Familial hemophagocytic lymphohistiocytosis (FHL), an inherited, fatal disorder of infancy. We report here a 17-day-old female infant who presented with high fever, hepatosplenomegaly, hypertriglyceridemia, hypofibrinogenemia, thrombocytopenia, and liver failure. Leukocytosis was detected with circulating "atypical" lymphoid cells. Flow cytometric studies revealed expanded subpopulations of CD8+ T cells with unusual immunophenotypic features, including a subset that lacked CD5 expression. A liver biopsy showed hemophagocytic lymphohistiocytosis with exuberant infiltrates of CD8+ T cells that lacked perforin. Mutational studies revealed a 666C→A (H222Q) missense mutation in the perforin gene. T-cell receptor studies on flow-sorted T-cell subpopulations revealed no evidence of monoclonality. Analysis of T-cell receptor excision circle levels indicated long proliferative history in the aberrant CD8+ T-cell subsets. This case provides an instructive example of uncontrolled reactive proliferation of CD8+ T cells in FHL, resulting in atypical morphology and unusual immunophenotypic features that might suggest malignancy in other clinical settings. (Blood. 2004;104:2007-2009)

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was added to 1.0 µL ROX500 molecular markers and 24 µL deionized formamide, denatured at 95°C for 5 minutes, and immediately cooled on ice. Analysis on an ABI310 Genetic Analyzer using GeneScan software (Applied Biosystems, Foster City, CA) followed.

**Perforin gene mutational analysis**

Sequencing of the perforin (PRF1) gene was performed for mutational analysis.4

**T-cell receptor excision circle (TREC) levels**

TREC levels were evaluated by using quantitative real-time PCR assays13 with the albumin gene being used as a control for quantification.14

**Photomicrography**

Photomicrographs were obtained using an Olympus BX50 microscope equipped with an Olympus PM-C35DX camera (Olympus America, Melville, NY). Liver sections and Wright-stained blood smear micrographs were viewed with 50×/0.90 and 100×/1.30 oil objectives, respectively (Olympus, Tokyo, Japan). Kodachrome slides (35 mm; Eastman Kodak, Rochester, NY) were scanned in using Adobe Photoshop software (Adobe, San Jose, CA).  

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**Results and discussion**

**Morphology and flow cytometry**

The peripheral blood contained numerous medium-sized to large lymphoid cells that showed abundant basophilic cytoplasm with intense peripheral basophilia, oval to irregular nuclear contours, slightly dispersed chromatin, and occasional nucleoli (Figure 1A).

Flow cytometry (Figure 1B) revealed that 78% of events were mature, surface CD3+ T lymphocytes with a predominance of CD8+ T cells (CD4/CD8 ratio = 0.35:1). CD4+ T cells were composed of a CD45RO− subset (presumably naive) and an expanded CD45RO+ subset (memory/effector). CD8+ T cells were composed of 3 major subpopulations (blue, black, and red in Figure 1B) with a CD2+, CD7+, CD45 bright+, CD25 predominantly−, CD56 predominantly−, TCR-αβ+, and TdT− immunophenotype. TCR-γδ+ T cells were also present and were distinct from these populations.

The smallest subpopulation (~ 2.5% of total events; blue) was composed of small cells (forward light scatter characteristics) and exhibited a CD4−, HLA-DR−, CD45RO−, CD45RA bright−, CD5 bright− phenotype and most likely represented normal, naive CD8+ T cells. The other 2 subpopulations showed partial dim CD4 and slightly bright CD8 expression and were predominantly CD57−, uniformly CD45RO+, and CD45RA dim− to −−, bearing some similarity to a previously described case.15 The population indicated in black (~ 43% of total events) showed variable cell size with dim CD5, variable HLA-DR, and bright CD8 expression. The population indicated in red (~ 12% of total events) was composed of small- to medium-sized HLA-DR+ cells that showed complete lack of CD5 expression. Thus, the CD8+ T cells consisted of a small proportion of naive T cells and 2 unusual expanded subpopulations with an “effector/memory phenotype” and underexpression of CD5.

**Liver biopsy**

The liver biopsy showed an exuberant lymphocytic infiltrate within portal triads, with occasional foci within lobules. The infiltrates were composed predominantly of CD3+ T cells (Figure 2), including mainly CD8+ and a few CD4+ cells with a high degree of pleomorphism, enlarged nuclei, eosinophilic nucleoli, and abundant mitoses. Scattered macrophages were observed within sinusoids with occasional phagocytosis of neutrophils. Kupffer cell hyperplasia was noted on CD4 and CD68 staining. The infiltrating cells showed positive staining for granzyme B and T-cell intracellular antigen-1 (TIA-1). However, they were negative for perforin. Scattered cells showed weak diffuse cytoplasmic staining, which was distinct from the perforin staining pattern seen in positive controls.7 These findings are consistent with a diagnosis of FHL arising from a perforin mutation.7 Of interest, CD5 staining revealed few positive cells, likely corresponding to the CD4+ T cells. Thus, the infiltrating CD8+ T cells in the liver corresponded to the CD5dim or CD5+ subpopulations identified in blood.

**Clonality, proliferation history, and mutational analysis**

To evaluate for potential monoclonality in the T cells, we flow-sorted 4 subpopulations (Figure 1B) and performed PCR reactions for TCR-γ rearrangement. All sorted populations and bulk blood leukocytes showed oligoclonal or polyclonal electrophoretograms (Figure 1C). Thus, despite the aberrant immunophenotype and atypical morphology, there was no evidence of a monoclonal or
neoplastic lymphoproliferative disorder. This finding suggested a dysregulated reactive proliferation of CD8+ T cells in the setting of perforin deficiency, leading to expansions of phenotypically unusual subsets.

To test this hypothesis, we assessed proliferative history by measuring TREC, which are episomal DNA fragments (excised from the TCR genes during thymic development) that are mitotically diluted during proliferation.13,14 As predicted, the highest TREC levels were observed in naïve CD8+ T cells (population II). Importantly, there were no detectable TREC in the 2 aberrant subpopulations (III and IV), confirming their prolonged proliferative history (Figure 1C).

To clarify the underlying genotype, we performed mutational analysis of the perforin gene and discovered a homozygous missense point mutation in exon 3 (666C→A[H222Q]). This point mutation was recently described in a study of North American families with HLH.16

This case is an example of FHL associated with perforin deficiency and dysregulated proliferation of CD8+ T cells, leading to atypical morphology and unusual immunophenotype. In other clinical settings (eg, in an adult patient), these findings would be suspicious for a clonal or malignant T-lineage proliferation. However, there was no evidence of monoclonality in any subpopulation, indicating that this was a reactive proliferation, probably driven by antigenic stimulation of CD8+ T cells with impaired cytoxic and apoptotic functions.3,9,17

This case study provides several valuable clinical lessons. First, it depicts the wide spectrum of morphologic and immunophenotypic variability in reactive (nonmalignant) T cells. Second, it provides a detailed description of the morphology and immunophenotype of FHL-associated, dysregulated CD8+ T cells. In future studies, it might be worthwhile to test whether this phenotype is predictive of impaired cytotoxicity in the setting of HLH, thereby making it an indicator of focused diagnostic tests. Finally, this case re-emphasizes the need for a multimodal approach to the diagnosis of hematolymphoid disorders, in which data from morphology, immunophenotyping, and molecular or genetic tests contribute to the diagnostic process.

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**References**

Unusual immunophenotype of CD8+ T cells in familial hemophagocytic lymphohistiocytosis

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