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Proposed Guidelines for Diagnosing Chronic Active Epstein-Barr Virus Infection

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Since the initial report of unusual manifestations possibly associated with chronic active Epstein-Barr virus (EBV) infection (CAEBV), nearly three decades have passed. During this period, reported cases with this entity have dramatically increased in the world. Additionally, recent development of diagnostic procedures, including molecular biological and immunological techniques, have provided us with the ability to define certain diseases, especially malignant disorders. Guidelines, derived mainly from the current literature and recent experiences with CAEBV in Japan, for diagnosing CAEBV are proposed to clarify this enigmatic disease. Am. J. Hematol. 80:64–69, 2005. © 2005 Wiley-Liss, Inc.

Key words: guidelines; diagnosis; chronic active Epstein-Barr virus infection

Epstein-Barr virus (EBV), one of eight known human, ubiquitous herpesviruses, often causes symptomatic diseases [1]. These diseases include infectious mononucleosis (IM) and the lymphoproliferative disorder (LPD) in immunologically compromised individuals. Additionally, EBV is etiologically linked to human malignancies such as endemic Burkitt lymphoma (BL) and undifferentiated nasopharyngeal carcinoma (NPC). Recently, EBV association with various etiologically unknown diseases has been widely documented because of the presence or increase of viral genomes in affected lesions, whose detection has been made possible by the development of molecular, biological, and immunological methods [2].

In 1975, Horwitz et al. first described cases with unusual or protracted clinical manifestations having high IgG antibody titers against EBV-replicating antigens such as viral capsid antigen (VCA) and early antigen-restricted molecules (EA-R) [3]. The patients had IM-like chronic symptoms characterized by persistent or intermittent fever and lymphadenopathy. In 1982, Tobi et al. further reported similar atypical illnesses associated with serological evidence of persistent EBV infection [4]. Following these

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reports, Dubois et al. proposed the term "chronic mononucleosis syndrome" to describe such cases [5]. The authors emphasized the absence of malignancy, autoimmunity, or profound immunodeficiency in patients with this syndrome. Jones et al. and Straus et al. reported large numbers of patients as part of this entity [6,7]. Eventually this enigmatic disease was defined as chronic, symptomatic EBV infection [8].

In contrast, in 1978, Virelizier et al. reported a case with severe lymphoproliferation having extraordinarily elevated IgG antibody titers against EBV–VCA and EA [9]. The affected girl had a chronic disease characterized by persistent high fever, massive lymphadenopathy, interstitial pneumonitis, thrombocytopenia, and polyclonal hypergammaglobulinemia. In 1988 and in 1991, diagnostic criteria were independently proposed for this unique type of serious illness as severe, chronic EBV infection, or as severe chronic active EBV infection syndrome (SCAEBV), respectively [10,11]. The majority of patients with this syndrome have a poor prognosis, and some cases are now thought to be linked to the neoplastic smoldering process highly associated with certain EBV-associated T-cell lymphomas [12–14]. Severe, chronic EBV infection or SCAEBV could be diagnosed by using the previously proposed criteria with minor revisions [15].

However, in cases diagnosed as CAEBV, confusion persists as to diagnosis because of varying clinical manifestations, outcomes, and association with certain underlying diseases, mainly LPD derived from T-cell or NK-cell lineages [16–19]. We recently demonstrated in a national Japanese survey that patients with CAEBV had poorer outcomes when associated with late onset of disease, thrombocytopenia, and EBV infection of T cells [20].

New diagnostic methods for EBV infection have now been developed that define more precise pathogenetic mechanism(s) of EBV-associated diseases [2]. These diagnostic methods identify the presence of EBV variants, distinct target cell populations associated with different viral gene expressions, and various abnormalities of immune surveillance, including the involvement of certain cytokines.

Here, we propose diagnostic criteria for CAEBV, according to our current state of knowledge and the recent experience in Japan, to improve the diagnostic specificity of this enigmatic disease. In particular, underlying diseases should be diagnosed accurately, and when the associated disease is defined, the name of each disease should be used rather than "CAEBV."

Proposed diagnostic guidelines and recommended specific laboratory tests are shown in Tables I and II, respectively.

Additionally, we briefly review clinical features, virological studies, pathological findings, immunological studies, etiology, and possible treatment in patients with CAEBV to promote further recognition and definition of this disease.

