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Short communication

Synergistic defects of novo FAS and homozygous UNC13D leading to autoimmune lymphoproliferative syndrome-like disease: A 10-year-old Chinese boy case report

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ABSTRACT

Autoimmune lymphoproliferative syndrome (ALPS) usually presents in childhood with fever, nonmalignant splenomegaly and lymphadenopathy along with hemocytopenia. This case report describes a 10-year-old boy presenting with signs of autoimmune disease, splenomegaly, hepatomegaly and resistant hemocytopenia. Sirolimus controlled the relapsed thrombocytopenia after splenectomy. Sequencing of the FAS gene identified two spontaneous heterozygous mutations (c.234 T > G, p.D78E) (c.236dupA, p.P80Tfs*26). The boy's homozygous missense variation (c.2588G > A, p.G863D) (rs140184929) in UNC13D gene had been identified as being related to familial hemophagocytic lymphohistiocytosis (FHL). TCRαβ + CD4/CD8 double-negative T cells (markers of ALPS) were not significantly increased from the outset. Elevated cytokines, such as interferon (IFN)-γ, interleukin (IL)-6 and tumor necrosis factor α decreased to normal levels after splenectomy whereas IL-10 remained high. Immunological analysis of the patient revealed a marked depletion of forkhead-box $P3^+$ expressing regulatory T cells (Treg) and Th17 cells. The obtained data demonstrate that mutations to FAS and UNC13D which result in overwhelming T-cell and macrophage activation, one associated with inhibited Treg cell development and a severe ALPS-like symptom. Therefore, we propose that variations of *UND13D* may be a risk factor of ALPS development.

1. Introduction

ALPS is due to defective function of the Fas death receptor, which results in defective apoptosis of activated lymphocytes, and often involves autoimmune manifestations (Oliveira et al., 2010; Teachey, 2011). Deleterious heterozygous mutations in the FAS gene are the most common cause of failed lymphocyte apoptosis, termed ALPS-FAS (Price et al., 2014). DNTs, which may accumulate with either somatic or germ-line FAS mutations, are important mediators of disease (Price et al., 2014). ALPS-FAS is most frequently caused by heterozygous mutations that generate mutant FAS proteins, often with defective death domains (Fisher et al., 1995). The mutations in FAS gene exon 3 belong to extracellular portion (Singh et al., 2009). Most extracellular-region FAS mutations induce low FAS expression due to nonsense-mediated RNA decay or protein instability, resulting in defective death-inducing signaling complex formation and impaired apoptosis, although to a lesser extent as compared with intracellular mutations (Kuehn et al., 2011).

Numerous studies have established that variation on UNC13D affecting the Munc13-4 protein, a critical effector of the exocytosis of cytotoxic granules priming cytotoxic granule fusion, results in Familial hemophagocytic lymphohistiocytosis (FHL) (Sieni et al., 2011; Chandrakasan and Filipovich, 2013; Zhang et al., 2016). Munc13-4 is a tethering, docking, and fusion regulator known to participate in the secretory pathway of several cellular systems (Elstak et al., 2011). It is highly expressed in hematopoietic cells and also in lungs, kidneys, and other organs with secretory functions (Koch et al., 2000).FHL is a rare autosomal recessive disorder of immune dysregulation associated with defective perforin-mediated cytotoxicity, leading to ineffective immune hyperactivation upon viral infection, with tissue damage and fatal outcomes (Gholam et al., 2011).However, much less is known about the effects of UNC13D in the development of ALPS. In the case report, we

Abbreviations: ALPS, Autoimmune lymphoproliferative syndrome; FHL, familial hemophagocytic lymphohistiocytosis; HLH, hemophagocytic lymphohistiocytosis; DNTs, double-negative T cells; IFN, interferon; IL, interleukin; Treg, regulatory T cells; Th, T helper cell; $Foxp3^+$, frokhead-box $P3^+$; IVIG, intravenous immunoglobulin; DALD, Dianzani Autoimmune/lymphoproliferative Disease

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describe the clinical and laboratory findings of FAS-exon 3 mutation associated with homozygous UNC13D variant, presenting with features of severe ALPS-like symptom while exhibiting normal number of DNTs and a low percentage of Treg and Th17.

