Infectious mononucleosis caused by EBV infection with clonal cytotoxic T cells in an infant patient

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Abstract: Infectious mononucleosis (IM) is a self-limiting lymphoproliferative disease usually caused by Epstein-Barr virus (EBV) infection, which predominantly infects B lymphocytes in adults. IM that occurs in T cells infected with EBV is rare and only a few cases have been reported worldwide. We describe a rare case of IM in an infant with an EBV infection predominantly in clonal cytotoxic T cells. A cervical node taken from a 10-month-old male infant was retrospectively analyzed. The architecture of the lymph node was effaced mostly with focal to extensive coagulative necrosis. In some areas, numerous infiltrating lymphocytes were detected, expressing CD2, CD3, and granzyme B, but lacked CD5 and CD56. EBV-encoded small RNAs were detected in the majority of lymphocytes. Furthermore, the T cell presented with T-cell receptor rearrangements. The patient did not do any radiation and chemotherapy. Thirty-two months follow up showed that the patient was well. We speculate IM can occur in infants through EBV infection of clonal cytotoxic T cells.

Keywords: Infectious mononucleosis, herpesvirus 4, human, T-lymphocytes, genes, T-cell receptor, infant

Introduction

Infectious mononucleosis (IM) is a self-limited lymphoproliferative disease usually caused by Epstein-Barr virus (EBV) infection, characterized clinically by fever, pharyngitis, cervical lymphadenopathy, and lymphoproliferation of prominent cytotoxic T cells and partial B cells, as assessed using histology and immunophenotyping. EBV infection in IM is usually reputed to occur predominantly in B rather than T cells [1, 2]. However, in this report we describe a case of IM in an infant with an EBV infection predominantly in clonal cytotoxic T cells.

Case report

A ten-month-old male infant began a fever with a temperature of 38.2°C on October 16, 2013. His lymph nodes were enlarged on the left side of his neck 4 days after onset. His blood test showed that he had a white blood cell (WBC) count of 3.76 × 10^9/L, a hemoglobin level of 106 g/L, and a platelet count of 256.4 × 10^9/L. Therefore, he was admitted to hospital 7 days after onset with a preliminary diagnosis of acute lymphadenitis. The liver and spleen were not palpable. Laboratory tests revealed both an elevated aspartate aminotransferase (61.60 U/L) and lactate dehydrogenase (754 U/L). After admission, He was treated with an anti-infective and his temperature returned gradually to normal. However, his temperature rose again. A computed tomography (CT) scan showed multiple enlarged lymph nodes with an unclear border on the left side of the neck with some of the nodes merging into a single group, and extending down to the left anterior superior mediastinum and mildly compressing the trachea. A cervical node was biopsied on the 17th day after onset. The infant’s fever returned and reached 39°C just after he was biopsied, but it resolved after supportive treatment. The patient was discharged on the 24th day after onset.

Follow-up

Since being discharged, the infant has not had fever or other symptoms for 20 months.
7 months following discharge (June 21, 2014), the patient was reexamined. The CT scan showed that there were several enlarged lymph nodes on the left side of his neck, which were smaller than before, and no mass or enlarged lymph nodes in the mediastinum.

**Materials and methods**

The removed cervical node biopsied sample was round, with an unsmooth surface, and was approximately 1 cm in diameter. The sample was cut and fixed in 10% neutral formalin solution and embedded in a paraffin block. Four-micrometer sections were cut from the paraffin block and stained with hematoxylin and eosin for morphological analysis.

**Immunohistochemistry (IHC)**

IHC staining was performed manually for immunophenotypic analysis. Briefly, after being deparaffinized in xylene, sections were hydrated in graded ethanol. Endogenous peroxidase activity was then blocked with 0.5% H₂O₂/methanol. For antigen retrieval, sections were pretreated in EDTA buffer (pH 8.0) under high pressure. Four-micrometer sections were cut from the paraffin block and stained with hematoxylin and eosin for morphological analysis.