TABLE I. Proposed Guidelines for Diagnosing CAEBV*,†

(1) Persistent or recurrent IM-like symptom
(2) Unusual pattern of anti-EBV antibodies with raised anti-VCA and anti-EA, and/or detection of increased EBV genomes in affected tissues, including the peripheral blood
(3) Chronic illness which cannot be explained by other known disease processes at diagnosis[a]

*A case of CAEBV must fulfill each category.
†Abbreviations: CAEBV, chronic active Epstein-Barr virus infection; IM, infectious mononucleosis; VCA, viral capsid antigen; EA, early antigen; LPD, lymphoproliferative disorder.
[a]An EBV-associated disease such as hemophagocytic lymphohistiocytosis or LPD/lymphoma mainly derived from T-cell or NK-cell lineage often develops during the course of illness. Some patients also suffer from cutaneous lesions, such as hypersensitivity to mosquito bites.

TABLE II. Supplemental Findings and Recommended Specific Laboratory Tests*

(1) IM-like symptoms generally include fever, swelling of lymph nodes, and hepatosplenomegaly; additional complications include he matological, digestive tract, neurological, pulmonary, ocular, dermal, and/or cardiovascular disorders (including aneurysm and valvular disease) that mostly have been reported in patients with IM
(2) Anti-EBV antibodies with raised anti-VCA and anti-EA ordinarily consist of VCA-IgG $\geq$ 1:640 and EA-IgG $\geq$ 1:160; positive IgA antibodies to VCA and/or EA are often demonstrated
(3) Recommended specific laboratory tests
  (a) Detection of EBV DNA, RNA, related antigens and clonality in affected tissue including the peripheral blood
    (i) PCR (quantitative, qualitative)
      More than $10^{2.5}$ copies/μg DNA are generally detected in peripheral blood mononuclear cells; healthy individuals occa sionally show positive results by qualitative PCR analysis
    (ii) *In situ* hybridization (e.g., EBERs)
    (iii) Immunofluorescence etc. (e.g., EBNA, LMP)
    (iv) Southern blotting (including clonality of EBV)
    (v) Clarifying target cells of EBV infection
      Double staining of EBNA or detection of EBER or EBV DNA with each marker for B, T, NK cells or monocytes/ macropharge/histiocytes is recommended by using such methods as immunofluorescence, immunohistological staining, or magnetic beads
  (b) Histopathological and molecular evaluation
    (i) General histopathology
    (ii) Immunohistological staining
    (iii) Chromosomal analysis
    (iv) Rearrangement studies (e.g., immunoglobulin, T-cell receptor)
  (c) Immunological studies
    (i) Generalized immunological studies
    (ii) Marker analysis of peripheral blood (including HLA-DR)
    (iii) Cytokine analysis

*Abbreviations: IM, infectious mononucleosis; EBV, Epstein-Barr virus; VCA, viral capsid antigen; EA, early antigen; PCR, polymerase chain reaction; EBERs, EBV-encoded RNAs; EBNA, EBV-determined nuclear antigen; LMP, latent membrane protein; HLA, human leukocyte antigen.

CLINICAL FEATURES

The following clinical features are commonly noted in patients with CAEBV [19–21]. Symptoms are persistent and chronic and generally consist of prolonged or intermittent fever, lymphadenopathy, and/or hepatosplenomegaly. Other symptoms, such as recurrent or continuous debilitating fatigue, sore throat, lymph node tenderness and pain, headache, myalgia, and arthralgia, can be encountered in these patients. Many other complications, which have been reported in patients with IM, can also be seen, such as hematological, digestive tract, neurological, pulmonary, ocular, dermal, and/or cardiovascular disorders [2,19]. In cardiovascular disorders, coronary aneurysms or valvular disease observed in some patients with CAEBV are of particular interest [21]. No apparent features of a known underlying disease are noted at the time of diagnosis. However, during the course of illness, certain diseases such as EBV-related hemophagocytic lymphohistiocytosis, T-cell or NK-cell LPD/lymphomas occasionally develop [19], although EBV-related hemophagocytic lymphohistiocytosis generally occurs during the primary infection with EBV [22,23]. Caution should be exercised in diagnosing CAEBV initially in the presence of these diseases. Skin lesions such as hypersensitivity to mosquito bites were characteristic in some patients with CAEBV who may suffer from the granular lymphocyte proliferative disorder or clonal T-cell lymphoproliferation [21,24–26], and various cutaneous manifestations, including hydroa vacciniforme-like eruptions, were recently reported to be associated with T-cell or NK-cell LPDs having chronic activated EBV infections [27].

