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Abstract

We report on a 9-year-old boy who presented with acute encephalopathy and hemophagocytic lymphohistiocytosis (HLH). The patient was referred to our hospital because of fever, seizures, and decreased consciousness. He showed moderately elevated levels of proinflammatory cytokines in the cerebrospinal fluid and plasma, and clonal expansion of highly activated $CD8^+$ T cells in the peripheral blood. These $CD8^+$ T cells were found to be larger cells that stained positive for T-cell receptor Vβ13.6, and decreased shortly after steroid therapy. Our findings suggest that his acute encephalopathy was likely a clinical manifestation of HLH, and that immunophenotypic analysis may be helpful for early recognition of HLH in such rare encephalopathy.

Key words: acute encephalopathy; hemophagocytic lymphohistiocytosis; clonal T-cell expansion; CD8.

Introduction

Encephalopathy is a heterogeneous group of disorders of the brain that are characterized by altered states of consciousness, altered cognition or personality, and seizures [1]. It is distinguished from encephalitis by the absence of cerebrospinal fluid pleocytosis. The onset of acute encephalopathy is frequently associated with viral infection in children [2]. Viral infection also causes hemophagocytic lymphohistiocytosis (HLH) that is characterized by fever, cytopenia, hepatosplenomegaly, liver dysfunction, coagulation abnormalities, and hemophagocytosis [3]. Patients with HLH frequently develop central nervous system (CNS) involvement during the disease course [3]. The initial presenting neurological symptoms of HLH rarely mimic acute encephalopathy, however, it may be difficult to identify such a condition especially before appearance of systemic symptoms of HLH [4]. Herein, we describe the identification of clonal T-cell expansion and its contribution to early diagnosis of HLH in a case of acute encephalopathy accompanied by HLH.

Case report

A previously healthy 9-year-old boy was hospitalized because of fever for 3-days, coughing, vomiting, and generalized tonic seizures. Neurologic examination exhibited decreased consciousness (Glasgow Coma Scale E3, M6, V2). There were no pyramidal, cerebellar, or extrapyramidal signs. Laboratory studies revealed cytopenias (white blood cells, 2630/μL; hemoglobin, 12.0 g/dL; and platelets, 7.3 x 10^4/μL) and coagulopathy. Other abnormal findings included hyponatremia (126 mEq/L), mild

liver dysfunction (aspartate aminotransferase 78 IU/L), hyperferritinemia (5514 ng/mL), and elevated serum-soluble interleukin (IL)-2 receptor levels (3230 U/mL). Hyperammonemia and hypoglycemia were not detected. Head computed tomography scan revealed mild cerebral edema, and electroencephalography exhibited global slowing. Magnetic resonance imaging showed slight contrast medium enhancement of the meninges but no parenchymal lesions. Lumbar puncture showed protein and glucose levels in the normal range with no pleocytosis, however, the levels of IL-6, IL-8 and neopterin were elevated to 144 pg/mL, 380 pg/mL and 31 nmol/L, respectively. Plasma concentrations of IL-6, IL-8, and neopterin were 2.8 pg/mL (normal, <5 pg/mL), 14 pg/mL (normal, <31 pg/mL), and 45 nmol/L (normal, 2-8 nmol/L), respectively. Elevated concentrations of soluble tumor necrosis factor receptors were noted in the plasma: type I, 3300 pg/mL (normal, 484-1407 pg/mL); type II, 11300 pg/mL (normal, 829-2262 pg/mL). A bone marrow aspirate exhibited a hypocellular marrow and accumulation of hemophagocytic cells. Then, acute encephalopathy, disseminated intravascular coagulation [5], and HLH [6] were diagnosed, and the patient was treated with mannitol, low molecular weight heparin, anticonvulsant, and methylprednisolone pulse therapy followed by dexamethasone. He was also treated empirically with intravenous acyclovir. Two days later, the patient recovered consciousness except for mild drowsiness. No seizures were seen after admission. Magnetic resonance imaging on hospital day 8 exhibited no parenchymal lesions. His abnormal laboratory manifestations approached the normal range within 3 weeks of hospitalization. No neurological sequelae were found at discharge. The patient exhibited only a 4-fold titer increase in neutralizing antibody for adenovirus type 3 with a convalescent-phase titer 32, and results were negative for a series of other microbiological tests.

