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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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Running title: CD5 down-regulation on CD8⁺ T cells in FHL2

Abstract

Hemophagocytic lymphohistiocytosis (HLH) is characterized by uncontrolled activation of T cells and macrophages with overproduction of cytokines. Familial HLH type2 (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 5 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 V β 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.

Keywords

CD5; CD8⁺ T cells; perforin; familial hemophagocytic lymphohistiocytosis.

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.

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.

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 5 patients with FHL2 from 5 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 mo, 2 yr, 5 mo and 5 mo, respectively [13]. Patients P1 and P3 are alive with no evidence of disease, but patient P4 died of acute graft-versus-host disease 5 months later. In patient P5, the transplantation was performed recently. We also investigated 10 cases of EBV-HLH and 13 cases of infectious mononucleosis (IM) as disease controls [5, 14]. All patients with EBV-HLH showed typical clinical features of HLH and exhibited high viral copy numbers. It was noted that CD8⁺ T cells were the major cellular targets of EBV in all EBV-HLH patients by *in situ* hybridization for EBV-encoded small RNA1 in the available samples. No detectable mutations within the *SH2D1A* or *XIAP/BIRC4* genes were observed in the 5 male patients

with EBV-HLH [5]. The diagnosis of IM was clinically determined. All patients with IM exhibited self-limited disease. Primary EBV infection was serologically confirmed for all cases of EBV-HLH and IM. Approval for the study was obtained from the Human Research Committee of Kanazawa University Graduate School of Medical Science, and informed consent was provided according to the Declaration of Helsinki.

2.2. Flow cytometry

For the analysis of perforin expression, peripheral blood mononuclear cells (PBMCs) were isolated from patients and controls by Ficoll-Hypaque gradient centrifugation. PBMCs were stained with fluorescein isothiocyanate-conjugated anti-CD56 and peridinin chlorophyll protein-conjugated anti-CD3 monoclonal antibodies (mAbs). After washing, cells were fixed and permeabilized with Cytotfix/Cytoperm Plus kit and incubated with phycoerythrin (PE)-conjugated anti-perforin mAb or control Ab (BD, San Diego, CA). Perforin expression was evaluated in CD3⁺ CD56⁺ NK cells with a FACSCalibur flow cytometer using CellQuest software (BD Bioscience, Tokyo, Japan). For the analysis of CD5 expression, whole blood was stained with fluorescein isothiocyanate-conjugated anti-CD5, PE-conjugated anti-human leukocyte antigen (HLA)-DR (both from BD), and R-PE-Cy5-conjugated anti-CD4 or anti-CD8 (Dako, Glostrup, Denmark) mAbs. CD5 expression on CD8⁺ T cells was considered negative if it was similar to levels of the NK cells and most of the B cells that do not express CD5 [5]. Analysis of differences among the data groups was performed using Student's unpaired t-test. P-values less than 0.05 were considered significant. Flow cytometric analysis of the TCR V β repertoire was performed as previously described [15].

2.3. Cytokine determination

Serum or plasma concentrations of cytokines were determined using the following enzyme-linked immunosorbent assay kits: interferon- γ and interleukin-6 (R&D systems, Minneapolis, MN); and neopterin (IBL, Hamburg, Germany) [14].

2.4. Mutation analysis

DNA was extracted from blood samples using standard methods. The *PRF1* gene was amplified from genomic DNA using specific primers [16]. Sequencing was performed on purified polymerase chain reaction products using the ABI Prism BigDye Terminator Cycle sequencing kit on an ABI 3130 automated sequencer (Applied Biosystems, Foster, CA).

2.5. Cell cultures

To stimulate T cells, PBMCs were incubated for 72 h with 5 $\mu\text{g/mL}$ phytohemagglutinin, with 1 $\mu\text{g/mL}$ anti-CD3 (OKT3) plus 5 $\mu\text{g/mL}$ anti-CD28 mAb, or with 20 ng/mL phorbol myristate acetate plus 250 ng/mL ionomycin in RPMI 1640 medium containing 10% fetal calf serum and antibiotics [17].

