Lack of evidence of Epstein–Barr virus infection in patients with Castleman's disease

Molecular genetic analysis

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ABSTRACT

Objective: Epstein-Barr virus (EBV) infection is associated with a diverse group of malignancies and many lymphoproliferative disorders. Castleman's disease (CD) is atypical lymphoproliferative disorder. The role of EBV in the pathogenesis of CD is not clear yet. The objective of this study is to investigate the EBV status in CD.

Methods: We searched medical records for cases of CD at the Toronto General Hospital, Toronto, Canada and King Abdulaziz University Hospital, Jeddah, Saudi Arabia. Twenty cases were found. The presence of EBV was analyzed using polymerase chain reaction. Polymerase chain reaction were performed at the Department of Pathology and Laboratory Medicine, Toronto General Hospital. The study started in 2001 and completed in 2005.

Results: The age range was 16-90 years. Seventeen patients manifested the localized form of CD. There were 11 males 9 females. Epstein-Barr virus genome was detected only in 2 cases; both were males and have plasma cell type. One is a localized type and the other is of a multicentric type. One patient revealed clonal rearrangement of the immunoglobulin H.

Conclusion: The number of cases is small; however it appears that EBV is less likely to play a significant role in the pathogenesis of CD; however, it seems to be associated with clonal progression.

Saudi Med J 2006; Vol. 27 (8): 1125-1129

Epstein-Barr virus (EBV) infection is associated with a diverse group of malignancies including nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's disease, Nasopharyngeal carcinoma, and lymphoproliferative disease. The EBV is ubiquitous worldwide, with greater than 80% of people over the age of 30 having been infected. Once EBV infection has occurred, it remains for the lifetime of the individual, making EBV one of the most persistent viruses that infect humans.[1] Castleman's disease (CD) (angiofollicular lymphoid hyperplasia) is a polyclonal heterogeneous group of lymphoproliferative disorders of uncertain cause.[2-4] Three histologic variants (hyaline vascular, plasma cell, and mixed) and 2 clinical types (localized and multicentric) of CD have been described.[5] Unlike the localized type, for which surgical excision is curative regardless of the histologic type, multicentric disease

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Received 21st February 2006. Accepted for publication in final form 26th April 2006.

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often necessitates aggressive systemic therapy and portends a poorer outcome.[5] In the few previously published studies discussing the involvement of EBV in the etiology of CD, the role of EBV was not clear. However, only few patients have been examined in those series. Whether there is any role of EBV in the pathogenesis of CD is still a matter of controversy.[6] In this study, we examined the presence of EBV in a relatively large number of patients with this rare disease.

Methods. Medical records were examined for cases of CD at the Toronto General Hospital (now University Health Network), Toronto, Canada, and King Abdulaziz University Hospital (KAUH), Jeddah, Saudi Arabia. Twenty cases were selected (18 from Toronto General Hospital, 2 from KAUH) from over a period of 8 years (1988 to 2004). Patients with HIV lymphadenopathy have been excluded. The clinical histories were reviewed. Blocks were re-cut and sections stained with hematoxylin and eosin. Frozen tissue specimens were available for 15 patients. Epstein-Barr virus status was assessed using the polymerase chain reaction (PCR), which was performed on DNA from the frozen tissue. In 5 cases DNA for PCR was extracted from paraffin embedded tissue, using methods previously described.[7] Polymerase chain reaction analysis for EBV was performed on lymph node tissues involved by CD. All PCRs were performed under standard conditions as previously described.[3,4] For PCR analysis, 500 ng was amplified using the appropriate primer electrophoresed through a 2% agarose gel and visualized using ethidium bromide. Appropriate positive, negative and internal controls were run with each specimen. Polymerase chain reactions were performed at the Department of Pathology and Laboratory Medicine, University of Toronto, Toronto, Canada.

Results. The age range was 16-90 years. There were 9 female and 11 males. Seventeen patients manifested the localized form of CD and ranged in age from 16-65 years. The clinical data of the cases and the molecular results were summarized in **Table 1**. Biopsies from patients with the localized disease showed the morphologic features of the classical hyaline-vascular variant of CD in 14 patients and the remainder 3 patients showed morphologic features characteristics of the mature plasma cell variant of CD (**Figure 1**). The multicentric CDs were all of the plasma cell type. Epstein-Barr virus genome was detected only in 2 cases out of 20 patients (**Figure 2**). Patients with EBV infection were males and aged 65 and 90 years. The first patient presented with paratracheal lymphadenopathy and the other with an axillary mass. Both patients were of the plasma cell type. The first patient was of the localized and the second patient revealed multicentric variant of CD. The first patient revealed clonal rearrangement for immunoglobulin H (IgH).

