The same samples were further investigated by nested PCR in 5 independent experiments, with and without the application of Poisson’s assumption. Sixteen PCNSL and 10 systemic B-NHL scored repeatedly negative in 5/5 experiments, suggesting absent HHV-8 infection. Fourteen PCNSL and 20 systemic B-NHL yielded a positive PCR product in a fraction (< 5) of nested PCR experiments, suggesting a viral load ≤ 1 viral copy/200,000 HHGE. Only 1 PCNSL scored positive in 5/5 nested PCR experiments, consistent with a viral load comprised between 1 and 100 viral copies/200,000 HHGE. The degree of viral load detected in systemic B-NHL and PCNSL differs dramatically from the viral load detected in BCBL and KS (approximating 1-2 × 10^7 viral copies/200,000 HHGE in the case of BCBL) and rules out a significant pathogenetic role of HHV-8 in systemic B-NHL and in PCNSL.

Conversely, the low levels of HHV-8 infection detected in systemic B-NHL and PCNSL are consistent with infection of sporadic normal cells infiltrating the tumor biopsy. Our data also mandate extreme caution in the interpretation of PCR results in diagnostic studies aimed at defining the distribution of HHV-8 infection in human tumors and tissues.