Letter to the Editor
Diagnostic Hematology

Ann Lab Med 2015;35:155-158
http://dx.doi.org/10.3343/alm.2015.35.1.155
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

First Report on Familial Hemophagocytic Lymphohistiocytosis with an Abnormal Immunophenotype and T Cell Monoclonality in Korea

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Hemophagocytic lymphohistiocytosis (HLH) can be divided into primary (genetic or familial) and secondary (acquired or reactive) forms. Abnormal immunophenotyping and downregulation of CD5 or CD7 in T cells have been well characterized in Epstein-Barr virus (EBV)-associated HLH [1, 2]. A few studies have also reported abnormal immunophenotyping of T cells in patients with familial HLH (FHL) [1, 3]. Here, we report a case of FHL with immunophenotypically abnormal T cells and monoclonal T cells confirmed by a *T-cell receptor* (*TCR*) gene rearrangement test.

A 2-month-old infant was referred to our hospital for further evaluation and treatment of persistent fever and thrombocytopenia. Hepatosplenomegaly and petechiae were observed on physical examination. The total leukocyte count was 1.64×10^9/L, consisting of 65% lymphocytes, with 1% atypical lymphoid cells. The Hb level was 9.1 g/dL, and the platelet count was 20×10^9/L. Blood chemistry results were as follows: 570 IU/L AST, 454 IU/L ALT, 657 IU/L lactate dehydrogenase (LDH), 11.4 mg/dL total bilirubin, and 147 mg/dL fibrinogen. The prothrombin time (PT) and activated partial thromboplastin time (aPTT) was 15.3 sec (reference range; 12.6-14.9 sec) and 40.3 sec (reference range; 29.1-41.9 sec), respectively. A serological test to detect any underlying viral infection showed (+, positive; –, negative): anti-CMV IgG (+), anti-CMV IgM (–), anti-EB-VCA IgG (+), anti-EB-VCA IgM (–), EBV-EA (–), and anti-EB-NA IgG (+). The results of a chromosome study were normal (46,XY). Atypical lymphocytes were observed on peripheral blood smear (Fig. 1A). Hemophagocytic histiocytes were observed on bone marrow biopsy (Fig. 1B, C, and D). EBV was not detected by *in situ* hybridization. We performed mutation analysis of the FHL-related genes *UNC13D* and *PRF1* using DNA isolated from peripheral blood. Sequencing analysis revealed compound heterozygous mutations in *UNC13D* [c.118-308 C>T (;)754-1G>C] (Fig. 2A and B). These mutations are frequently observed in Korean FHL patients [4]. Immunophenotyping using bone marrow aspirate revealed an abnormal population of $CD8^+$ T cells with downregulated levels of CD5 or CD7 (14.61% of total events; Fig. 2C, D, and E). A *TCR* gene rearrangement study using paraffin-embedded tissue revealed T cell monoclonality [5] (Fig. 2F). The patient was treated with

Received: May 19, 2014
Revision received: July 16, 2014
Accepted: October 29, 2014