VIROLOGICAL STUDIES

Great progress has been made in virological studies of CAEBV, primarily due to the development of molecular biological procedures [1,2]. For example, in the past decade immunofluorescence was used to detect EBV-related antigens such as EBV-determined nuclear antigens (EBNA). Now, detection of the EBV genome by molecular techniques such as the polymerase chain reaction (PCR) and in situ hybridization targeted EBV-encoded RNAs (EBERs) has become available. Although the presence of the EBV genome does not always identify it as the causative agent, these sensitive methods of EBV detection have allowed us to define many diseases of unknown etiology as being the result of EBV infections. Moreover, viral loads of more than $10^{2.5}$ copies/μg DNA have been demonstrated in the peripheral blood mononuclear cells of patients with CAEBV [28,29]. Because CAEBV has been thought to be linked to viral replication, quantitative analysis of genes responsible for the lytic part of the life cycle of the virus may be useful in the study of the disease in the future [30]. Distinct targeting of cells involved in EBV infections other than human B lymphocytes or epithelial cells is now possible using techniques such as double-staining, in situ/immunohistochemistry, and magnetic beads procedure [2,31]. To date, no disease-specific virus has been isolated or recognized, although some variants were reported including variants of EBNA-1, EBNA-2, or latent membrane protein (LMP)-1 coding regions, or lytic viral strains [1,2]. Regarding virus variants, when data are available from disease-derived virus strains in the absence of geographically matched control isolates, caution must be exercised to confirm the disease-specific virus [32].

Unfortunately, antibody titers obtained by different laboratories are not comparable because the immunofluorescence tests are subjective and depend upon such factors as the quality of the fluorescence microscope used and the source of reagents. Nevertheless, EBV serologic tests generally reveal high IgG antibody titers against EBV VCA ($\geq$1:640) and EA ($\geq$1:160) in each laboratory in patients with CAEBV, although an increase of only circulating EBV DNA was shown in some patients [19]. The patients often had IgA antibodies against VCA and/or EA. Various antibody titers against EBNA were reported, ranging from nondetectable to increased levels. Additionally, a homogeneous, episomal population of EBV is often seen in affected lesions and/or the peripheral blood, suggesting a single progenitor cell being infected by EBV, especially in cases with a poor prognosis, possibly due to a neoplastic process [33].

Six different EBNAs, three LMPs, and two EBERs have been thought to be associated with latent EBV infections [1,2]. Interestingly, expressions of these latent proteins and RNAs were different in each EBV-associated disease at tissue levels [2]. Only EBNA-1 and EBERs are expressed in EBV genome-positive BL (latency I). In cases with EBV genome-positive NPC, Hodgkin disease (HD), or T-cell or NK-cell lymphoma, EBNA-1, EBERs, and LMPs are generally expressed (latency II). In contrast, EBV-LPD, which occurs in immunologically compromised individuals, expresses all series of EBNAs, LMPs, and EBERs (latency III). EBNA-2, the EBNA-3 family, and LMP-1 are thought to be major targets of EBV-specific cytotoxic T lymphocytes (EBV-CTL) [2]. In cases of CAEBV, especially of the severe type, products of latency II or III were shown using immunohistochemical and/or immunoblotting methods in affected tissues, although heterogeneous restricted patterns were also reported [15,34],

suggesting both abnormal cellular regulation and defective or unbalanced immunosurveillance in patients with CAEBV.

PATHOLOGICAL FINDINGS

Patients with CAEBV need not show any malignant pathological abnormalities in affected tissues at the time of diagnosis. Some patients have increased numbers of large granular lymphocytes positive for CD16, CD56, and human leukocyte antigen (HLA)-DR or increased T cells positive for CD4 or CD8 and HLA-DR in their circulation [24,25]. Additionally, some patients develop oligoclonal or monoclonal lymphoproliferation, including T-cell or NK-cell proliferation, eventually resulting in T-cell or NK-cell malignant lymphomas [2,11,16–18]. Anecdotally, malignancies such as B-cell neoplasms and HD-like disease were also reported [34–36]. Although no specific chromosomal abnormality was observed in analyses of patients' peripheral blood mononuclear cells [19], unique abnormalities in the 6q region were reported frequently in affected tissues or cell lines derived from patients with CAEBV, in particular by those who developed NK cell LPD [37,38]. Recently, novel mutations in both alleles of the perforin gene were shown in a patient with CAEBV who developed hemophagocytic lymphohistiocytosis, suggesting that mutations may be responsible in some cases [39]. There were no responsible gene mutations in X-linked lymphoproliferative diseases in the patients that were studied [40].