As part of the clinical evaluation, we performed immunophenotypic analysis of lymphocytes on hospital day 2 [7]. We noted an increased percentage of $CD3^+$ T cell (81.5%) with an inverted proportion of $CD4^+$ to $CD8^+$ T cells (0.49), when the lymphocyte region was gated (Fig. 1A). More importantly, an unusual cell subset of $CD8^+$ T cells was increased significantly among the monocyte region in which no lymphocytes existed generally. The vast majority of those cells expressed the activation marker, HLA-DR (Fig. 1A) and CD45RO (data not shown). Flow cytometry analysis of the T-cell receptor (TCR) Vβ repertoire demonstrated that the cells were detectable only with TCR Vβ13.6, although the $CD8^+$ T cells within the lymphocyte region expressed the polyclonal repertoire (Fig. 1B). CDR3 spectratyping of the TCR Vβ13.6 segments amplified from the $CD8^+$ T cells exhibited a single peak, whereas the results from a healthy control demonstrated a Gaussian distribution (Fig. 1C). The junctional amino acid sequence of TCR Vβ13.6 segments from the $CD8^+$ T cells also exhibited a monoclonal profile (CASS LVVGA NTGELFFGEG, 93.8% of 16 clones). The expanded cells (Fig. 1D) declined shortly after initiation of the treatment.

Discussion

Acute encephalopathy is one of the most serious complications of viral infections and may be caused by any virus infections [2]. The pathogenesis of acute encephalopathy associated with viral infections is not entirely understood but may involve metabolic disorders, proinflammatory cytokines, excitotoxicity, or genetic susceptibility [2, 8]. In addition, acute encephalopathy may develop in children with secondary HLH caused by viral infection [4, 9]. In this study, we report on a patient

with acute encephalopathy, disseminated intravascular coagulation, and HLH who showed clonal T-cell proliferation in the peripheral blood. Because the patient showed only moderate elevation of levels of proinflammatory cytokines in the cerebrospinal fluid and the CNS is often involved in HLH [3], it seems reasonable to presume that his acute encephalopathy might be a clinical manifestation of HLH but not a consequence of cytokine storm in the CNS.

The patient exhibited a 4-fold titer increase in neutralizing antibody for adenovirus type 3. Adenoviral infections are common events among the pediatric population, and the spectrum of disease can be quite broad [10]. However, adenovirus-associated HLH has seldom been reported except for bone marrow transplant recipients [11]. Because adenovirus infection was not confirmed by viral isolation or by a direct antigen-detection assay, an association between HLH and adenovirus type 3 infection was unclear in our patient.

Although uncontrolled activation of T cells and macrophages might account for disease development in HLH [3], the clonality and immunophenotypic characteristics of T cells are unknown in most cases of HLH. Exceptions to this would be familial HLH, in which clonal expansion of αβ-T cells with skewed Jβ1 usage has been demonstrated [12], and Epstein-Barr virus (EBV)-associated HLH, in which clonal proliferation of EBV-infected $CD8^+$ T cells with CD5 down-regulation has been reported [13]. The present study clearly demonstrated T-cell activation and clonal expansion of TCR $V\beta13.6^+$ $CD8^+$ T cells in the patient. The TCR $V\beta13.6^+$ $CD8^+$ T cells expressed HLA-DR and CD45RO and were generally larger, suggesting a highly activated state. In addition, the cells decreased shortly after the therapy concomitant with the decrease in plasma cytokine levels (data not shown). These results suggest that the clonally-expanded TCR $V\beta13.6^+$ $CD8^+$ T cells might be

associated with HLH, and contributed our early recognition of HLH in this case. Because of the predominance of larger cells, the total sample of mononuclear cells, but not lymphocytes, required further investigation in order to avoid underestimation. This fact together with the timing of sample collection could be associated with the infrequent occurrence of clonal T-cell proliferation in HLH.