2. Methods

Peripheral blood samples were taken from the patient during different treatment (before\after splenectomy, oral sirolimus). For phenotypic characterization, 100 µl of whole blood were incubated with antibody mixtures for 30 min and subsequently fixed and washed before analysis. The antibodies used were peridinin chlorophyll protein (PerCP)-labelled antiCD3 (clone SK7, BD Pharmingen, San Jose, CA, USA), fluorescein isothiocyanate (FITC)-labelled anti-CD4 (BD Pharmingen, San Jose, CA, USA), allophycocyanine (APC)-labelled antiCD8 (clone SK1, BD Pharmingen, San Jose, CA, USA), APC-labelled anti-CD25 (clone 2A3, BD Pharmingen, San Jose, CA, USA), BD FastImmune™ IFN-γ/IL-4 (BD Pharmingen, San Jose, CA, USA), PE-labelled anti-Foxp3 (clone:PCH101, eBioscience, San Diego, CA, USA), APC-eFluor 780-labelled anti-IL-17A (clone:eBio64DEC17, eBioscience, San Diego, CA, USA). We tested for perforin, granzyme, X-linked inhibitor of apoptosis protein (XIAP), SAP, CD107a and NK cell activity to exclude HLH. Th1, Th2 and Tregs were identified in surface and intracellular stainings as $CD3^+CD8^-$ IFN-γ^+, $CD3^+CD8^-$ IL-4^+ and $CD4^+CD25^+$ $FOXP3^+$ respectively. To measure IL-2, IL-4, IL-6, IL-10, TNF and IFNγ protein levels in a single sample, we used the BD™ CBA Human Th1/Th2 Cytokine Kit II (Catalog No.551809). All samples were analyzed in a FACS Canto II flow cytometer using the FACSDiva software (BD Biosciences) or in a Navios cytometer (Beckman Coulter).

3. Results

3.1. Patient presentation

This article presents a case study of a patient with clinical and genetic characteristics of ALPS and FHL disease despite initial absence of his TCRαβ + CD4/CD8 double-negative T cells (markers of ALPS) elevation. From 2016.9, post-fever he showed unexplained large-scale splenomegaly and lymphadenopathy, along with severe thrombocytopenia, neutropenia and anemia. The patient experienced multiple cytopenia and was unresponsive to steroids, immunoglobulins, platelet transfusions, and recombinant human platelet growth factor. The negative phenotype of TCRαβ + CD4/CD8 double-negative T cells (DNTs) (markers of ALPS) (Boggio et al., 2013) and lack of clinical and laboratory diagnostic criteria of HLH (Chandrakasan and Filipovich, 2013) were of great interest to investigators. His immunoglobulin levels were within normal range, but positive for anti-platelet antibodies, anti-EpsteinBarr viruses, rubella viruses, cytomegalovirus, herpes simplex virus immunoglobulin G and antinuclear antibodies. In addition, the analysis of markers for measles virus, parvovirus B19, schistosomiasis and *Leishmania donovani* infection was negative. In peripheral blood samples, T lymphocytes (CD3+ CD19−) and T helper cells (CD3+ CD4+) were found to be slightly increased. Moreover, the patient displayed amplified proportions of cytokine IFN-γ, IL-10, IL-6. Histological analysis of the bone marrow and spleen did not show signs of hemophagocytosis.

The patient's spleen was palpable in the left iliac fossa, following splenectomy and recovery, the patient was discharged. Unfortunately, the thrombocytopenia returns after a week. At that time, we obtain spleen morphological results which suggested ALPS; in peripheral blood samples, CD3+ CD19− and CD3+ CD8+ cells were significantly elevated. Based on the application of glucocorticoids, we added the immunosuppressive agents sirolimus, after which platelet count was restored.