3. Results

3.1. Perforin expression and PRF1 mutations

We first analyzed perforin expression by flow cytometry. As shown in Fig. 1, perforin was not detectable in NK cells from patients P1, P2, P4 or P5. NK cells from patient P3 showed residual expression of the mutated perforin, which might be associated with the later age of onset, compared with the other patients. Table 2 presents the sequencing data for each patient. Patient P1 was a compound heterozygote bearing Leu364fs and Tyr521Cys mutations in *PRF1*. The effect of the novel missense mutation Tyr521Cys was evaluated using a web-based analysis tool, and was found to be deleterious on the basis of the SFIT program [18]. Two distinct frameshift mutations, Pro333fs and Leu364fs, were demonstrated in patient P2. The former is a novel deletion mutation. The 1090_1091delCT (Leu364fs) mutation which was found in patients P1, P2, P4 and P5 has been repeatedly reported in Japanese patients with FHL2 [3]. All patients had at least one nonsense or frameshift mutation.

3.2. Increased subpopulation of activated CD8⁺ T cells with down-regulation of CD5

Immunophenotypic analysis of the lymphocytes demonstrated an increased percentage of CD8⁺ T cells expressing the activation marker HLA-DR in the acute phase of the disease (Table1, Fig. 2A). Compared with CD8⁺ T cells, CD4⁺ T cells were less activated, except for patient P3 who had primary EBV infection. More importantly, the activated CD8⁺ T cells exhibited down-regulation of CD5, which was normally expressed on both CD4⁺ and CD8⁺ T cells from normal individuals (Fig. 2A, 2B). In contrast to the

CD8⁺T cells, CD4⁺T cells from the patients with FHL2 exhibited normal expression of CD5. Although patients with IM have been reported to exhibit marked immune responses to regulate EBV-infected B cells and an increased subpopulation of highly activated CD8⁺T cells, down-regulation of CD5 was not detected in these CD8⁺T cells (Fig. 2B) [5].

The percentage of this unique subset (CD5⁻HLA-DR⁺CD8⁺T cells) decreased after treatment with the HLH-2004 protocol in patient P1 (22.1% to 3.9%), P4 (35.2% to 0.4%) and P5 (20.3% to 0.8%) concomitant with the relief of clinical symptoms and serum levels of neopterin and IFN- γ (Fig. 2C). We did not perform serial analysis in patient P3 due to the availability of sample. In contrast, patient P2 did not respond well to the treatment and continued to exhibit a high percentage of the subset (58.3% to 63.0%) and died at 12 days of age. CD107 cytotoxicity assay was not performed in our patients.

3.3. CD5 expression on normal T cells after in vitro stimulation

To assess whether down-regulation of CD5 was induced on normal T cells after stimulation, PBMCs from 3 normal individuals were cultured for 72 hours with phytohemagglutinin, anti-CD3 plus anti-CD28 mAbs, or phorbol myristate acetate plus ionomycin. Although normal CD8⁺T cells expressed activation marker HLA-DR after any of the stimulation, CD5 was not down-regulated (Fig. 3). We were not able to analyze expression of CD5 after *in vitro* stimulation using PBMCs from patients with FHL2 because no appropriate samples were available, except for from patient P4. No down-regulation of CD5 was observed on the stimulated CD8⁺T cells from patient P4 (data not shown).

3.4. TCR V β repertoire of CD8⁺ T cells

We have previously demonstrated that activated and EBV-infected CD8⁺ T cells with down-regulation of CD5 proliferated clonally in patients with EBV-HLH [5]. To assess clonality of the CD8⁺ T cells from patients with FHL2, we investigated the diversity of the TCR V β repertoire in CD8⁺ T cells by flow cytometry (Fig. 4A).

Although a massive expansion of a specific TCR V β has often been demonstrated for CD8⁺ T cells from patients with EBV-HLH, CD8⁺ T cells from patients with FHL2 exhibited oligoclonal or polyclonal proliferation. Oligoclonal expansion was more prominent in CD5⁻ CD8⁺ T cells than in CD5⁺ CD8⁺ T cells from patient P2 at 12 days of age (Fig.4B).