In summary, our results demonstrate the presence of clonal T-cell expansion in acute encephalopathy accompanied by HLH. Immunological evaluation may be helpful for early diagnosis of HLH in patients presenting with acute encephalopathy as a primary manifestation of HLH.

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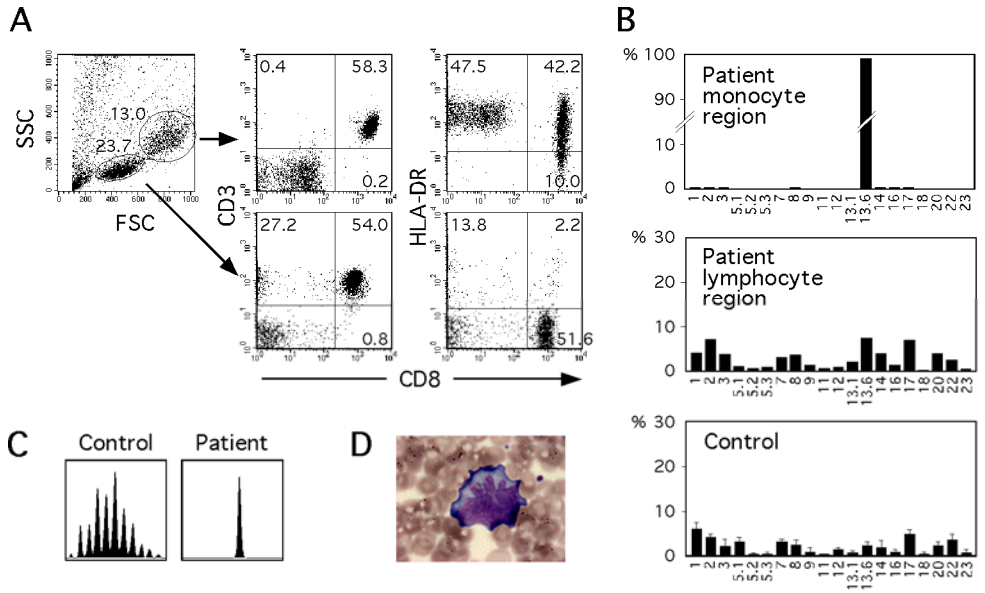
Figure Legends

Figure 1. Characterization of clonally-expanded $CD8^+$ T cells.

(A) Regions of lymphocytes and monocytes were gated for the analysis of CD3, CD8 and HLA-DR expression. The percentage of cells gated in each cell population is shown. (B) Expression profiles of T-cell receptor (TCR) Vβ subfamilies. Peripheral blood samples were stained with monoclonal antibodies for the individual TCR Vβ subfamilies together with anti-CD8. The percentage of the expression of each TCR Vβ within the $CD8^+$ T cells was analyzed by FACS. (C) CDR3 spectratyping, showing the size distribution of the polymerase chain reaction products of TCR Vβ13.6 of $CD8^+$ T cells. (D) Peripheral blood smears (May-Grünewald-Giemsa staining, original magnification x 400). FSC, forward scatter; SSC, side scatter.

Figure 1


A
SSC
FSC
13.0
23.7
CD3
CD8
0.4
58.3
0.2
27.2
54.0
0.8
HLA-DR
47.5
42.2
10.0
13.8
2.2
51.6
B
% 100
90
10
0
Patient monocyte region
% 30
20
10
0
Patient lymphocyte region
Control
C
Control
Patient
D