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LETTER TO THE EDITOR

Course of IL-2-inducible T-cell kinase deficiency in a family: lymphomatoid granulomatosis, lymphoma and allogeneic bone marrow transplantation in one sibling; and death in the other

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IL-2-inducible T-cell kinase (ITK) is an intracellular protein expressed in T lymphocytes. It has a critical role in proximal TCR signaling, T cell differentiation and natural killer (NK)T cell maturation/survival. It participates in antiviral and antitumor immunity.[1,2]

Deficiency of ITK was first reported in two siblings in 2009 as an autosomal recessive form of primary immunodeficiency (PID).[3] Ghosh *et al.*[4] reviewed the total of 9 cases of ITK deficiency. Recurrent febrile episodes associating EBV, hemophagocytosis and lymphoproliferation have been identified, and hematopoietic stem cell transplantation (HSCT) was performed in two of them.

Here, we report two siblings with ITK deficiency who presented with history of recurrent infections and progressive CD4+ lymphocytopenia. Patient 1, a 7-year-old boy, after admitted with chronic persistant productive cough, was referred for pulmonary nodules, unresponsive to antibacterial therapy. He was the first child of healthy first degree consanguineous parents and had a history of sibling death with features of lymphoproliferative disease at the age of 8 months (Figure 1). He was treated for bronchial hyperreactivity in the early childhood. On physical examination, he had bilateral multiple mobile submandibular and axillary lymph nodes up to 1×1 cm, bilateral coarse crackles on both lung fields, hepatomegaly (2 cm below the costal margin). His complete blood count and immunological findings are presented in Table 1. CMV and EBV DNA were negative (CMV IgM, negative; CMV IgG positive; Anti-EBV viral capsid antigen (VCA) IgM, anti-EBV early antigen (EA) IgG were negative; anti-EBV VCA IgG, anti-EBV Epstein Barr nuclear antigen IgG were positive (before IVIG therapy)). For T cell deficiency, monthly IVIG therapy was started. Chest X-ray and computed tomography (CT) showed areas of nodular consolidation and mediastinal lymphadenopathy (Figure 1). During hospitalization, he had severe hypoxemia and tachypnea. Bronchoscopic evaluation was normal, no microorganism was isolated. Open lung biopsy revealed lymphomatoid granulomatosis (LG) stage 1. Lymphoplasmocytic cell infiltration, few cytomegalic cells having inclusions were seen in the alveolar epithelium, EBV latent membrane protein (LMP) was positive in small number of cells. Thoracic nodules regressed after corticosteroids. Sequence analysis for ITK gene revealed the same homozygous missense mutation (C1085T (R335W) in exon 11) reported by Huck *et al.*[3] in both affected siblings (Figure 1). The parents were heterozygous carriers for this mutation. Donor screening was started for HSCT.

He was admitted again at age 8 with the complaints of vomiting and abdominal pain. Abdominal ultrasound and CT images showed hepatosplenomegaly, multiple mesenteric, parailiac lymphadenopathies and $4.5 \times 5.5 \times 6$ cm pelvic mass indenting the bladder from left upper side. Tru-cut biopsy showed mature B cell lymphoma (Burkitt Lymphoma). Bone marrow (BM) aspiration smear showed L3 type blasts, FAB LMB-96 GroupC regimen (central nervous system-negative) was started. With prophase and induction courses, complete response in abdominal disease was achieved. For stable thoracic nodules, open lung biopsy performed showed LG that 3 cycles of rituximab treatment (375 mg/m^2 3-weekly) was given. A new 24 mm solid mass detected after 3 months in the left adrenal gland was totally removed, and was also found to be compatible with LG type 1. Interferon alpha-2a (10 000 000 IU/m^2 per day 3 times a week) was given instead of rituximab due to the relapse, and complete regression of the thoracic nodules was recorded after 3 months of therapy.