**Epstein-Barr virus-encoded small RNA (EBER) in-situ hybridization**

The EBV Probe In Situ Hybridization Kit (Triplex International Biosciences (China) Co. Ltd, Fuzhou, China) was used to detect EBERs according to the following steps: (1) deparaffination and dehydration of the paraffin sections using xylene and a series of graded ethanol solutions; (2) pretreatment with proteinase K for 5 min; (3) hybridization with a digoxigenin-conjugated EBV (EBERs) probe at 37°C for 4 h; (4) signal detection using a peroxidase-conjugated anti-digoxigenin antibody and 3,3'-diaminobenzidine; and (5) counterstaining the sections with hematoxylin solution. Positive signals were brownish-yellow and localized within the nuclei.

**T-cell receptor (TCR) gene clonality analysis**

Analysis of TCR gene rearrangements was performed using polymerase chain reaction (PCR) based on the ‘Biomed-2’ primers (Invivolscribe Technologies, San Diego, CA, USA) [3]. The DNA was extracted from the formalin-fixed, paraffin-embedded tissue using a TIANamp FFPE DNA Kit (DP331) (Tiangen, Beijing, China) in accordance with the manufacturer’s instructions. For the gene arrangement assay, PCR was carried out in a 25 µL volume taken from a master mix containing 22.5 µL master mix, 0.13 µL AmpliTaq Gold DNA polymerase (0.23 µL for TCRβ:Vβ + Jβ1/2 and Vβ + Jβ2), and 100 ng genomic DNA. For each assay, three controls (positive, negative, and no template control) were added. For the positive control of each master mix, the template was used as the product insert. The template of the negative control was the IVS-0000 polyclonal control. The cycling profile used for all reactions was as follows: 95°C for 7 min; 35 cycles at 95°C for 45 s, 60°C for 45 s, and 72°C for 90 s; and a 10-min final extension at 72°C. After amplification, PCR products were denatured at 94°C for 5 min. PCR products were then quick chilled at 4°C for at least 60 min. PCR products were electrophoresed in 6% polyacrylamide gels in 1 x TBE buffer at 120 V for approximately 65 min. The gel was then soaked for 20 min in 100 mL

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**Table 1. Primary antibodies and conditions used for immunohistochemical staining**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>Dilution</th>
<th>Antigen Retrieval</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td>L26</td>
<td>1:200</td>
<td>HP EDTA</td>
<td>Maxim-Bio</td>
</tr>
<tr>
<td>CD2</td>
<td>AB74</td>
<td>1:50</td>
<td>HP EDTA</td>
<td>Maxim-Bio</td>
</tr>
<tr>
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<td>SP7</td>
<td>1:100</td>
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<td>Maxim-Bio</td>
</tr>
<tr>
<td>CD5</td>
<td>SP19</td>
<td>1:50</td>
<td>HP EDTA</td>
<td>Maxim-Bio</td>
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<tr>
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<td>SP35</td>
<td>1:80</td>
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<td>Maxim-Bio</td>
</tr>
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<td>1:100</td>
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<td>Maxim-Bio</td>
</tr>
<tr>
<td>CD56</td>
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<td>Maxim-Bio</td>
</tr>
<tr>
<td>CD68</td>
<td>KP1</td>
<td>1:30</td>
<td>HP EDTA</td>
<td>Maxim-Bio</td>
</tr>
<tr>
<td>CD123</td>
<td>BR4MS</td>
<td>1:100</td>
<td>HP EDTA</td>
<td>Maxim-Bio</td>
</tr>
<tr>
<td>CD163</td>
<td>10D6</td>
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</tr>
<tr>
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<td>GZB01</td>
<td>1:25</td>
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<td>Maxim-Bio</td>
</tr>
<tr>
<td>Ki-67</td>
<td>MiB-1</td>
<td>1:200</td>
<td>HP EDTA</td>
<td>Maxim-Bio</td>
</tr>
</tbody>
</table>

HP EDTA: Boiling with EDTA (1 mM, pH 8.0) under high pressure.

