Periodic Illness Associated with Epstein-Barr Virus Infection


A 15-year-old boy with a 13-year history of periodic fevers, lymphadenopathy, and leukocytosis showed virological, serological, immunohistologic, and molecular evidence of persistent, active, Epstein-Barr virus (EBV) infection. Acyclovir and several other agents failed to alter his clinical course. Comprehensive immunological studies could not identify a defined immune deficiency syndrome to explain the persistent infection, although he does continue to have circulating polymeric EBV-specific immunoglobulin type A, as is seen in individuals during acute EBV infections. In vitro work suggests that this polymeric antibody prevents B cell infection by EBV. Cumulative data suggest that this patient suffers from a novel form of EBV infection.

"Periodic disease" was defined by Reimann in the 1940s as a heterogenous group of "benign syndromes which last for several days and recur for years" in otherwise healthy individuals [1]. Reimann remarked that the adjectives periodic, cyclic, episodic, and recurrent are used interchangeably, denoting syndromes of both fixed and varying cycle length [1].


Subsets of this syndrome were described further as familial Mediterranean fever and the similar syndrome familial hibernian fever, cyclic neutropenia, hyperimmunoglobulinemia D and periodic fever syndrome, hereditary angioedema, and episodic angioedema [2-6]. FAPA, an acronym for the syndrome of recurrent fever, aphthous stomatitis, pharyngitis, and adenitis, was described as occurring in 13 children who developed normally despite frequent illness [7]. The pathogenesis of that syndrome is unknown, and no effective treatment has been established, although there are reports of the successful use of prednisone and cimetidine in some cases [7, 8]. Also to be considered in the evaluation of these syndromes is psychogenic fever, which can manifest as periodic febrile episodes elicited by stressful situations and hyperventilation [9].

We present a case in which episodes of documented fever, generalized lymphadenopathy, and occasional splenomegaly developed at intervals averaging every 2-3 weeks for more than 10 years. The clinical presentation of this patient does not fit well into the recognized categories of periodic febrile illnesses. Diverse serological, virological, and molecular data are provided that reveal an association between the illness of this child and active Epstein-Barr virus (EBV) infection.

Case Report

The patient is a 15-year-old male. He is of Western European ethnicity, and the family history is unremarkable. Specifically, there is no history of leukemia, lymphoma, or X-linked lymphoproliferative disorder. The patient has two half-siblings who are healthy. His mother's pregnancy was uncomplicated, and the patient received all routine immunizations. Prior to 18 months of age, he had several brief but unremarkable illnesses. At age 19 months he had a 2-day illness marked by fever (temperature to 104°F), diffuse adenopathy that was most pronounced in the right cervical chain, and minor splenomegaly. Reexamination 10 days later revealed complete recovery. Since that time, the patient has had recurrent episodes of fever accompanied by adenopathy and splenomegaly, occurring at fairly regular intervals, averaging 2-4 weeks and never exceeding 12 weeks.

Typically, these episodes are heralded by 24 hours of malaise, irritability, and abdominal discomfort, followed by the acute onset of spiking fever (peak temperature, 102°F to 104°F), tender diffuse lymphadenopathy, and splenomegaly, all lasting only 2-3 days. During untreated episodes, leukocytosis (with a shift toward band forms), mild anemia, and reticulocytosis develop. No atypical lymphocytes, no thrombocytopenia, and no abnormalities in electrolyte levels, creatinine concentration, or liver function are discerned. Results of repeated bacterial, viral, and fungal cultures and numerous serological tests have been unremarkable, with the exception of findings of EBV-specific studies (table 1).
Table 1. EBV-specific serological findings for a child with recurrent fever, adenopathy, and splenomegaly.

<table>
<thead>
<tr>
<th>Year</th>
<th>VCA IgM</th>
<th>VCA IgG</th>
<th>VCA IgA</th>
<th>EA-R</th>
<th>EBNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>32</td>
<td>320</td>
<td>640</td>
<td>40</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>1987</td>
<td>32</td>
<td>320</td>
<td>160</td>
<td>20</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>1989</td>
<td>32</td>
<td>320</td>
<td>ND</td>
<td>20</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>1991</td>
<td>16</td>
<td>640</td>
<td>ND</td>
<td>20</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>1992</td>
<td>16</td>
<td>320</td>
<td>ND</td>
<td>20</td>
<td>&lt;2.5</td>
</tr>
</tbody>
</table>

NOTE. Numbers shown are reciprocal values. EA-R = early antigen-R component; EBNA = Epstein-Barr virus nuclear antigen; EBV = Epstein-Barr virus; ND = not determined; VCA = viral capsid antigen.