Discussion. Epstein-Barr virus was discovered 40 years ago from examining electron micrographs of cells cultured from Burkitt's lymphoma, a childhood tumor that is common in sub-Saharan Africa.[8] Epstein-Barr virus infection is associated with a diverse group of malignancies and many lymphoproliferative disorders. Despite of their diverse cellular origin, most of these malignancies share common features, including the expression of either some or all of the EBV latent proteins.[9-11] In these tumors, every tumor cell carries the virus in a latent infection but the number of normal cells infected is very low. The significance of detecting this virus in certain disease is not only as causative or prognostic factor but also because treatment that could somehow cause the elimination of EBV infected cells would be very specific for the cancer in such cases.[10,12,13] The EBV can choose between 2 alternative lifestyles; latent or lytic replication.[14] It has been demonstrated that in Hodgkin's lymphoma, EBV tumor cell presence is associated with better survival in young patients and poorer survival in older patients, independent of other factors indicating that EBV is a meaningful prognostic marker in certain tumors.[15] Epstein-Barr virus was detected in all the nasopharyngeal carcinoma samples by several techniques including PCR, in situ hybridization, and immunohistochemical methodologies.[11] Castleman's disease (angiofollicular lymphoid hyperplasia) is a heterogeneous group of lymphoproliferative disorders of uncertain cause. Recently, we demonstrated that CD is not a clonal disease and represent a reactive rather than a neoplastic disease.[2] Three histologic variants (hyaline vascular, plasma cell, and mixed) and 2 clinical types (localized and multicentric) of CD have been described.[5] Authors reported variable results regarding the role of EBV in CD.[16-25] Epstein-Barr virus genomes were detected in the small lymphocytes of 2 of the 3 cases examined by in situ hybridization studies.[23] Epstein-Barr virus was not detected by some authors.[22] In the current study, EBV genome was detected only in 2 cases out of 20. One patient with multicentric CD and the other one had a localized CD that was associated with clonal IgH rearrangement. This may suggest that EBV is associated with clonal progression in CD. Copies of EBV genome were also detected in 2 of 3 patients with CD with clonal rearrangement.[16] No

Table 1 - Summary of clinical and laboratory results of the patients with Castleman's disease.

Patient's number	Age	Gender	Location	Diagnosis	EBV
1	65	Male	Paratracheal	Localized plasma cell variant	+
2	26	Female	Paratracheal	Localized hyaline-vascular variant	-
3	26	Female	Mediastinum	Localized hyaline-vascular variant	-
4	90	Male	Axilla	Multicentric plasma cell variant	+
5	26	Female	Mediastinum	Localized hyaline-vascular variant	-
6	38	Female	Axilla	Localized hyaline-vascular variant	-
7	33	Male	Inguinal	Localized hyaline-vascular variant	-
8	28	Male	Axilla	Multicentric plasma cell variant	-
9	32	Female	Mediastinum	Localized hyaline-vascular variant	-
10	62	Female	Neck	Localized plasma cell variant	-
11	54	Male	Axilla	Localized hyaline-vascular variant	-
12	41	Male	Mesenteric	Localized hyaline-vascular variant	-
13	51	Male	Pericolic	Localized hyaline-vascular variant	-
14	15	Male	Submandibular	Localized hyaline-vascular variant	-
15	66	Female	Omentum	Localized hyaline-vascular variant	-
16	52	Female	Axilla	Multicentric plasma cell variant	-
17	30	Female	Abdomen	Localized hyaline-vascular variant	-
18	38	Female	Abdomen	Localized hyaline-vascular variant	-
19	42	Male	Abdomen	Localized hyaline-vascular variant	-
20	39	Male	Neck	Localized hyaline-vascular variant	-

