Analysis of the Human Herpesvirus 8 (HHV-8) Genome and HHV-8 vIL-6 Expression in Archival Cases of Castleman Disease at Low Risk for HIV Infection

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Abstract

Lymph nodes from 44 patients with Castleman disease (CD) without risk factors for HIV infection were analyzed with polymerase chain reaction (PCR), in situ hybridization (ISH), and immunohistochemical analysis for human herpesvirus 8 (HHV-8) and viral interleukin-6 (vIL-6). PCR detected HHV-8 genome in 2 of 4 cases; ISH detected it in 9 of 16 cases. HHV-8 vIL-6 peptides were detected in 2 of 44 cases. vIL-6– and ISH-positive cells were found in large transformed and small lymphocytes of the follicular mantle, respectively. Of 9 cases of plasma cell (PC) CD that demonstrated HHV-8 genome by PCR or ISH, 1 expressed vIL-6. Clonal populations of PCs in CD by immunohistochemical analysis or immunoelectrophoresis of serum and urine were associated with neuropathy. HHV-8 vIL-6 detection was associated with poor survival and lack of HHV-8 IL-6, with low risk for subsequent lymphoma. Although HHV-8 genome was detected in a considerable number of patients with PC CD, vIL-6 expression was infrequent. Expression of HHV-8 vIL-6 in CD may indicate poor prognosis in patients at risk for lymphoma who may prospectively require more aggressive treatment. The lack of vIL-6 expression in CD with HHV-8 genome suggests that human IL-6 rather than vIL-6 may be the principal pathogenic cytokine.

Castleman disease (CD) is an atypical lymphoproliferative disorder of lymphoid follicles and interfollicular lymph node regions that produces increased interleukin-6 (IL-6) in patients with systemic symptoms.1 IL-6 is a multifunctional cytokine that is responsible for systemic symptoms in patients with CD1 and also may locally promote plasma cell proliferation,2,3 resistance of plasma cells to apoptosis,4 and plasma cell differentiation from regional B-cell precursors.5-7 B-cell precursors of plasma cells have been hypothesized to accumulate in the concentric expanded mantle zone regions of lymphoid follicles in CD, demonstrate a distinctive immunophenotype, and show a selective propensity for lambda immunoglobulin light-chain rearrangement, as has been demonstrated for the immunoregulatory murine Ly-1 B-cell lymphocytes.8

A viral surrogate of IL-6 (vIL-6) is produced by human herpesvirus 8 (HHV-8) infection in immunocompromised patients with CD.9 vIL-6 is produced by transformed mantle zone lymphocytes latently infected with HHV-8 in CD in immunocompromised patients10 but may not be as important as human IL-6 (hIL-6) in the pathogenesis of CD because of the low affinity of vIL-6 for the hIL-6 receptor,11 because of the induction of hIL-6 production by HHV-8 infection,12 and because clinical symptoms resolve in HIV-infected patients with CD and hIL-6 neutralizing monoclonal antibodies.13

We retrospectively studied a large series of patients with CD for the feasibility of detection of HHV-8 genome and vIL-6 protein in archival specimens and for the presence and cellular compartment of HHV-8 genome and vIL-6 expression. CD was characterized by a distinctive mantle zone immunophenotype. CD with the HHV-8 genome or vIL-6 expression was correlated with clinical findings, including...
the POEMS syndrome (peripheral neuropathy, organomegaly, endocrinopathy, monoclonal protein [paraprotein], and skin changes [hyperpigmentation, hypertrichosis]), development of lymphoma, and survival.