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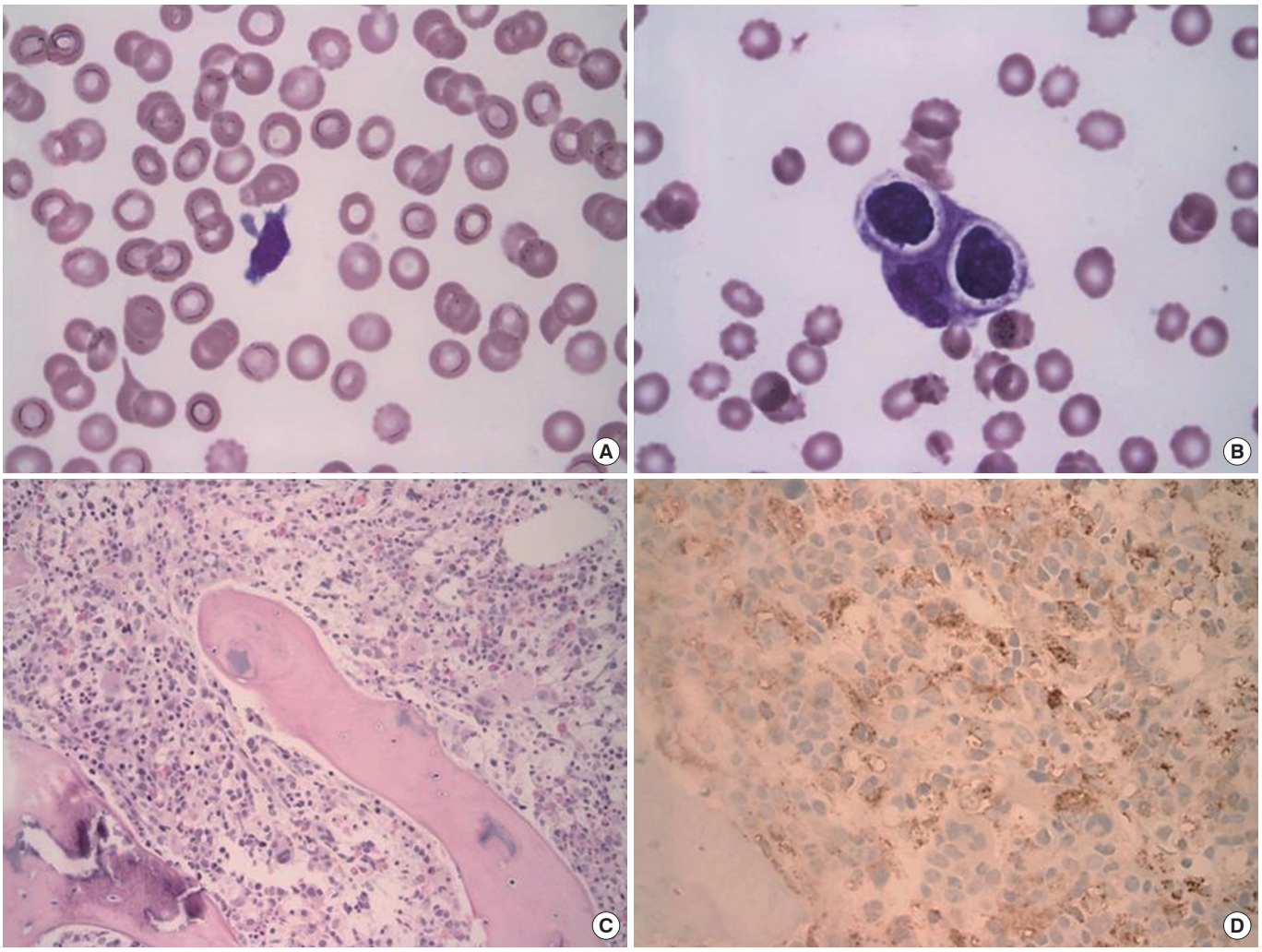

Fig. 1. Peripheral blood smear, bone marrow biopsy and aspirate. (A) Pancytopenia and atypical lymphoid cells were observed in peripheral blood smear (Wright-Giemsa stain; magnification, ×1,000). (B) Hemophagocytic histiocytes were frequently observed in bone marrow aspiration (Wright-Giemsa stain; magnification, ×1,000). (C) Histiocytes were high in number in the biopsy section (Hematoxylin-Eosin stain; magnification ×200). (D) CD68 immunization (magnification, ×400).

HLH-2004 chemotherapy and allogenic bone marrow transplantation. Bone marrow studies were performed before and after allogenic bone marrow transplantation. Hemophagocytic histiocytes were not observed in the follow-up bone marrow study. The patient remained in remission (follow-up time: 12 months after diagnosis). A familial study for *UNC13D* gene mutation was not performed.

Abnormal immunophenotype of $CD8^+$ T cells with downregulation of CD5 is a common finding in HLH associated with viruses, especially EBV-associated HLH [1, 6, 7]. McCall *et al*. [1] reported that 6 of 9 cases (76%) of EBV-associated HLH had expansion of $CD8^+$ T cell populations with variable downregulation of CD5, CD7, and/or CD3, whereas 1 of 8 cases (13%) of non-EBV-associated HLH showed downregulation of CD7 expression [1]. There are few reports on abnormal immunophenotype of T cells in FHL [1, 3, 8]. Karandikar *et al*. [3] reported a case of FHL associated with a perforin gene mutation that showed $CD8^+$ T cells with loss of CD5 expression. Wada *et al*. [8] also reported a case of FHL associated with a perforin gene mutation that showed $CD8^+$ T cells with loss of CD5 expression. Downregulation of CD5, CD7, CD3 and aberrant expression of HLA-DR is a common finding in both FHL and EBV-associated HLH or other acquired HLH [1, 3]. In this case, downregulation of CD5 and CD7 was found, as reported previously [1, 3]. The cause of downregulation of CD5 expression in acquired HLH or FHL remains unclear. Some authors have suggested a link be-