HLA compatible related or unrelated donor could not be found, and during follow-up period, mother had unplanned pregnancy. Amniocentesis and molecular analysis showed that the fetus had no ITK mutation and was HLA fully-compatible with Patient 1. Cord blood transplantation was planned after the treatment of LG and lymphoma. Conditioning regimen including rituximab (375 mg/m^2 at days -11), fludarabin (30 mg/m^2/day at days $-10, -9, -8, -7, -6, -5$ days), melphalan (140 mg/m^2/day at day -4), and anti-thymocyte globulin (rabbit) (7.5 mg/kg/day at days $-4, -3, -2, -1$) were given. 1.1×10^5/kg CD34+ stem cells from cord blood and additionally 2.8×10^6/kg CD34+ stem cells from bone marrow (donor was 5 months old) were given to the patient. Neutrophil and platelet engraftment were achieved at day +14 and +17 respectively. Acute GvHD prophylaxis (cyclosporine A 3 mg/kg/day) was given. CMV reactivation during post-transplantation course was successfully treated with gancyclovir. Now, +17 months after HSCT he is doing well, and full donor chimerism was achieved.

Patient 2, sister of index patient, was admitted at age 3 with recurrent pulmonary infection. As she had intermittant fever and pulmonary reticular infiltration on chest X-ray, she was hospitalized at the same time as the brother and given antibacterial therapy. Ten days after her discharge, she readmitted with fever. On physical examination she had perforated left eardrum, bilateral submandibular (2×2 cm), cervical (1×1 cm), axillary (1.5×1.5 cm) lymphadenopathies and hepatosplenomegaly (liver and spleen were both palpable about 6–7 cm below the respective costal margins). Complete blood count and immunologic findings are given in Table 1. EBV serology was consistent with recent EBV infection; EBV VCA IgM, 4.3 (0–1.1); EBV EBNA IgG, 41.7 (0–20); EBV VCA IgG, 35.1(0–20); EBV EA IgG, 35.2 (0–20). CMV IgM was 1.79 IU/mL (0–0.9) and CMV IgG was 14 IU/mL (0–6), which may be due to cross reaction as CMV PCR was negative. BM aspiration smear revealed increased number of histiocytes. Parenchymal nodules, bilateral hilar and mediastinal lymphadenopathies were seen on chest CT (Figure 1). She had recurrent fever episodes associated with lymphadenopathy. After definite diagnosis, monthly IVIG therapy and matched unrelated donor screening was started. However, an HLA compatible donor was not found. Several months after the diagnosis she died from respiratory

insufficiency a few days after being admitted with severe dyspnea and lymphadenopathies to a local hospital.

It is difficult to control EBV infections when there is defect in T cell function,[5] T cell cytotoxicity and disrupting the interactions between B and T cells. Also in secondary immunodeficiencies, such as in HSCT and AIDS, severe EBV infections may develop.[6,7] EBV has a predilection for B cells and mechanisms involved in its control involve invariant NKT (iNKT) cells, EBV-specific cytotoxic CD8+T lymphocytes, and virus-specific humoral components (8,9). Depletion of iNKT cells are shown to cause deficiency in