Materials and Methods

The tissue files from the Mayo Clinic for the years 1955 through 1996 were searched for cases with the diagnosis of CD that had paraffin block material for analysis and clinical follow-up. The date of a lymph node biopsy diagnosis of CD, size of lymph nodes, and clinical and laboratory findings were recorded. Clinical risk factors for HIV infection were assessed without HIV serologic testing (unprotected sex with multiple partners, transfusion before 1985, intravenous drug use, other sexually transmitted diseases). The phenotypes of the mantle zone lymphocytes were analyzed with Kiel-derived B-lymphocyte–specific monoclonal antibodies KiB3\(^{14}\) and KiB5 (by M.T. and R.P.), which distinguished CD from nonspecific hyperplasia in cases that were difficult to diagnose by conventional histologic criteria of Keller et al.\(^{15}\) CD was typed as hyaline vascular (HV) or plasma cell (PC), according to the morphologic definitions proposed by Keller et al.\(^{15}\) In addition, a rare type of CD defined by a mantle zone immunophenotype only (lacking diagnostic features of HV or PC disease) was studied (mantle zone CD).\(^{8}\) Plasma cell clonality was assessed with cyttoplasmic immunoglobulin light-chain staining in paraffin tissue samples (\(>4:1\) kappa/lambda or \(<1:1\) lambda/kappa), and clinical analyses of serum and urine samples for paraproteins were done with immunoelectrophoresis. Lymphocyte clonality was assessed with polymerase chain reaction (PCR)–amplified immunoglobulin heavy-chain gene rearrangement\(^{16,17}\) in paraffin tissue samples. B-cell chronic lymphocytic leukemia served as a positive control, and placental DNA served as a negative control. Clinical findings included staging of lymph node involved (localized or systemic), results of bone marrow examination and skeletal survey, evidence of the POEMS syndrome, and evidence of effusions (pericardial, pleural, or abdominal). Clinical follow-up was used to determine survival, response to therapy, and whether lymphoma developed.

HHV-8 genome was detected with in situ hybridization (ISH) that used biotinylated probes for the open reading frame 72 region.\(^{18}\) DNA from a B-1 cell line served as a positive control, and placental DNA served as a negative control. PCR for detection of HHV-8 was performed with Kaposi sarcoma–specific 330233-primer.\(^{19}\) PCR for detection of immunoglobulin heavy-chain gene rearrangements analyzed the hypervariable CDR3 region of the immunoglobulin heavy-chain gene (by M.T. and R.P.).\(^{16,17}\) KiB3, KiB5, and HHV-8 vIL-6 stains were performed according to standard immunoperoxidase techniques.\(^{20,21}\)

Immunostains for HHV-8 vIL-6 used a polyclonal antibody (provided by E.C.) without antigen retrieval and then with antigen retrieval if no product was identified. Antigen retrieval was performed by microwave heating for 15 minutes in citrate buffer (pH 6.0). Immunohistochemical analysis was performed on the Techmate 500 Automated Immunostainer (Ventana Medical Systems, Tucson, AZ) with the modified MIP protocol (Ventana Medical Systems). Expression of vIL-6 was demonstrated with the ChemMate ABC peroxidase secondary system (Ventana Medical Systems). A primary effusion lymphoma cell line served as a positive control, and a primary effusion lymphoma cell line without antibody served as a negative control.

Immunostains for KiB3 and KiB5 were performed with the following APAAP (alkaline phosphatase and monoclonal antialkaline phosphatase) technique. No antigen retrieval was used for KiB3, whereas antigen retrieval was used for KiB5. Sections were deparaffinized as follows: 3 times in xylene for 5 minutes each, 2 times in 100% ethanol for 10 minutes each, 2 times in 95% ethanol for 10 minutes each, and washed with tap water. For antigen retrieval, the slides were placed in glass slide holders and submerged in a plastic jar containing a 0.01-mol/L citric acid solution adjusted to pH 6.0 with a 2-mol/L concentration of sodium hydroxide. They were covered with a loose lid and heated in a microwave oven (750 W) for 10 minutes at the highest power setting and then an additional 15 minutes at medium power (425 W). The slides were removed from the jar and cooled in tap water. The slides then were washed in Tris-buffered saline (TBS) for 5 minutes and incubated for 30 minutes with the primary antibody. They were washed for 5 minutes and then incubated for 30 minutes in secondary antibody, rabbit antimouse DAKO Z295 (DAKO, Carpinteria, CA), dilution 1:20, in TBS/human serum, 1:10. This was followed by a 5-minute wash in TBS and then incubation with APAAP-Complex (DAKO) for 30 minutes. The slides then were washed in TBS. The rabbit antimouse and APAAP incubations were repeated twice for 10 minutes each followed by a TBS wash. All incubations were at room temperature. The following buffers and stains were used for development:

- Buffer A: 0.05 mol/L Tris 8.7 g NaCl + 1.5 g Tris-HCl + 4.9 g Tris base added to 1,000 mL distilled water
- Buffer B: 0.2 mol/L propandiol 21 g added to 1,000 mL distilled water, pH 9.7
- Mix 50 mL of buffer A and 18 mL of buffer B + 28 mg levamisole (Sigma L 9756, Sigma, St Louis, MO) to block endogenous alkaline phosphatase

- Solution 1: 35 mg naphthol-AS-BI phosphate (Sigma N 2250) + dimethylformamide 420 µL
- Solution 2: 350 µL 4% sodium nitrate in distilled water + 140 µL 5% new fuchsin in 2 mol/L HCl
Mix solutions 1 and 2, then put into buffer A and B, pH 8.7-8.8. Filter the solution and fill a Coplin jar and incubate the slides for 15 to 20 minutes. Wash in tap water. Counterstain with Hämalaun (Mayer) or hematoxylin.

KiB3 stains were interpreted relative to KiB5 staining of the mantle cell lymphocytes. Monoclonal antibodies KiB3 and KiB5 were obtained at the Lymph Node Registry in the Institute of Hematopathology, Kiel, Germany. Plasma cell kappa and lambda light-chain staining used polyclonal antibodies against immunoglobulin light chains, applying the streptavidin-biotin complex (DAKO). Statistical correlations between groups were performed with chi-square analysis. Double labeling for KiB3, KiB5, and HHV-8 ISH or vIL-6 expression was not possible.

Results

We identified 44 cases of CD that had paraffin tissue samples for laboratory analysis and clinical follow-up. Forty cases could be classified as CD by conventional histologic criteria; 4 cases of CD were diagnosed only by immunophenotypic abnormalities of the mantle zone. All conventional cases of CD showed decreased mantle cell KiB3 staining and either had clusters or lacked clusters of interfollicular plasma cells, findings that permitted categorization into PC and HV types of CD. The ages of the tissue blocks for HV and PC cases were similar (not significantly different) [Table 1] (duration of clinical follow-up).

HHV-8 genome status was determined successfully in 15 of 25 cases of PC CD but in only 3 of 15 archival cases of HV CD (P = .01). HHV-8 genome was determined in 2 cases of HV CD by ISH and in 1 case of HV CD by PCR. HHV-8 genome was determined in 13 of 25 PC CD cases by ISH and in 3 of 25 PC CD cases by PCR [Image II]. Only 1 patient with HV CD had HHV-8 genome (ISH only) [Table 2]. This 61-year-old patient had localized CD in the retroperitoneum and neuropathy but no skin changes, other components of the POEMS syndrome, or effusions. The patient had typical histologic features of HV CD without interfollicular clusters of plasma cells by H&E staining or kappa and lambda immunostains. The 6-cm mass was removed, and the patient was alive without disease or development of lymphoma after 4.2 years of follow-up.

Nine patients with PC CD had HHV-8 genome, detected by ISH in 8 and by PCR in 1. None of these patients had development of lymphoma. Of the 9 patients with HHV-8 genome, 5 had one or more components of the POEMS syndrome (by definition, all had neuropathy, and 4 showed skin hyperpigmentation, the next most frequently
demonstrated component of the syndrome,\(^2^2\) and 4 did not have the POEMS syndrome (neuropathy, a cardinal feature of the POEMS syndrome, was absent).\(^2^2\) Four HHV-8 genome–positive cases of PC CD had evidence of clonal populations of plasma cells (lambda restricted) by immunohistochemical analysis (1 case) or immunoelectrophoresis (3 cases), whereas 5 HHV-8 genome–negative cases of PC CD had evidence of clonal populations of plasma cells (lambda restricted) by immunohistochemical analysis (1 case) or immunoelectrophoresis (4 cases). Of 9 cases of PC CD with HHV-8 genome, 2 showed immunoglobulin heavy chain (IgH) rearrangements and 7 did not, whereas of 6 cases of PC CD without HHV-8 genome, 4 did not show an IgH rearrangement and 2 could not be analyzed for IgH by PCR. This difference was not significant \((P = .3)\). Of the 9 patients with PC CD and HHV-8 genome, 6 are alive and 3 have died. Of the 6 patients with PC CD who did not have HHV-8 genome, 3 are alive and 3 have died. This difference was not significant \((P = .51)\). Only 1 patient with PC CD and HHV-8 genome (PCR only) showed HHV-8 vIL-6 expression. This 76-year-old patient had systemic lymphadenopathy and fever but no POEMS syndrome and died 20 days later of sepsis. The lymph node showed typical features of PC CD without lymphoma or IgH rearrangement.