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The gel was then soaked for 20 min in 100 mL
Infectious mononucleosis with T cell infected EBV in an infant

Results

Histology

The sample removed for biopsy was a lymph node with architecture that was mostly effaced. The cortex and medulla were not clear. Few follicles and subcapsular sinuses existed (Figure 1A). The lesion appeared to have focal to extensive coagulative necrosis, a variable number of apoptotic bodies and focal fibrosis. In some areas, numerous infiltrating lymphocytes of medium size, round with mildly irregular nuclei, inconspicuous or small nucleoli, and dispersed chromatin were detected (Figure 1B). Mitotic figures were readily apparent. There were some macrophages with abundant pale cytoplasm containing nuclear debris (Figure 1C). The angiocentric growth pattern of lymphocytes could also be seen in the lesion. Neutrophils, eosinophils, and plasma cells were not remarkable.

Immunophenotype

The majority of lymphocytes in the lesion expressed CD2, CD3 (Figure 1D), and granzyme B, but lacked CD5 (Figure 1E) and CD56. The number of CD4-positive cells was similar to that of CD8. There were only a few scattered lymphocytes that expressed CD20 (Figure 1F). The scattered larger cells were weakly CD30 positive. Ki-67 staining showed that about 60% of cells were positive. Some histiocytes and macrophages expressed CD68 and CD163, but did not express CD123 or MPO.

EBER detection

EBERs were detected in the majority of lymphocytes, approximately 70-80% of all cells (Figure 2A). Most EBER-positive cells were seen in an area with well-preserved morphology. In contrast, only a few EBER-positive cells in the necrotic area were found.

TCR gene clonality

T-cell clonality analysis using BIOMED-2 PCR separate protocols revealed that TCR Vb/Jb2 presented with monoclonal rearrangements (Figure 2B), and TCR Vγ1-8 and TCR Vγ10/multiple Jγ regions showed monoclonal rearrangements as well.

Discussion

IM is reputed to be a self-limiting lymphoproliferative disease usually caused by EBV infec-
Infectious mononucleosis with T cell infected EBV in an infant

Clinically, it presents with a typical triad of fever, pharyngitis, and cervical lymphadenopathy. There are several methods used that demonstrate EBV infection at different levels, including a serum antibody test that shows a primary EBV infection composed of VCA-IgM+, VCA-IgG+, and EBNA-IgG- [4], with EBV DNA copies increased in peripheral blood, and EBV protein or RNA detected in tissue using IHC for LMP1 or EBNA, and in situ hybridization for EBERs [5]. Laboratory findings include increased lymphocytes in peripheral blood and several parameters of liver metabolic abnormality that may exist. In the present case, the infant had a fever, pharyngeal hyperemia, and cervical and anterior superior mediastinal lymphadenopathy. Numerous EBER-positive lymphoid cells were found in his biopsied lymphoid tissue. There was an increased percentage of lymphocytes in his peripheral blood (highest 68.04%, normal 40-60%) and increased aspartate aminotransferase (61.6 μ/L, normal ≤40 u/L). Finally, the infant recovered spontaneously and has been disease free for 20 months. Thus, the present case was consistent with a diagnosis of IM, although the EBV serum antibody data were not available.

In IM, EBV-infected cells are reputed to be mostly B cells because the surface of B cells harbor the C3d receptor (CD21). CD21 is an EBV receptor, which EBV interacts with, thereby entering the cells [1]. However, EBV-infected cells in the present case were cytotoxic T cells expressing CD3 and granzyme B, but lacking CD20, CD5, and CD56. This is the first reported case of IM in which EBV prominently infected cytotoxic T cells in the patient’s lymph nodes. There have been other similar cases reported in the literature. He et al. reported an IM case of a 32-year-old woman with a lesion in the nasal cavity in which the lymphoid cells were EBER positive and expressed CD2, CD3, CD5, CD7, and CD4/CD8, but not CD20 or CD56. Data on cytotoxic molecules were not available [6]. Arai et al. described another IM case of a 22-year-old man with an EBV infection in T cells detected in a liver needle core biopsy sample, and peripheral blood [7]. The infiltrating lymphocytes in the sinusoid were positive for CD8, but not for CD4, CD20, or CD56. Although the EBV-DNA load in peripheral blood was markedly elevated to 2.3 × 10^5 copies/μg, EBER-positive cells were very rare, with only one or two EBER-positive cells identified in the biopsy sample (see Figure 1C in the article [7]). Furthermore, Malik et al. described an atypical case of IM with clonal rearrangement of the TCR gene, and EBERs showed positive staining in approximately 1% of lymphocytes [8]. However, the article did not provide details of the EBV-infected lymphocyte’s phenotype. In the present case, most of the infiltrating lymphoid cells were T lymphocytes that were infected with EBV and had lost their T-cell antibodies. Malignant T-cell lymphoma was highly suspected but because the infant recovered spontaneously, the diagnosis was consistent with IM.