4. Discussion

HLH is a heterogeneous group of diseases that are characterized by uncontrolled proliferation of activated macrophages and T cells with overproduction of pro-inflammatory cytokines [1, 2]. Activated CD8⁺ T cells are frequently observed during the acute phase of HLH. We have recently described the clinical significance of down-regulation of CD5 on activated and clonally-expanded CD8⁺ T cells that were predominantly infected by EBV in patients with EBV-HLH [5]. However, down-regulation of CD5 is likely a general consequence of the dysregulated proliferation of CD8⁺ T cells. Increased subpopulations of CD5⁻ CD8⁺ T cells have been reported in patients with allogeneic bone marrow transplantation, human immunodeficiency virus-1 infection, acute herpes virus infections and peripheral T-cell neoplasma [19-22]. In addition, a 17-day-old patient with FHL2 has been reported to exhibit uncontrolled proliferation of CD5⁻ CD8⁺ T cells that showed massive infiltration into the liver [11]. We therefore investigated whether this unusual subset of CD5⁻ CD8⁺ T cells was generally present in FHL2, which represents most common form of FHL.

In the present study, we demonstrated a significant increase in the subpopulation of CD5⁻ CD8⁺ T cells in all patients with FHL2, compared with control participants. These cells expressed HLA-DR antigen, indicating an activated phenotype. In contrast to EBV-HLH in which expanded CD8⁺ T cells often reacted with a specific TCR V β mAb reflecting clonal proliferation of EBV-infected cells [5], CD8⁺ T cells from patients with FHL2 exhibited much milder restriction in TCR V β repertoire suggesting reactive proliferation. This unique subset of CD5⁻ HLA-DR⁺ CD8⁺ T cells in FHL2 was detectable

only in the acute phase of HLH in which patients exhibited hypercytokinemia, and declined progressively after successful treatment concomitant with the levels of serum ferritin, soluble IL-2 receptor and pro-inflammatory cytokines. Thus, serial analysis of CD5 expression and activation markers on CD8⁺ T cells may represent an additional valuable tool for the follow-up of patients with FHL2. Moreover, this profile might provide us with clinical clues to suspect FHL2 during the initial flow cytometric assessment of lymphocyte subsets with a small amount of peripheral blood.

The mechanism underlying down-regulation of CD5 on activated CD8⁺ T cells from patients with FHL2, as well as with EBV-HLH, remains to be elucidated. It is also unknown whether CD8⁺ T cells from other HLH cases exhibit similar profiles during the acute phase. Failure of the down-regulation of CD5 on normal CD8⁺ T cells after *in vitro* stimulation suggests that this profile indicates the highly dysregulated activation and proliferation of CD8⁺ T cells *in vivo* in the setting of HLH. On the other hand, studies of T cells from CD5-deficient mice have reported hyperresponsiveness of CD5⁻ T cells to TCR stimulation, suggesting that down-regulation of CD5 might contribute to the uncontrolled proliferation of CD8⁺ T cells in FHL2. Further studies are necessary to address these issues. Because a variety of primary and secondary causes of HLH lead to similar clinical and biological features, and HLH can range from a self-limited episode to a rapidly fatal course [11], it is necessary to identify a parameter that can predict severe cases of HLH, for which the timely initiation of life-saving therapy would be required. Further investigations are underway to determine whether down-regulation of CD5 on activated CD8⁺ T cells could distinguish severe cases of HLH from other inflammatory conditions, including mild cases of HLH.

In summary, our studies demonstrate the increased subpopulation of activated CD8⁺ T cells with down-regulation of CD5 in the acute phase of FHL2, and point to an additional aspect of the immune dysregulation in this disease.

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References

- [1] Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL: How I treat hemophagocytic lymphohistiocytosis. *Blood* 2011;118:4041.
- [2] Usmani GN, Woda BA, Newburger PE: Advances in understanding the pathogenesis of HLH. *British journal of haematology* 2013.
- [3] Nagai K, Yamamoto K, Fujiwara H, An J, Ochi T, Suemori K et al. : Subtypes of familial hemophagocytic lymphohistiocytosis in Japan based on genetic and functional analyses of cytotoxic T lymphocytes. *PloS one* 2010;5:e14173.
- [4] Ishii E, Ohga S, Imashuku S, Yasukawa M, Tsuda H, Miura I et al. : Nationwide survey of hemophagocytic lymphohistiocytosis in Japan. *International journal of hematology* 2007;86:58.
- [5] Toga A, Wada T, Sakakibara Y, Mase S, Araki R, Tone Y et al. : Clinical significance of clonal expansion and CD5 down-regulation in Epstein-Barr Virus (EBV)-infected CD8+ T lymphocytes in EBV-associated hemophagocytic lymphohistiocytosis. *The Journal of infectious diseases* 2010;201:1923.
- [6] Bierer BE, Nishimura Y, Burakoff SJ, Smith BR: Phenotypic and functional characterization of human cytolytic T cells lacking expression of CD5. *The Journal of clinical investigation* 1988;81:1390.
- [7] Lozano F, Simarro M, Calvo J, Vila JM, Padilla O, Bowen MA et al. : CD5 signal transduction: positive or negative modulation of antigen receptor signaling. *Critical reviews in immunology* 2000;20:347.