All archival CD cases could be analyzed for HHV-8 vIL-6 expression. HHV-8 vIL-6 was not identified in any case of HV CD and in only 2 cases of PC CD. Lymphoma developed in 1 of the 2 patients with HHV-8 vIL-6 PC CD and in only 1 of the 23 patients with HHV-8 vIL-6–negative PC CD \((P = .02)\). Both of the patients with PC CD and expression of HHV-8 vIL-6 died: 1 of diffuse immunoblastic B-cell lymphoma unresponsive to chemotherapy and 1 of sepsis. Of 23 patients with HHV-8 vIL-6–negative PC CD, 15 are alive. Patients with PC CD that did not express HHV-8 vIL-6 had better survival \((P = .07)\). Of 23 cases of PC CD that did not express HHV-8 vIL-6, 15 showed evidence of a clonal proliferation of plasma cells (4 by immunohistochemical analysis and 11 by immunoelectrophoresis, lambda restricted), whereas 2 of cases of PC CD that expressed HHV-8 IL-6, both were without evidence of a clonal proliferation of plasma cells \((P = .07)\). Evidence of a clonal proliferation of plasma cells was associated with the clinical finding of neuropathy \((P = .008)\). Of 15 patients with PC CD and evidence of clonal proliferation of plasma cells, 13 had neuropathy, 10 had skin hyperpigmentation, 9 had organomegaly, and 8 had sclerotic bone lesions. Of 15 patients with PC CD and evidence of a clonal population of plasma cells by immunohistochemical analysis or immunoelectrophoresis and lymph node analysis of IgH rearrangement by PCR, 10 of 12 (3 could not be evaluated by PCR) showed no evidence of rearrangement. Of 10 patients with PC CD and no evidence of a clonal population of plasma cells by immunohistochemical analysis or immunoelectrophoresis, 7 of 9 (1 could not be evaluated by PCR) showed no evidence of gene rearrangement. Thus, there was no significant correlation between IgH rearrangement and the finding of a clonal population of plasma cells.

Of the 2 patients in whom lymphoma developed, 1 was negative for IgH and the other did not have amplifiable DNA. Of the 23 patients in whom lymphoma did not develop, 20 had amplifiable DNA: 4 showed an IgH rearrangement and 16 showed no IgH rearrangement. There was no significant correlation between the development of lymphoma and the finding of IgH by PCR \((P = .6)\). PC CD was associated with a poorer survival than HV CD \((P = .02)\).

The differences between the frequency of HHV-8 vIL-6 expression and the detection of HHV-8 genome within HV and PC CD were significant. In HV CD, 0 of 15 cases were HHV-8 vIL-6 positive and 1 of 3 cases were HHV-8 genome positive \((P = .04)\). In PC CD, 2 of 23 cases were HHV-8 vIL-6 positive and 9 of 15 were HHV-8 genome positive \((P = .001)\). The differences in frequency of HV vIL-6 expression and detection of HHV-8 genome between HV CD and PC CD were not significant.

The distribution of HHV-8 genome in HV CD and PC CD was in scattered lymphocytes, histiocytes, and stromal cells throughout the lymph nodes, with increased numbers of positive cells in lymphoid follicles (germinal center and predominantly in mantle zone) [Image 21]. HHV-8 IL-6 was seen in large transformed lymphocytes of the follicular lymphoid follicles (germinal center and predominantly in mantle zone).
Comparison of serial sections of HV CD that contained HHV-8 genome by ISH and serial sections of PC CD that contained HHV-8 genome or expressed HHV-8 vIL-6 suggested that the genome-positive lymphocytes and the vIL-6–expressing large transformed lymphocytes of the follicular mantle were negative for KiB3 and positive for KiB5. The lambda light-chain restriction of the small or large transformed lymphocytes of the follicular mantle could not be identified even when interfollicular clusters of lambda-restricted plasma cells were identified. Tissue from the lymphoma arising in PC CD was not available for detection of HHV-8 genome or analysis of vIL-6 expression.