(+) - Positive, (-) - Negative, EBV - Epstein-Barr virus

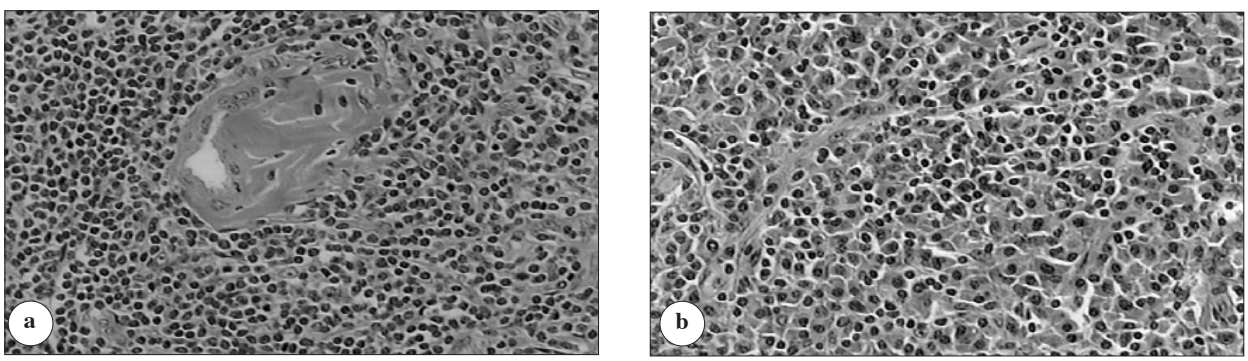

Figure 1 - Microscopic images **a)** Section from patient number one lymph node revealed plasma cell infiltrate in the interfollicular regions encasing the germinal center that revealed hyaline deposits in the germinal center. (Hematoxylin and eosin stain, original power x200). **b)** Another field from the same patient revealed sheets of plasma cells in the interfollicular regions (Hematoxylin and eosin stain, original power x200).

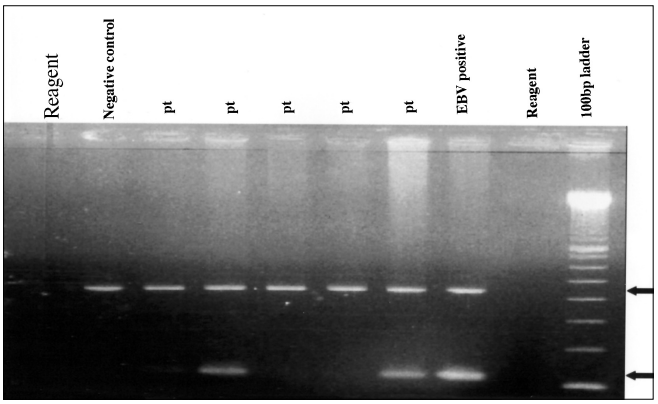

Figure 2 - Polymerase chain reaction for detection of Epstein-Barr virus (EBV) genome revealing positive results in patient 1 (to the left of the EBV positive control). The top arrow represents the internal control that is used to ensure the presence of amplifiable DNA in each sample. The rest of the patients in the figure do not belong to this series.

linkage between CD and EBV could be demonstrated immunohistochemically using an anti-latent membrane protein-1 monoclonal antibody (n = 16) or by RNA in situ hybridization with an EBV-encoded RNA transcript-specific probe (n = 13).[18] Recently, CD especially the plasma cell or the mixed type, suggested to be related to excess interleukin-6 (IL-6)-like activity. Kaposi's sarcoma- associated herpes virus (KSHV or HHV8), which encodes a functional cytokine (vIL-6) (IL-6), has been found in some patients with Castleman's disease.[26-32] Furthermore Leger-Ravet et al[30] suggested that the variable pattern of IL6 gene expression and clinicopathologic heterogeneity of CD indicating the different immune mechanism. Rare association between CD and Kaposi's sarcoma has been reported.[32-36] In Kaposi's sarcoma cells, HHV-8 is mainly latent with the expression of latent nuclear antigen-1 (LNA-1), whereas in multicentric Castleman's disease both lytic and latent antigens are produced by lymphoid cells.[27,37] Foss et al[38] demonstrated that in CD, germinal centres containing small vessels show expression of vascular endothelial growth factor (VEGF) and suggested that vascularization of germinal centres in CD may be a consequence of abnormal local expression of VEGF. It is evident from these studies that CD is characterized by 2 distinct entities. Multicentric plasma cell variant is strongly associated with infection by human herpesvirus 8 (HHV-8), but the pathogenesis of the localized hyaline vascular variant is still unknown. Our study failed to demonstrate EBV in any case of the localized hyaline vascular variant. Our current results indicate that EBV is less likely to play a significant role in the pathogenesis of CD; however, it seems to be associated with clonal progression. This is in keeping with other authors.[16,18,21,39]

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