- [8] Friedlein G, El Hage F, Vergnon I, Richon C, Saulnier P, Lecluse Y et al. : Human CD5 protects circulating tumor antigen-specific CTL from tumor-mediated activation-induced cell death. *Journal of immunology* 2007;178:6821.
- [9] Dalloul A: CD5: a safeguard against autoimmunity and a shield for cancer cells. *Autoimmunity reviews* 2009;8:349.
- [10] Brossard C, Semichon M, Trautmann A, Bismuth G: CD5 inhibits signaling at the immunological synapse without impairing its formation. *Journal of immunology* 2003;170:4623.
- [11] Karandikar NJ, Kroft SH, Yegappan S, Rogers BB, Aquino VM, Lee K et al. : Unusual immunophenotype of CD8+ T cells in familial hemophagocytic lymphohistiocytosis. *Blood* 2004;104:2007.
- [12] Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S et al. : HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatric blood & cancer* 2007;48:124.
- [13] Nishi M, Nishimura R, Suzuki N, Sawada A, Okamura T, Fujita T et al. : Reduced-intensity conditioning in unrelated donor cord blood transplantation for familial hemophagocytic lymphohistiocytosis. *American journal of hematology* 2012;87:637.
- [14] Wada T, Muraoka M, Yokoyama T, Toma T, Kanegane H, Yachie A: Cytokine profiles in children with primary Epstein-Barr virus infection. *Pediatric blood & cancer* 2013.
- [15] Okajima M, Wada T, Nishida M, Yokoyama T, Nakayama Y, Hashida Y et al. : Analysis of T cell receptor Vbeta diversity in peripheral CD4 and CD8 T

- lymphocytes in patients with autoimmune thyroid diseases. *Clinical and experimental immunology* 2009;155:166.
- [16] Keerthikumar S, Raju R, Kandasamy K, Hijikata A, Ramabadran S, Balakrishnan L et al. : RAPID: Resource of Asian Primary Immunodeficiency Diseases. *Nucleic acids research* 2009;37:D863.
- [17] Wada T, Seki H, Konno A, Ohta K, Nunogami K, Kaneda H et al. : Developmental changes and functional properties of human memory T cell subpopulations defined by CD60 expression. *Cellular immunology* 1998;187:117.
- [18] Hijikata A, Raju R, Keerthikumar S, Ramabadran S, Balakrishnan L, Ramadoss SK et al. : Mutation@A Glance: an integrative web application for analysing mutations from human genetic diseases. *DNA research : an international journal for rapid publication of reports on genes and genomes* 2010;17:197.
- [19] Bierer BE, Burakoff SJ, Smith BR: A large proportion of T lymphocytes lack CD5 expression after bone marrow transplantation. *Blood* 1989;73:1359.
- [20] Indraccolo S, Mion M, Zamarchi R, Coppola V, Calderazzo F, Amadori A et al. : A CD3+CD8+ T cell population lacking CD5 antigen expression is expanded in peripheral blood of human immunodeficiency virus-infected patients. *Clinical immunology and immunopathology* 1995;77:253.
- [21] Borthwick NJ, Bofill M, Hassan I, Panayiotidis P, Janossy G, Salmon M et al. : Factors that influence activated CD8+ T-cell apoptosis in patients with acute herpesvirus infections: loss of costimulatory molecules CD28, CD5 and CD6 but relative maintenance of Bax and Bcl-X expression. *Immunology* 1996;88:508.