Patients with EBV-associated peripheral T-cell and NK-cell LPD/lymphomas have clinical and laboratory findings similar to those of CAEBV [41]. Plasma EBV DNA was reported to be significantly increased in patients with EBV-positive T-cell and NK-cell lymphomas [42]. Indeed, EBV association is clearly noted in some T-cell and NK-cell LPD/lymphomas [43,44]. Therefore, in pathologically confirmed cases, it is strongly recommended to refrain from using the CAEBV nomenclature and to assign the "name to the underlying disease associated with chronic activated EBV infection" instead. "CAEBV" should refer only to cases without an underlying disease at the time of diagnosis.

IMMUNOLOGICAL STUDIES

Immunosurveillance against EBV infection includes neutralizing antibodies, NK cells, antibody-dependent cell-mediated cytotoxicity, and EBV-CTL [1,2]. Among them, EBV-CTL is considered the most important in the regulation of infected cell proliferation. There have been many approaches to clarify the underlying immunological abnormalities in patients with CAEBV. No consistent immunological abnormalities, however, have been noted to date. Nevertheless, as described previously, the degree of expression of latent EBV genes in tissues suggests both cellular and immunological abnormalities in patients with CAEBV. Some patients demonstrated increased interleukin (IL)-10 activity in their sera, indicating that T-helper (Th)2 cells were predominantly activated [45]. In contrast, activated T cells having high levels of EBV DNA were associated with an increased expression of Th1 and Th2 type cytokines, suggesting the presence of an unbalanced cytokine profile [46]. Additionally, recent studies showed that cells infected with EBV in patients with CAEBV were not positive for CD8, suggesting a deficiency of cytotoxicity as the causative role of the immunological disturbance [17,31].

ETIOLOGY

Despite many studies, the cause of CAEBV remains unclear. The magnitude of the EBV antibody response to EBV-replicating antigens and/or the increased viral load in tissues, including peripheral blood, suggest the dual possibilities of unusual EBV replication and aberrant cell proliferation of EBV-infected cells as major factors in the development of this disease. Therefore, the study of the pathogenetic mechanisms responsible for EBV activation and/or lymphoproliferation of distinct EBV-infected cells seems to be a major key to our understanding of the pathogenesis of CAEBV. Additionally, mechanisms that should be investigated include the presence of different EBV gene products involved in cellular transformation, variation of EBV strains, distinct target cell populations, and/or selective recruitment of other supportive cell types as cofactors in the heterogeneity of CAEBV. It has recently been reported that the trans-synaptic acquisition of the EBV receptor, CD21, from EBV-infected B cells might be responsible for the unusual mode of infection for NK cells [47]. Furthermore, it has been shown that a recombinant EBV has the capacity to enter human NK cells in vitro, associated with both latent and lytic infections [48]. These novel observations are attractive but require further valid investigation.

TREATMENT

The majority of the patients with CAEBV suffer from an IM-like chronic illness. Some develop serious diseases such as EBV-related hemophagocytic lymphohistiocytosis or T-cell or NK-cell LPD/lymphomas during the course of illness described previously.

Many therapies including antiviral agents, immunomodulative therapy such as interferon gamma, IL-2, corticosteroids, cyclosporin A or immunoglobulins, and/or chemotherapeutic drugs have been tried without obvious effect on morbidity and outcome [49]. Recently, autologous EBV-CTL were given successfully in a series of patients with CAEBV [50]: 4 of 5 patients did not show a relapse of their disease during the period of observation. Eventually, allogeneic peripheral blood or bone marrow stem-cell transplantation may be the treatment of choice because successful results were recently reported in patients with severe disease [16,35,51]. Further clinical studies should be done to evaluate and improve on these approaches.

CONCLUDING REMARKS

The development of CAEBV seems to be linked to activated EBV infections, which result initially in an IM-like illness, although the precise pathogenetic mechanisms underlying the progression to CAEBV remain unclear. Furthermore, certain underlying diseases may lead to a chronic activated EBV infection, resulting in a misdiagnosis of CAEBV instead of the underlying disease. This paper provides guidelines for the diagnosis of CAEBV and the recognition of its relationship to other disease entities.

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