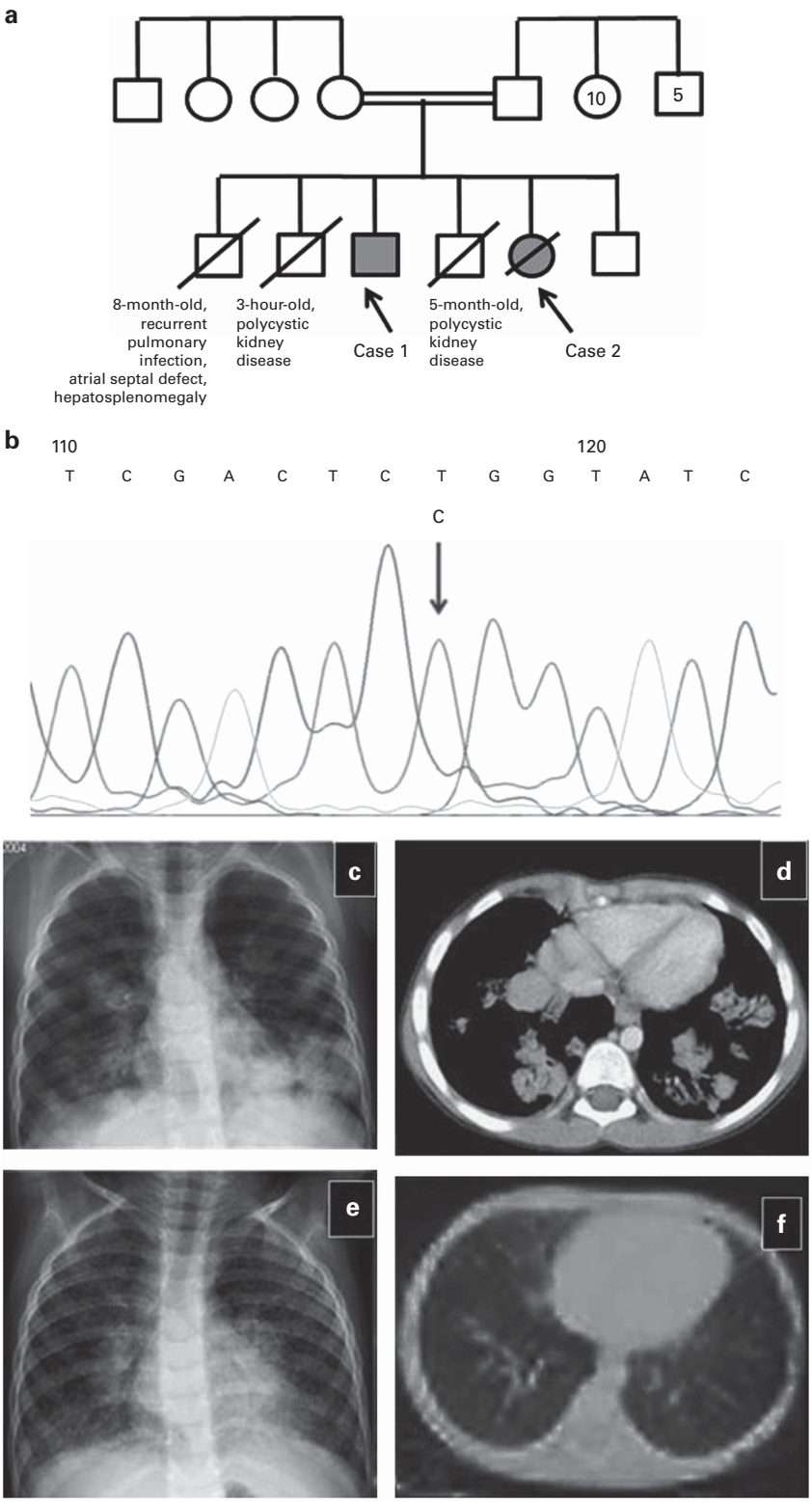

Figure 1. (**a**) Pedigree (Numbers indicate number of siblings). (**b**) Homozygous missense mutation (C1085T (R335W) in exon 11 of the ITK gene. Chest X-Rays and thorax CT sections of Patient 1(**c**, **d**) and Patient 2 (**e**, **f**).

Table 1. Immunologic findings of Patients 1 and 2

		Patient 1	*Patient 2*
Age (year)		7	3.5
Complete Blood Count			
Hb (g/dL)		13.2 (11.5–15.5)	9.3 (11.5–13.5)
WBC (/mm^3)		15 600 (5500–15 500)	10 400 (5500–15 500)
Plt (/mm^3)		158 000 (150 000–400 000)	136 000 (150 000–400 000)
ALC (/mm^3)		5 800 (1100–5900)	2600 (1700–6900)
ANC (/mm^3)		8600 (1500–8000)	6600(1500–8500)
Immunoglobulins (Ig)			
IgA (mg/dL)		40 (70–303)	56.7 (44–244)
IgG (mg/dL)		1770 (764–2134)	1200 (640–2010)
IgM (mg/dL)		77 (69–387)	455 (52–297)
IgE (IU/L)		13.8	2.74
Anti A/Anti B		1/64 (+)	(−) (Blood group: BRh+)
Anti Hbs (mLU/mL)		59.82	(−)
Specific Ab response	(Pneumococcal polisaccharide Ab response)	NT	Normal
Lymphocyte subsets			
CD3 (%) (/mm^3)		60 (60–76) 3480 (1200–2600)	68 (56–75) 1742 (1400–3700)
CD4 (%) (/mm^3)		14 (31–47) 436 (650–1500)	27 (28–47) 728 (700–2200)
CD8 (%) (/mm^3)		43 (18–35) 2494 (370–1100)	36 (16–30) 936 (490–1300)
CD19 (%) (/mm^3)		32 (13–27) 1856 (270–860)	24 (14–33) 624 (390–1400)
CD16+56 (%) (/mm^3)		16 (4–17) 928 (100–480)	7 (4–17) 182 (130–720)
Lymphocyte transformation (SI) (P/C)	PHA	Low (1/2 control)	Low (1/2 control)
	Con A	Low (1/2 control)	—
	PMA+Ion	Low (1/2 control)	Low (1/2 control)