At three years of age (1982), a heterophile screen was weakly positive during one typical episode. At age 3 1/2 years, immunoglobulin levels, complement levels, and findings of mitogen stimulation studies were normal. Findings of a lymph node biopsy were considered unremarkable. The patient continued to have monthly episodes until the age of 5 years, when he underwent tonsillectomy, adenoidectomy, and placement of bilateral tympanostomy tubes. Symptoms resolved for 3 months but then returned at biweekly intervals. Evaluation at another hospital revealed normal serum protein electrophoresis and normal phagocyte chemotaxis. A quantitative immunoglobulin determination, however, showed abnormally high serum IgA levels, ranging from 254 mg to 880 mg, as measured on three occasions. A repeated lymph node biopsy revealed reactive hyperplasia with focal neutrophilic microabscesses as well as perinodal plasma cellular infiltration and chronic inflammation. Special stains of nodal tissue showed no evidence of acid-fast bacilli or of fungal or bacterial elements. A bone marrow biopsy revealed moderate hypercellularity with all elements present and mild myeloid hyperplasia. A diagnosis of variant chronic granulomatous disease was suggested, and the patient began taking trimethoprim/sulfamethoxazole three times a week, but this had no apparent impact on his illness.

At age 6 1/2 years, the patient was referred to the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (NIH) for further evaluation. Examination revealed a healthy-appearing child whose height and weight were at the fifth percentile for his age. He had no fever, lymphadenopathy, or hepatosplenomegaly. Routine laboratory studies were again performed, but the results were normal. Hepatitis B surface antigen, heterophile, and antinuclear antibodies were not detected; rheumatoid factor was detected at a dilution of 1:20. T lymphocyte helper and suppressor subsets were normal in number, ratio, and function. Abdominal ultrasonography showed no abnormalities of the spleen, pancreas, kidneys, liver, or gallbladder. Treatment with colchicine was initiated empirically and continued for 1 month; however, there was no change in the patient’s course, and the therapy was discontinued.

At age 7 years the patient had a typical episode that was witnessed during a routine NIH follow-up visit. Examination revealed a temperature of 104°F, diffuse tender lymphadenopathy that was most pronounced in the right cervical chain, and tender splenomegaly extending 2 cm below the left costal margin. Laboratory measures during the episode showed leukocytosis (17.8 x 10^9 leukocytes/L; 53% mature neutrophils, 28% band forms, 6% monocytes, and 13% lymphocytes) and no atypical lymphocytes. All other chemistry values and cell counts were normal. A lumbar puncture was performed, and the CSF was unremarkable; bacterial, viral, and fungal cultures all yielded no growth. A cervical lymph node biopsy performed during the same episode revealed reactive lymphoid hyperplasia with mixed immunologic phenotype, with 1%–5% of cells positive for EBV nuclear antigen (EBNA) (see below). Repeated throat cultures performed during and in between episodes yielded EBV. On the basis of these and EBV-specific serological findings, a trial of acyclovir (Burroughs Wellcome, Research Triangle Park, NC) was begun, with dosage escalation to 600 mg (30 mg/kg) three times a day. At this dosage the patient’s mother reported a change in the character of his episodes: they no longer included lymphadenopathy. However, the biweekly occurrence of fever, malaise, and abdominal pain continued. Acyclovir levels were not determined. Treatment ended after 4 months.

At age 7 1/2 years (December 1986), the patient developed pneumonia complicated by respiratory failure. No causative infectious agent was discovered, and the patient’s recovery was associated with the concomitant use of high-dose steroids and intravenous immunoglobulins. Monthly intravenous immunoglobulin therapy was continued until October 1987; no benefit was noted. After recovery, therapy with prednisone was continued, with dosings every other day until December 1988. During this time, cyclic fevers and adenopathy continued; however, discrete episodes appeared to be abbreviated by the use of higher-dose pulses of prednisone with a 3-day taper to the alternate-day schedule. After the discontinuation of the every-other-day prednisone regimen, the patient continued to use pulsed steroids during each episode, and he reported a reduction in the severity and duration of symptoms. Data recorded in a diary and noted during episodes witnessed at the NIH while the pulsed prednisone regimen was in use showed peak temperatures of only 100.5°–101°F.