IM is a benign disease because most patients can spontaneously recover in approximately 1 month. This is the result of cytotoxic T-cell reactive proliferation after EBV antigen stimulation, which then attacks and destroys EBV-positive B cells. Genetic evidence arising from clonality analysis of gene rearrangements of IgH and
Infectious mononucleosis with T cell infected EBV in an infant

TCRγ in typical IM cases [9], showed no monoclonal T- or B-cell populations in 17 of 18 cases of IM and only one case had an oligoclonal pattern of TCR rearrangements. However, monoclonal TCRγ gene rearrangements were detected in our case using the BioMed-2 protocol. Similarly, the case from Arai et al. also showed a clonal T-cell proliferated population in peripheral blood mononuclear cells using PCR of the TCRβ gene rearrangement [7]. These findings showed that clonal T cells could be present in IM and further supported that the clonality of cells is not the only evidence of malignancy.

To the best of our knowledge, IM occurs generally in adolescents and young adults in Western countries because of delayed EBV infection. However, it usually occurs earlier in children in Eastern countries because of earlier EBV exposure [10, 11]. Data from Hong Kong indicated that the age-specific seroprevalence of antibodies to EBV rose rapidly between 1 and 6 years, reaching 80% by 6 years, and by 10 years almost 100% of children had seroconverted [12], with similar data reported in mainland China [13]. Despite this, it was rare that IM occurred in infants less than 1 year [10]. We presumed that this was because of the maternal antibody effect. Surprisingly, the patient in our paper was only 10 months old. He had been continuously breast fed in the first year. The reason for early EBV infection in this infant is still unclear.

In the present case, there were numerous lymphoid cells, with marked necrosis, expressing the cytotoxic T-cell antigen; they were EBER positive and had a clonal T-cell genotype, which mimicked extranodal NK/T-cell lymphoma (ENKTL), EBV+ T-cell lymphoproliferative disease (EBV + T-LPD), and Kikuchi’s disease. Therefore, the following diseases should be taken into consideration for differential diagnosis. ENKTL generally occurs in adults and rarely in children, and no infant case has been reported to date. This tumor occurs predominantly in the nasal cavity, followed by the gastrointestinal tract, skin, and soft tissue, and only in rare cases does it occur primarily in lymph nodes [14]. The majority of cases of the disease are CD56 positive. Finally, ENKTL is an aggressive disease that may not regress spontaneously. All of these features are not consistent with our case. EBV + T-LPD has a longer recurrent clinical manifestation, usually more than 3 months [15, 16]. In our case, the infant had an acute clinical appearance, which continued for 1-2 months and from which he recovered spontaneously without recurrence. Although Kikuchi’s disease is a self-limiting disease with necrosis, involving predominantly the lymph nodes, it occurs mostly in young female individuals with expression of CD68, CD163, CD123, and MPO detected in lesions, but usually lacks EBV infection and clonal T-cell rearrangements. These features were not manifested in our case.

Conclusion

Although primary EBV infections occur in children who usually present with mild symptoms, this usually leads to IM during adolescence. Our report shows that in rare situations, IM can occur in infants with EBV infection of clonal cytotoxic T cells.

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Disclosure of conflict of interest

None.

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Infectious mononucleosis with T cell infected EBV in an infant