Abbreviations: AEC = absolute eosinophil count; ALC = absolute lymphocyte count; con A = concanavalin A; Hb = haemoglobin; NT = not tested; P/C = patient/control; PHA = phytohemaglutinin; Plt = platelet; PMA+Ion = phorbol myristate acetate+ionomycine; SI = stimulation index. Reference ranges for lymphocyte subsets are taken from reference number 15.

controlling EBV infection during the earliest stages of B-cell infection, and increase both viral titers and EBV-infected B cells.[8,9]

Chronic active EBV infection with persistent symptomatic viremia, dysgammaglobulinemia, hemophagocytic lymhohistiocytosis, or epithelial, mesenchymal, and lymphoid malignancies may develop in these patients.[10] Several PID, such as STK-4, CD27, MAGT1 and ITK deficiencies, have been discovered to associate EBV induced lymphoproliferation.[11]

Although the majority of patients with ITK deficiency develop serious and uncontrolled EBV associated lymphoproliferation leading to death, presentation may be in form of persistent symptoms of infectious mononucleosis, such as recurrent febrile episodes and lymphadenopathies, and CD4 lymphopenia with only recurrent infections.[3,5,11] Unless HSCT is performed, the disease is fatal in most patients.[12] In the course, patients need therapy of antiviral agents, corticosteroid, rituximab or other chemotherapeutic agents especially for LG and Hodgkin lymphoma (HL). Patients with ITK deficiency have progressive hypogammaglobulinaemia, loss of CD4+ T cells with a declining proportion of naive cells, and low iNKT cell counts.[3,4] Both Patient 1 and 2 had normal IgG, IgM and low IgA levels with normal Ab responses; mildly low CD4+ T cells (low percentage, low range of normal CD4+ T cell counts) and decreased lymphoproliferative response to mitogens.

LG is a progressive EBV-driven lymphoproliferative disease.[13] The most common site of involvement is the lung. Other extranodal sites such as skin, kidney, liver, and central nervous system are less frequently involved.[14] Cells in LG are shown to be EBV-infected B cells, monoclonal, and they can progress to lymphoma in 12–47% of patients.[13,14] Low incidence of LG contribute to frequent delays in diagnosis. Biopsy or EBV DNA study is necessary for diagnosis. For granulomatous pulmonary involvement, patient 1 underwent open lung biopsy. Biopsy was needed for definite diagnosis.

B cell neoplasia is the most common type of lymphoma in patients with ITK deficiency,[4] but BL has not been reported in previous patients. The presentation of immunodeficiency-associated BL, is frequently nodal, commonly with bone marrow involvement.[7] Despite its aggressiveness, BL is highly sensitive to chemotherapy. BL after LG was seen in patient 1. He had nodal BL with bone marrow involvement, and remission was achieved with chemotherapy. Rituximab has been shown to be useful in patients with EBV associated lymphoproliferative disorders.[12] The patient benefited from rituximab which was used in both chemotherapy and conditioning regimen in HSCT.

As far as we know, 7 out of 8 previously reported IITK-deficient patients developed lymphoproliferative disease such as HL, B-cell lymphoproliferative disorder, LG and large B cell lymphoma. Patient 1 is the third patient with ITK which was succesfully treated by HSCT.[4]

Patients presenting with recurrent lymphoproliferation and relapsing lymphoma should be regularly screened for EBV and CMV, and evaluated especially for PID associating with EBV induced lymphoproliferation. Steroids, rituximab and antiviral therapies may be used in ITK deficiency for LG and lymphoma. Hematopoietic stem cell transplantation corrects the primary immune defect and lymphoproliferation.[12]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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