From July through September 1990, the patient was prescribed cimetidine on the basis of its reported value in cases of the FAPA syndrome; however, there was no change in the frequency or character of the patient’s episodes [8]. In March 1991 therapy with recombinant INF-α was tried, with dose escalation to 2 million units subcutaneously three times a week. No benefit was noted, and the therapy was discontinued in May 1991. During June through September 1992, the patient again received acyclovir (800 mg four times a day), in addition to prednisone (taken as needed), but there was no obvious further change in the number or character of his episodes. The patient continues to take pulses of prednisone (40 mg per day for 3
days) at the onset of episodes, which now occur at irregular intervals of 1–4 weeks.

Methods

Immune Studies

Lymphocyte phenotype, monocyte chemotaxis, and phagocytosis studies were conducted and enzyme level determinations were made as previously described [10, 11].

EBV Studies

Titers of EBV-specific antibodies to the viral capsid antigen (VCA), early antigen (EA) complex, and nuclear antigens were determined by standard methods [12]. Specimens were absorbed with either protein A or protein G prior to determination of IgM titers. Tests for antibodies to EBV-specific antigens EBNA-1 and EBNA-2 and to the viral latency-disrupting protein, ZEBRA, were performed as previously described [13, 14]. A touch imprint of a biopsied lymph node was tested for the presence of EBNA [12]. Dimeric anti-VCA IgA levels were determined as described elsewhere [15].

EBV was cultured from throat washings and peripheral blood mononuclear cells in RPMI 1640 media, with 10% fetal bovine serum and supplemental antibiotics, at 34°C. Cells were aged 10 days and then clarified for 30 minutes at 3,000 rpm at 5°C in a Beckman TJ6 centrifuge. Viral titers were determined as described [16].

In Situ Hybridization

The RNA in situ hybridization technique was recently described in detail [17]. In brief, 5-μm sections of paraffin-embedded tissue were prepared on sialanated slides. Tissue sections were deparaffinized, rehydrated, permeabilized with a nonionic detergent, and digested with proteinase K (10 μg/mL). An EBV-specific, digoxigenin-labeled riboprobe was applied in a formamide buffer, and the slides were hybridized overnight. Following stringent posthybridization washes, an anti-digoxigenin alkaline phosphate antibody conjugate was applied to a formamide buffer, and the slides were hybridized overnight. Following stringent posthybridization washes, an anti-digoxigenin alkaline phosphatase antibody conjugate was applied to each slide. The slides were then washed and placed in a color developing solution consisting of nitro blue tetrazolium and X-phosphate. The reaction was stopped by a brief washing of the slides in an appropriate buffer. The slides were counterstained with eosin, and coverslips were applied.

The integrity of the RNA in each tissue section was confirmed with a digoxigenin-labeled riboprobe (105 bp) directed at an abundant cellular mRNA transcript for a small nuclear ribonucleoprotein, snRNP U6 (courtesy of Dr. Richard Ambinder, Johns Hopkins University School of Medicine, Baltimore). Sections showing hybridization signal with the U6 probe were determined to be adequate for analysis with the EBV-encoded RNA (EBER1) probe. A control slide prepared from a paraffin-embedded tissue block (containing EBV-positive, nasopharyngeal carcinoma metastatic to lymph node) accompanied each hybridization run.

Immunophenotypic Studies

Immunophenotypic studies were performed with use of fixed, paraffin-embedded tissue sections and an avidin-biotin immunoperoxidase method previously described [18]. The antibody panel used for each biopsy included L26 (CD20), A6 (CD45RO), BERH2 (CD30), anti-CD3 (DAKO, Carpinteria, CA), and Leu22 (CD43) (Becton Dickinson, San Jose, CA). An extensive antibody panel directed at T cell–associated, B cell–associated, macrophage-associated, and natural killer cell–associated antigens was used in frozen section immunohistochemistry, as previously described [19].

