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Down-regulation of CD5 expression on activated CD8$^+$ T cells in familial hemophagocytic lymphohistiocytosis with perforin gene mutations

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ABSTRACT

Hemophagocytic lymphohistiocytosis (HLH) is characterized by uncontrolled activation of T cells and macrophages with overproduction of cytokines. Familial HLH type 2 (FHL2) is the most common form of primary HLH and is caused by mutations in *PRF1*. We have recently described a significant increase in the subpopulation of CD8$^+$ T cells with clonal expansion and CD5 down-regulation in Epstein-Barr virus associated-HLH, which represented a valuable tool for its diagnosis. However, this unusual phenotype of CD8$^+$ T cells has not been investigated fully in patients with FHL2. We performed immunophenotypic analysis of peripheral blood and measured serum pro-inflammatory cytokines in five patients with FHL2. All patients showed significantly increased subpopulations of activated CD8$^+$ T cells with down-regulation of CD5, which were negligible among normal controls. Analysis of T-cell receptor Vβ repertoire suggested the reactive and oligoclonal expansion of these cells. The proportion of the subset declined after successful treatment concomitant with reduction in the serum levels of cytokines in all patients except one who continued to have a high proportion of the subset and died. These findings suggest that down-regulation of CD5 on activated CD8$^+$ T cells may serve as a useful marker of dysregulated T cell activation and proliferation in FHL2.

1. Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal disease that is characterized by marked systemic inflammation and unregulated activation of macrophages and T cells [1,2]. Patients with HLH may present with fever, cytopenia, hepatosplenomegaly, liver dysfunction, coagulation abnormalities, and hemophagocytosis [1,2]. HLH is comprised of primary and secondary forms. Primary HLH includes familial HLH (FHL), which is caused by genetic defects related to granule-dependent cytotoxicity, and immunodeficiencies, such as X-linked lymphoproliferative syndrome. Mutations in the *PRF1*, *UNC13D*, *STX11*, and *STXBP2* genes cause FHL type 2 (FHL2), FHL3, FHL4, and FHL5, respectively. Perforin is a crucial effector molecule for cytotoxicity that is present in the granules of cytotoxic T lymphocytes and natural killer (NK) cells. FHL2 (perforin deficiency) accounts for more than half of the FHL cases in Japan [3]. Secondary HLH is associated with a variety of infections, autoimmune diseases and malignancies. Epstein-Barr virus (EBV)-associated HLH (EBV–HLH) is the most frequent subtype of HLH in Japan [4]. Establishing a diagnosis of HLH may be difficult when based solely on clinical and laboratory findings, because those findings are often present in severely ill patients. It is also difficult to differentiate between primary and secondary HLH and diagnose a specific subtype of HLH during the acute phase of HLH.

Abbreviations: EBV, Epstein-Barr virus; FHL, familial hemophagocytic lymphohistiocytosis; HLA, human leukocyte antigen; HLH, hemophagocytic lymphohistiocytosis; IM, infectious mononucleosis; mAb, monoclonal antibody; NK, natural killer; PE, phycoerythrin; PBMCs, peripheral blood mononuclear cells; TCR, T-cell receptor.

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We have recently reported the clonal proliferation of activated $CD8^+$ T cells with down-regulation of CD5 in patients with EBV–HLH [5]. This unique immunophenotype of $CD8^+$ T cells could be a valuable tool for the diagnosis of EBV–HLH [5]. However, the immunophenotypic features of T cells in other subtypes of HLH have not been fully characterized. Human CD5 is a membrane glycoprotein that belongs to the scavenger receptor cysteine-rich family of receptors [6–9]. It is expressed on thymocytes, mature peripheral T cells and a small population of B cells, and is involved in the modulation of antigen-specific receptor-mediated activation and differentiation signals [6–9]. It has recently been reported that CD5 is recruited and colocalized with CD3 at the immunological synapse and inhibits T-cell receptor (TCR) signaling in T cells without interfering with immunological synapse formation [10]. Although an expanded subpopulation of $CD8^+$ T cells lacking expression of CD5 has been reported in a single case of FHL2 [11], the nature of $CD8^+$ T cells with down-regulation of CD5 in FLH remains to be elucidated. In this report, we describe the down-regulation of CD5 on activated $CD8^+$ T cells in patients with FHL2 and discuss the relationship between down-regulation of CD5 and systemic inflammation.

2. Materials and methods

2.1. Patients

We studied five patients with FHL2 from five families, all of whom were born to non-consanguineous Japanese parents. Table 1 presents the clinical and laboratory data of the patients. All patients showed typical features of HLH, such as persistent fever, hepatosplenomegaly, cytopenia, liver dysfunction, and hypercytokinemia, i.e., neopterin, interferon-γ, and interleukin-6 at the onset of FHL2. Defective NK cell activity was a universal feature. In patient P3, HLH was triggered by a primary EBV infection, in which the major cellular target of EBV infection, as assessed by *in situ* hybridization for EBV-encoded small RNA1 was B cells (data not shown) but not $CD8^+$ T cells, resulting in marked lymphocytosis with atypical lymphocytes. The disease onset was during early infancy in all patients except for patient P4. Patient P2 did not respond to the HLH-2004 treatment protocol [12], and died at 12 days of age. Patients P1, P3, P4 and P5 underwent stem cell transplantation at the ages of 5 months, 2 years, 5 months and 5 months, respectively [13]. Patients P1 and P3 are alive with no

Table 1
Patient characteristics.

	P1	P2	P3	P4	P5	Normal range
Onset age	1 month	1 day	2 yr	3 months	1 month	
Fever	+	+	+	+	+	
Hepatosplenomegaly	+	+	+	+	–	
Hemophagocytosis*	+	NA	+	+	NA	
Stem cell transplantation	+	–	+	+	+	
Outcome	Alive	Deceased	Alive	Deceased	Alive	
Laboratory findings						
NK cell activity (%)	3.8	2.0	0.0	0.0	0.0	18–40
WBC (/μL)	3100	16,600	51,000	3330	5700	5000–19,500
Neutrophils (/μL)	580	13,600	4290	830	470	3150–6200
Lymphocytes (/μL)	2290	1660	40,800	2060	4380	1500–3000
Hemoglobin (g/dL)	9.2	18.6	8.0	7.8	6.4	9.0–14.0
Platelets (×103/μL)	86	75	50	18	81	150–350
Triglycerides (mg/dL)	356	105	600	129	158	30–149
Fibrinogen (mg/dL)	147	117	NA	59	NA	183–381
Ferritin (ng/mL)	9983	532	2400	1038	427	4.6–204.0
sIL-2R (IU/mL)	3306	11,209	31,000	18,355	4180	220–530
Lymphocyte subsets						
$CD3^+$ (%)	69.2	87.6	92.6	77.0	66.4	64.4–80.2
$CD4^+$ (%)	34.4	40.2	33.5	20.8	29.5	47.3–58.9
$CD8^+$ (%)	31.0	42.1	56.8	53.7	25.1	10.3–24.3
Serum cytokines						
Neopterin (nmol/L)	60	90	125	120	78	2–8
IFN-γ (pg/mL)	37	510	57	1200	205	<5
IL-6 (pg/mL)	122	18	<5	52	<5	<5

NK, natural killer; WBC, white blood cells; sIL-2R, soluble interleukin-2 receptor; IFN-γ, interferon-γ; IL-6, interleukin-6; NA, not available.
* Hemophagocytosis in bone marrow.