3.2. Laboratory findings

Routine blood results indicated anemia (hemoglobin 8.4–9.6 g/dL) and thrombocytopenia (0–5 × 10^9/L). One week after splenectomy, platelets (155 × 10^9/L) and hemoglobin (14.5 g/dl) were restored to normal. The following week, thrombocytopenia (5 × 10^9/L) relapsed. Four days after application of rapamycin, platelet count returned to 150 × 10^9/L. The patient displayed persisting T-cell lymphocytosis with an increased proportion of T-helper lymphocyte ($CD3^+CD4^+$), especially Th1 ($CD3^+CD8^-$ IFN-γ^+), while NK and Treg cells (CD3 + CD4 + CD25 + Foxp3 +) were reduced. Reduction of T-helper lymphocyte, Th1 and Treg was observed after splenectomy. After two months of rapamycin, Th1 reduced to normal proportion with an increased Treg to normal level. The serum levels of IFN-, TNF, IL-6 and IL-10 increased rapidly after two weeks of hospitalization. At the time of thrombocytopenia relapse, with the exception of IL-10, IFN-γ, TNF and IL-6 decreased to normal standards two weeks following splenectomy. After stimulation, CD107a expression on NK and CTL cells was increased significantly below the normal reference range. The expression of XIAP and PRF1 on NK and CTL cell membrane was slightly lowered. Investigators could not reconcile all findings with the finding that the proportion of DNTs failed to meet diagnostic criteria at time of hospitalization (Fig. 1).

3.3. Genetic findings

After receiving informed consent from the parents we sequenced the ALPS-related genes (FAS, FASLG, CASP8, CASP10) and genes known to cause common variable immunodeficiency. We identified two rare de novo spontaneous germline heterozygous mutations on the FAS gene (Fig. 1) and a homozygous missense variation (c.2588G > A, p.G863D) on the UNC13D gene. Both FAS mutations were occurred in exon 3 (Fig. 2). The first being missense mutation (c.234 T > G, p.D78E) resulting in an amino acid exchange and significant alpha-helix coefficient alteration (Fig. 3), the second being a frame shift mutation (c.236dupA, p.P80Tfs*26). Intriguingly, these two variants were not found in dbSNP database or ExAC database.

3.4. Clinical course

Medical history, clinical presentation, and results from laboratory tests led to the clinical diagnosis of autoimmune lymphoproliferative syndrome (ALPS), and treatment with sirolimus (1 mg/d, blood concentration 5–15 ng/L) was initiated. Subsequently, the patient's platelet count and clinical condition improved rapidly.

4. Discussion

In the present case report, we describe the phenotype of a 10-year-old boy presenting with splenomegaly, hepatomegaly and recurrent hemocytopenia. We identified two spontaneous germline heterozygous mutations (c.234 T > G, p.D78E) (c.236dupA, p.P80Tfs*26) in FAS and a homozygous missense variation (c.2588G > A, p.G863D) (rs140184929) in UNC13D. Immunological investigations using the patient's immune effector cells clearly demonstrated a selective T-cell defect with X-linked inhibitor of apoptosis protein (XIAP), Treg and Th17 cells. Moreover, the variation of UNC13D may play a role in disease pathogenesis, as both low expression of CD107a and perforin-1 (PRF1) on NK-cell and cytotoxic T lymphocyte was observed. Despite the fact that severe hemocytopenia can conventionally be controlled with blood transfusion, hematopoietic stimulating factor and immunomodulator including glucocorticoids and intravenous immunoglobulin (IVIG) infusions, the patient persisted in severe thrombocytopenia, autoimmune neutropenia, anemia and progressive splenomegaly. Combined FAS, UNC13D and XIAP mutation have been previously described in a 12-year-old boy who initially presented with