Results

The pattern of the child’s illness is revealed by the careful diaries maintained by his mother. For example, he had 21 febrile episodes recorded between February 1988 and February 1989. They averaged 2.1 ± 0.2 (SEM) days in duration and recurred at intervals of 16.0 ± 3.2 days. Routine cell counts and chemistry values before and following a typical febrile episode witnessed at the NIH Clinical Center in July 1986 were remarkable only for an elevated level of alkaline phosphatase (193 U/L and 222 U/L, respectively). Cell counts during a typical episode were notable for an elevated WBC count (17,800/mm³) and an increase in immature band forms (28%). Total immunoglobulin levels were normal, with the exception of a moderate elevation of IgA levels (398–631 mg/dL) that was sustained over the observed time period (1985–1992). Nasal secretions showed normal levels of total and secretory IgA (kindly assessed by Drs. Martha White and Michael Kaliner). Serum IgD and IgE levels were normal in 1985 at 74 mg/L and 280 mg/L, respectively. Lymphocyte phenotype studies performed in 1986 and 1987 reflected normal B, T, and NK cell subsets. Macrophage superoxide production and myeloperoxidase levels were normal. Monocytes demonstrated normal chemotaxis to Escherichia coli–activated serum, casein, and N-formyl-methionyl-leucyl-phenylalanine. Phagocytosis of latex beads and numbers of esterase- and myeloperoxidase-positive cells were normal.

Serial EBV-specific serum antibody titers are summarized in table 1. The initial determination in 1986 was remarkable for a low-positive titer of IgM antibodies to VCA and low titer or absence of antibodies to EBNA, consistent with a recent primary infection. The high titers of IgA antibody to VCA were unusual. Subsequent serological determinations were performed in parallel with anticomplementary immunofluorescence assay and showed persistently low-positive titers of IgM
antibody to VCA and EA and the absence of antibodies to EBNA, findings no longer compatible with a normal serological response to infection. Antibodies to EBNA-1 were detected, however, with use of cell lines overexpressing the antigen; antibodies to EBNA-2 and ZEBRA were absent. In addition, dimeric IgA antibody to VCA was detected in the patient’s serum in 1986 and 1990. Dimeric anti-VCA IgA was not detected in the patient’s throat washings on either occasion.

EBV was successfully isolated in 14 (93%) of 15 cultures of the patient’s throat washings, obtained during febrile episodes. Most throat cultures (seven of nine) were also positive during asymptomatic periods. Serial dilutions and cultivations of the patient’s peripheral blood mononuclear cells on one occasion revealed that ~1 per 10^7 cells would spontaneously yield EBV-transformed B cell lines, a normal value.

Lymph node biopsies were performed in 1985 and again in 1987. On both occasions, immunoperoxidase studies demonstrated preservation of the lymph node architecture and expansion of follicular, paracortical compartments, consistent with a reactive pattern (not shown). The lymph node follicles stained with L26 (CD20), whereas interfollicular lymphocytes stained primarily as T cells showing immunoreactivity for CD3, Leu22 (CD43), and A6 (CD45RO). BERH2 (CD30), a marker of activated lymphocytes, was negative. T cell subsets were identified in normal numbers and distribution.

EBV-specific RNA and antigens were detected in lymph nodes. By in situ hybridization, EBV-encoded RNA was identified in scattered, small interfollicular cells, focally numbering 2–4 per high-power field in paraffin-embedded tissues taken in 1985 and again in 1987. The integrity of the cellular RNA was confirmed by the observation of strong hybridization signals in all cells with use of the U6 riboprobe. A touch imprint of fresh lymph node tissue obtained in 1987 showed that 1%–5% of cells expressed EBNA (figure 1). Lymph nodes from healthy, EBV-seropositive individuals show little or no evidence of EBV-encoded RNA on in situ hybridization or of EBNA on touch preparations following convalescence from infection.

Discussion

Our patient’s recurrent and episodic manifestations of fever, malaise, and lymphadenopathy remain enigmatic in the face of normal immunologic indices. The child’s history, his family history, and extensive immunologic testing do not suggest a recognized immunologic disorder.

Findings of EBV-specific studies repeated on numerous occasions over the 9-year period of observation, though, are remarkable and reveal a unique pattern of abnormal host response to EBV. Abnormalities were documented in terms of serological responses to EBV, rates of virus shedding, and persistence of viral antigens and RNA in this child’s tissues.