- [22] Jamal S, Picker LJ, Aquino DB, McKenna RW, Dawson DB, Kroft SH:
Immunophenotypic analysis of peripheral T-cell neoplasms. A multiparameter
flow cytometric approach. American journal of clinical pathology 2001;116:512.

Table 1. Patient characteristics.

	P1	P2	P3	P4	P5	Normal range
Onset age	1 month	1 day	2 years	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)	3,100	16,600	51,000	3,330	5,700	5,000-19,500
Neutrophils (/μL)	580	13,600	4,290	830	470	3,150-6,200
Lymphocytes (/μL)	2,290	1,660	40,800	2,060	4,380	1,500-3,000
Hemoglobin (g/dL)	9.2	18.6	8.0	7.8	6.4	9.0-14.0

Platelets (x 10 ³ /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)	9,983	532	2,400	1,038	427	4.6-204.0
sIL-2R (IU/mL)	3,306	11,209	31,000	18,355	4,180	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	1,200	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.

Table 2. *PRF1* mutations

	Nucleotide mutation	Predicted effect
P1	c.1090_1091delCT	p.Leu364fs
	c.1562A>G	p.Tyr521Cys
P2	c.996_1000delGCCCG	p.Pro333fs
	c.1090_1091delCT	p.Leu364fs
P3	c.1246C>T	p.Gly416X
	c.1349C>T	p.Thr450Met
P4	c.1090_1091delCT*	p.Leu364fs
P5	c.1090_1091delCT*	p.Leu364fs

* homozygous mutation.

Figure Legends

Fig. 1. Perforin expression.

Intracellular expression of perforin in CD3⁻ CD56⁺ NK cells. Thin lines indicate control antibody; thick lines represent monoclonal antibody specific for perforin.

Fig. 2. Expression of CD5 by CD8⁺ T cells.

(A) Expression of CD5 and HLA-DR on the CD4⁺ and CD8⁺ T cells. The percentage of cells gated in each region is shown. (B) The frequency of CD5⁻ HLA-DR⁺ CD8⁺ T cells. Shown are the percentages of CD5⁻ HLA-DR⁺ cells among CD8⁺ T cells in controls ($n = 10$), IM ($n = 13$), EBV-HLH ($n = 10$), and FHL2 ($n = 5$). Bars represent the standard deviation. $*p < 0.01$. (C) Correlation between the percentages of CD5⁻ HLA-DR⁺ CD8⁺ T cells and serum levels of neopterin and IFN- γ . Open symbols indicate data of pre-treatment (Tx) time points; solid symbols represent those of post-Tx time points. EBV, Epstein-Barr virus; FHL2, familial hemophagocytic lymphohistiocytosis type 2; IM, infectious mononucleosis; HLH, hemophagocytic lymphohistiocytosis; IFN- γ , interferon- γ .

Fig. 3. Expression of CD5 after *in vitro* stimulation.

PBMCs from normal controls were cultured for 72 h with phytohemagglutinin (PHA), with anti-CD3 (OKT3) plus anti-CD28 mAb, or with phorbol myristate acetate (PMA) plus ionomycin. Expression of CD5 and HLA-DR on CD8⁺ T cells is shown.

Fig. 4. TCR V β repertoire.

(A) Peripheral blood samples were stained with monoclonal antibodies (mAbs) for individual TCR V β together with anti-CD4 and anti-CD8 mAbs. The percentage of TCR V β expression within the CD8⁺ T cells was shown. (B) Peripheral blood samples were stained with mAbs for individual TCR V β subfamilies together with anti-CD8 and anti-CD5 mAbs. The percentage of TCR V β expression within the CD5⁺ CD8⁺ and CD5⁻ CD8⁺ T cells from patient P2 at 12 days of age was shown.