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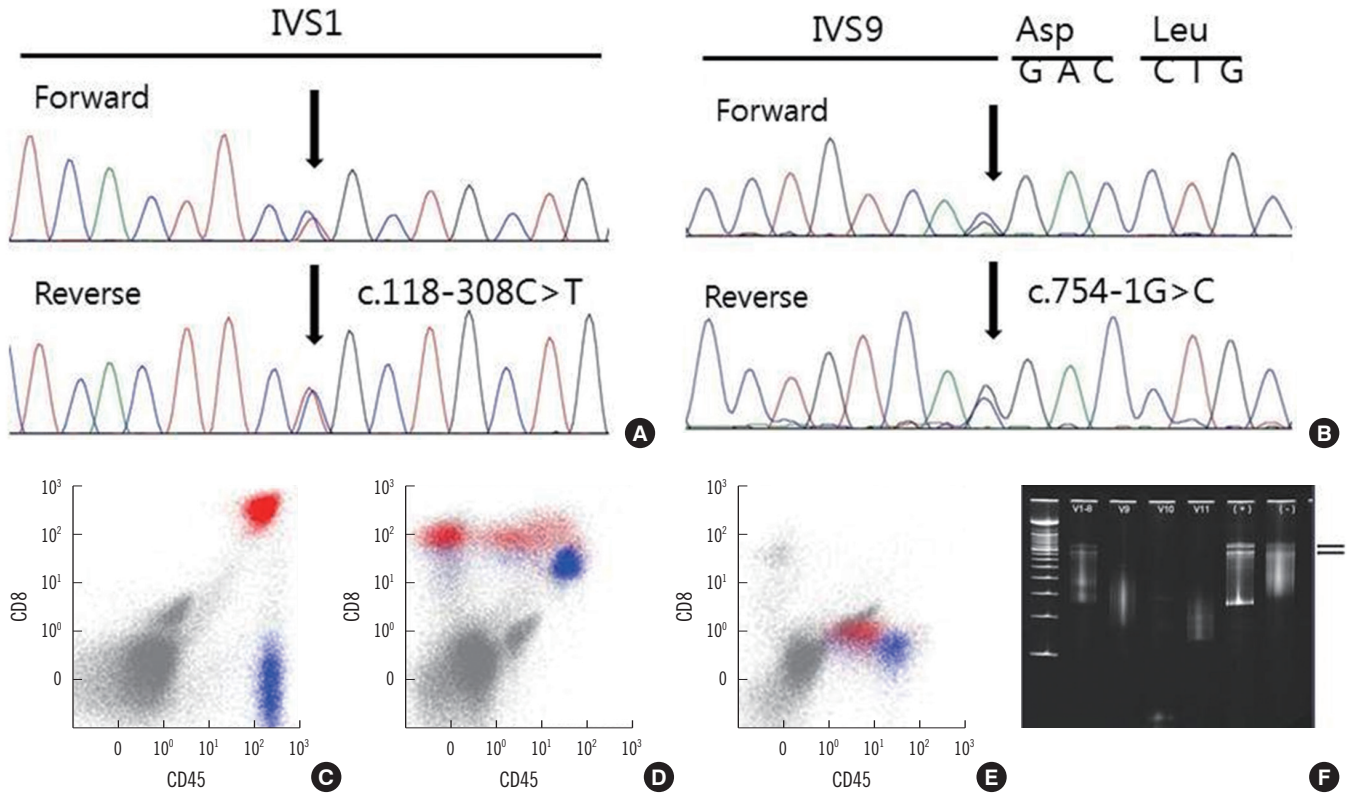

Fig. 2. Sequencing analysis, flow cytometry results, and single-strand conformational polymorphism (SSCP) analysis of the T cell receptor by using paraffin-embedded tissue samples. (A) Compound heterozygous mutations in the *UNC13D* gene were observed: C to T substitution at nucleotide –308 of intron 1, relative to the cDNA positioned between nucleotides 117 and 118 (118-308C>T). (B) G to C substitution at nucleotide –1 of intron 9, relative to the cDNA positioned between nucleotides 753 and 754 (754-1G>C). The $CD3^+/CD8^+$ T cells (red). (C) showed variable downregulation of CD5 or CD7 (lack of expression) compared with $CD3^+/CD4^+$ cells (blue, presumably normal T cells; D and E). (F) DNA was extracted from paraffin-embedded bone marrow tissue. Consensus primer for Vγ1-8, Vγ9-11 was used for amplification. PCR products were analyzed by SSCP, which separated DNA fragments according to nucleotide sequence. Well-defined, distinct bands were considered evidence of monoclonality. The two black arrows indicate the distinct monoclonal band at the level of the lowest smear in the polyclonal control (–). Monoclonality was observed in Vγ1-8, and polyclonality was observed in Vγ9-11.

tween CD5 downregulation in T cells and monoclonal proliferation [6].

Our case also showed monoclonality in the *TCR* gene rearrangement study. Monoclonality in the *TCR* gene rearrangement study is also a common finding in EBV-associated HLH [9]. Monoclonal T cell proliferation in FHL has also been reported [10]. Moreover, the study also reported clonal changes in T cell populations after treatment (monoclonal to polyclonal) [10]. These results might help shed light on the pathogenesis underlying clonal proliferation of T cells and downregulation of CD5 in HLH.

To the best of our knowledge, this is the first report on FHL with an abnormal immunophenotype and T cell monoclonality in Korea. Further studies are needed to characterize the immunophenotypic features and clonality of T cells in FHL and their diagnostic or clinical significance.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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http://dx.doi.org/10.3343/alm.2015.35.1.155