Serial EBV-specific antibody assays show continually low-positive titers of IgM antibody to VCA and low to absent titers of antibody to EBNA. The persistence of anti-EA responses at low titers, as seen in this child, is unremarkable [20]. The continued presence over the years of EBV-specific IgM and the inability to mount substantive titers of antibody to EBNA are recognized, however, in the context of chronic EBV infection [21–24]. EBNA-reactive antibodies were not detected by classic anti-complementary immunofluorescence assays, but responses to EBNA-1 were detected with use of cell lines overexpressing the protein [14]. Prior reports suggest that patients with chronic EBV infection preferentially lack antibodies to EBNA-1 rather than antibodies to EBNA-2, but this finding has not been consistent [25, 26]. This patient’s pattern of expression of antibody to specific EBNA and ZEBRA has been
seen in healthy persons and is distinct from the pattern in persons with classic EBV-associated lymphoproliferative disorders, who exhibit autoantibodies to ZEBRA and EBNA-2.

Persistence of IgA anti-VCA responses has been reported in association with chronic EBV infection but also in cases of nasopharyngeal carcinoma, oral hairy leukoplakia, and interstitial pneumonitis attributed to EBV in immunocompromised children, and it may reflect chronic viral antigen presentation at salivary or pulmonary mucosal surfaces [15]. The mere shedding of EBV in the oropharynx is not sufficient to sustain IgA anti-VCA responses [15].

Our patient's IgA anti-VCA response is all the more remarkable because the titer exceeded that of all other EBV-specific antibodies and also because of the persistence of polymeric immunoglobulin directed against EBV, an acute-phase reactant in serum. Polymeric IgA is usually found at the mucosa and, when complexed to EBV, facilitates epithelial infection via secretory component-mediated transcytosis [15]. Rates of pharyngeal excretion in our patient are extraordinarily high (21 of 24 instances), certainly exceeding the average shedding rate in healthy persons (~20%) and even exceeding those seen on average in transplant recipients and patients with AIDS [25, 27–29]. EBV infection of B lymphocytes in vitro is blocked by polymeric IgA [15]. These results could explain our patient's high rates of salivary excretion of the virus but normal proportions of peripheral blood mononuclear cells bearing EBV (1 per 10⁷ mononuclear cells).

On two widely separated occasions (1985 and 1987), the patient's lymph nodes were positive for EBER1 by in situ hybridization, and on one occasion a touch preparation revealed EBER staining as well. EBERs are expressed during latency and do not encode proteins. They can be detected, usually in a higher proportion of cells, in patients with Burkitt's lymphoma, nasopharyngeal carcinoma, lymphoproliferative disorders, and Hodgkin's disease [30]. EBNA-1 protein maintains EBV latency in B cells. It is detectable in those with Burkitt's lymphoma, nasopharyngeal carcinoma, and lymphoproliferative disorders [31]. Neither the EBERs nor the EBNA proteins are reliably detected in lymph nodes of healthy, EBV-seropositive people.

The cumulative data indicate that our patient suffers from a novel form of chronic EBV infection. No immune deficiency was detected, but virus-specific, cytotoxic T lymphocyte studies have not been done. NK cell number and function were normal in our patient, unlike in many other reported cases [32–34], which also is in keeping with the relatively low number of EBV-containing mononuclear cells detected in the patient's peripheral blood.

The periodicity of this patient's illness is a curious phenomenon and may be related to epithelial cell turnover in the oral mucosa or to B cell kinetics. Polymeric IgA present in the patient's serum may inhibit normal B cell infection and presentation of EBV, permitting recurrent, symptomatic oropharyngeal infection. One may postulate that the polymeric IgA found in this patient's serum does not completely preclude B cell immortalization and that, over time, this patient's disease manifestations will disappear.

The illness this patient exhibits, however, is compatible with a robust immune response rather than an immune deficiency. There is no progressive B cell proliferation. On the contrary, the child's lymph nodes are infiltrated primarily with reactive T cells, not unlike those seen in acute infectious mononucleosis. It is, perhaps, the immunoreactive component of his disease that permits corticosteroids to be of more benefit to him than acyclovir.

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References