Fig.1

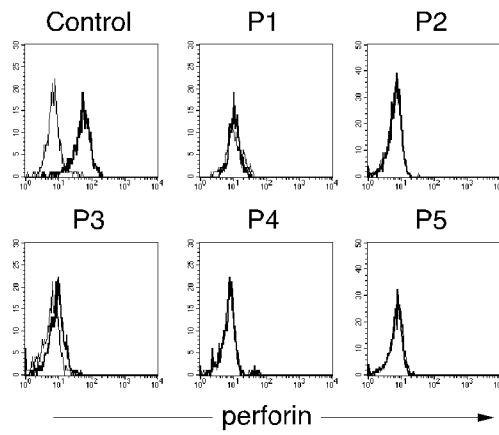


Fig.2

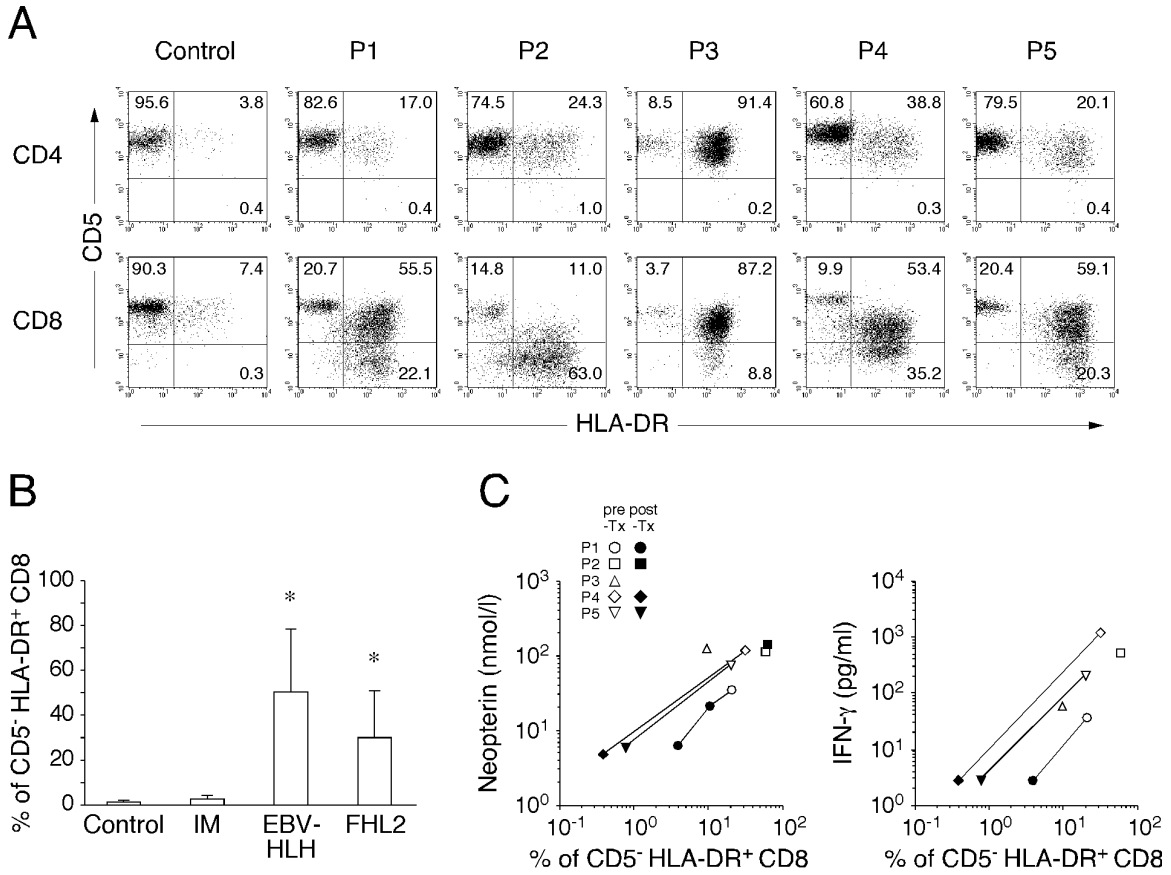


Fig.3

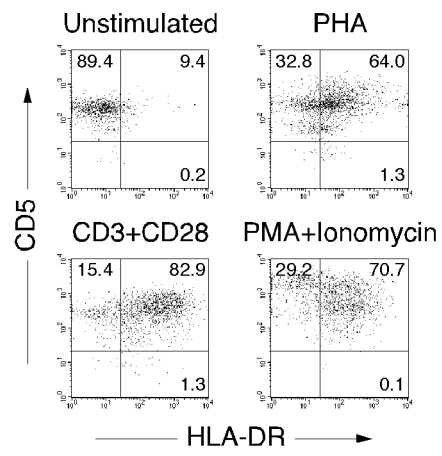


Fig.4