**Discussion**

Immunophenotyping and genotypic analysis of archival cases of CD for HHV-8 vIL-6 and HHV-8 genome revealed differences in the ability to detect HHV-8 vIL-6 and HHV-8 genome and the frequency of HHV-8 vIL-6 expression and HHV-8 genome within HV and PC types of CD. The restricted expression of HHV-8 vIL-6 rather than the detection of HHV-8 genome was correlated with the risk of development of lymphoma and poor survival in PC CD. The distributions of HHV-8 genome and expression of HHV-8 IL-6 in the follicular mantle were the same as those of KiB3-negative,
KiB5-positive lymphocytes in the follicular mantle. This finding suggests that HHV-8 may infect an immunophenotypically distinctive population of B lymphocytes that are important in the pathogenesis of CD.

The incidence and distribution of HHV-8 genome in CD reported herein are supported by the previous literature. The incidence of HHV-8 genome in HIV-negative patients with CD has been reported to be approximately 41%, detected only rarely in HV CD and commonly detected in PC CD. The predominant distribution of HHV-8 genome in lymphoid follicles by ISH in CD was reported recently by O’Leary et al., although our ISH findings suggest a more prominent mantle zone rather than germinal center distribution.

The incidence and distribution of HHV-8 vIL-6 in CD reported herein are similar and dissimilar to those reported in the literature. The lack of HHV-8 vIL-6 in HV CD was reported by Parravicini et al. They, however, found HHV-8 vIL-6–positive cells in all cases of HIV-negative PC CD that were positive for HHV-8 genome by PCR. HHV-8 vIL-6 also was found in transformed mantle zone lymphocytes and was associated clinically with poor survival. Although lymphoma developed in 2 of the CD cases (1 PC and 1 HV) reported by Parravicini et al., there was no correlation between expression of HHV-8 vIL-6 and the risk for development of lymphoma. Our findings of scattered transformed HHV-8 vIL-6–positive lymphocytes in the mantle zone of PC CD and their association with the risk of lymphoma suggest that HHV-8 vIL-6–positive cells may give rise to interfollicular immunoblastic (plasmablastic) lymphomas in patients at low clinical risk for HIV infection, similar to HIV-positive patients, as recently described by Dupin et al. In their series, plasmablastic (immunoblastic) lymphomas arising in HIV- and HHV-8–infected patients with CD were shown by microdissection to be genotypically derived from HHV-8 vIL-6–positive cells in the follicular mantle.

Parravicini et al. and others have shown that follicular dendritic cells are the primary source of IL-6, the principal pathogenic cytokine in CD. The work of Parravicini et al. showed that hIL-6 rather than HHV-8 vIL-6 was the principal type of IL-6 produced in HIV-negative patients. This finding corroborated earlier research by Leger-Ravet et al. who demonstrated that follicular dendritic cells produce hIL-6 and hIL-6 messenger RNA in PC CD and rare cases of HV CD associated with systemic symptoms. Other research that suggests that hIL-6 rather than HHV-8 vIL-6 is the principal pathogenic cytokine in CD is the finding of IL-6–related systemic symptoms (including the POEMS syndrome) in HHV-8–negative CD. hIL-6 also is likely to be of primary importance in HHV-8–positive CD because the systemic symptoms (including the POEMS syndrome) resolve with anti–hIL-6 therapy. hIL-6 not only is fundamentally responsible for systemic symptoms in CD but also may function as an important plasma cell differentiation factor in PC CD.

The distribution of HHV-8 genome and vIL-6 expression in KiB3-negative, KiB5-positive mantle zone lymphocytes suggests that HHV-8 may infect a distinctive B-lymphocytic population. Mantle zone lymphocytes show decreased KiB3 (CD45RA) expression compared with pan-B-cell antigen expression (KiB5 or CD20) in CD, unlike nonspecific lymphoid hyperplasia. Identification of this phenotype is necessary for recognition of rare cases that do not show diagnostic histopathologic features of CD but nevertheless are associated with the POEMS syndrome or for the identification of immunophenotypically abnormal mantle zone cells in regional lymphoid tissue in HV CD as a possible precursor lymphoid lesion. The KiB3 (CD45RA)–negative paraffin section or CD5+ frozen section mantle zone phenotype may be of pathogenic significance (Figure 1). B-1 (Ly-1) lymphocytes show this phenotype. Although characterized as fetal lymphocytes, they are found in adult patients, are important regulatory B lymphocytes in autoimmunity, preferentially undergo lambda immunoglobulin gene rearrangement, and show selective lambda light-chain expression. A large fraction of B-1 lymphocytes express a wide range of self-reactive immunoglobulins that are physiologically important for self-replication but may be associated pathologically with autoimmune diseases such as neuropathy by expressing antinuclear-associated glycoprotein specificity. Dysregulation of B-1–related lymphocytic immunity in CD also may account for other components of the POEMS syndrome, autoimmune hemolytic anemias, the predominance of lambda-restricted plasma cells, lambda-restricted paraproteinemia and proteinuria, and the
The predominance of lambda-restricted lymphomas observed in patients with PC CD (Figure 1).

The importance of B-1 lymphocytes in HV CD is suggested by a KiB3-negative mantle zone immunophenotype in lesional\textsuperscript{8,26} or regional lymphoid tissue,\textsuperscript{26} a clinical distribution of HV CD in midline anatomic areas consistent with the physiologic distribution zone of B-1 cells,\textsuperscript{34-37} and the predominance of HV CD in children.\textsuperscript{38} Additional findings that indirectly suggest a common histopathogenic link between HV CD and PC CD are the identification of rare cases of mixed HV CD and PC CD within the same lymph node or changes from one type of CD to another in sequential biopsy specimens.

Despite the suggestions of a histopathogenic link between HV CD and PC CD, their end-stage pathologic findings are fundamentally different. HV CD shows the inactive germinal centers composed of dysplastic follicular dendritic cells, altered germinal center T-lymphocytic subsets unlike the more functional, hyperplastic germinal centers of PC CD. HV CD shows an interfollicular sinus-obliterating proliferation of HV stromal and perivenular plasmacytoid monocytes, unlike the interfollicular plasmacytosis of PC CD. HV CD may be associated with the neoplastic proliferation of dendritic or vascular neoplasms rather than the development of lymphoma or plasma cell neoplasms in PC CD.

The role of HHV-8 in the pathogenesis of CD is intriguing. Our data indicate that the detection of HHV-8 genome and vIL-6 expression may not be concordant in CD and that vIL-6 expression rather than the detection of genome is important in the development of lymphoma and survival in HHV-8–infected patients with CD. The lack of correlation between HHV-8 and plasma cell monoclonality or the POEMS syndrome suggests that the oncogenesis of plasma cell neoplasia in PC CD may not be HHV-8 mediated.

Additional studies are needed that use microdissection and molecular analysis. Data presented by Dupin et al.\textsuperscript{10} indicated that plasmablastic (immunoblastic) lymphomas and single cell counterparts in the follicle mantle of HHV-8–infected patients with CD lacked somatic hypermutations of immunoglobulin variable regions, as is typical of B-1 lymphocytes.\textsuperscript{39} Whether these lymphomas or mantle cells lacked N-region diversity or restricted V-region use characteristic of B-1 cells\textsuperscript{40} was not reported. Microdissection and molecular analysis of interfollicular plasma cells in PC CD are needed to determine whether they lack evidence of somatic hypermutation, N-region diversity, or restricted V-region use, which would indicate whether they are derived from B-1 cells. If somatic hypermutations are found to be lacking in the plasma cells in CD, a derivation from B-1 lymphocytes would be suggested and would offer some explanation for the localized proliferation and limited (usually solitary) marrow spread of plasma cell neoplasms in CD compared with widespread marrow disease demonstrated by somatically hypermutated germinal center–derived plasma cells in multiple myeloma.

References


