Novel Insights from Clinical Practice

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IL-2-Inducible T-Cell Kinase Deficiency with Pulmonary Manifestations due to Disseminated Epstein-Barr Virus Infection

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Established Facts

- IL-2-inducible T-cell kinase (ITK) deficiency is a recently described, rare genetic disorder with various clinical presentations that mainly induces therapy-resistant Epstein-Barr virus (EBV)-positive B cell proliferation following uncontrolled EBV infection.
- Important characteristics of ITK deficiency are a low percentage of iNKT cells, a subset of innate T cells, and high levels of innate like CD8+ cells.
- Bone marrow transplant has previously been considered as the mainstay of treatment for this rare disease.

Novel Insights

- Here we illustrate an unusual presentation of ITK deficiency as predominant pulmonary B cell proliferation due to a novel homozygous frameshift mutation in ITK.
- Sustained complete remission after anti-CD20 mab treatment in this case outlines the role of anti-CD20 mab in the treatment concept of ITK deficiency (this may help in further treatment options and in recognition of the pathogenesis of this rare immunodeficiency).

Key Words
IL-2-inducible T-cell kinase deficiency · Epstein-Barr virus-positive lymphoproliferative disorder · Lung involvement · Rituximab

Abstract
IL-2-inducible T-cell kinase (ITK) deficiency is a rare inherited immunodeficiency disease characterized by homozygous mutations in the ITK gene and the inability to control Epstein-Barr virus (EBV) infection leading to EBV-associated

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lymphoproliferative disorders of B cell origin. Many aspects of its clinical presentation and immunologic phenotype are still unclear to clinicians. We report on a 14-year-old female patient with complaints of an 8-month history of cough and fever. Imaging studies revealed diffuse pulmonary nodules and mediastinal lymphadenopathy. Transbronchial lung biopsy showed nonmalignant polyclonal B cell proliferation. High titers of EBV DNA were detected by PCR analysis in bronchoalveolar lavage fluid, bone marrow, and blood. Genomic analysis revealed a homozygous single base pair deletion in exon 5 of the ITK gene (c.468delT) in this patient. Treatment with rituximab (anti-CD20 mab) resulted in complete clinical remission with resolution of pulmonary lesions and a negative EBV titer in serum. All patients with EBV-associated lymphoproliferative disorders should be analyzed for mutations in ITK.

Introduction

The IL-2-inducible T-cell kinase (ITK) gene encodes an intracellular tyrosine kinase protein expressed in T cells [1], which contains different domains including a PH, SH3, SH2 [2] and kinase domain. ITK was originally described as an important component of proximal TCR signaling pathways. Furthermore, it has been shown to play a sophisticated role in the development of conventional and innate type T cells in mice. Essentially, ITK provides important signals for the terminal maturation, efficient cytokine production, and peripheral survival of iNKT cells [3]. An interaction with signaling lymphocytic activation molecule (SLAM) has been discussed [4].

ITK deficiency has recently been described as a rare autosomal recessive genetic disorder with various clinical presentations [5, 6] including Epstein-Barr virus (EBV)-associated B cell proliferation and Hodgkin lymphoma following uncontrolled EBV infection, hypogammaglobulinemia, and hemophagocytosis. The clinical presentation of patients with ITK deficiency is very close to that of patients with X-linked lymphoproliferative syndrome (XLP) [7] harboring mutations in SAP (SLAM-associated protein) [8] or XIAP (X-linked inhibitor of apoptosis) [9].

Important immunologic characteristics of ITK deficiency are disturbances in the T cell compartment such as a decline of CD4+ T cells, a high number of innate-like, eomesodermin-expressing CD8+ T cells, and, as in XLP patients, a low percentage of Valpha24+Vbeta11+ iNKT cells, a subset of innate T cells [3, 5]. However, the exact mechanism as well as the pathogenetic role of those changes has not yet been elucidated [8]. ITK-deficient patients have been reported to develop EBV+ B cell proliferations and Hodgkin lymphoma involving lymphoid organs, the liver, kidneys, bone marrow, and the central nervous system. The lymphoproliferative diseases were fatal without bone marrow transplantation in several cases. Here we describe a novel ITK mutation in a patient with isolated, nonmalignant EBV-associated B-lymphocyte infiltration in the lung tissue which dramatically responded to treatment with rituximab.

Methods and Results

An unusual case of ITK deficiency was admitted and worked up in this referral center. EBV DNA PCR was performed according to a method described before with minor modifications [10]. ITK mutation analysis was performed according to the previously described method [4].

A 14-year-old girl, the third child of consanguineous healthy parents, was referred to our center with the chief complaint of an 8-month history of cough, fever, a 3-kg weight loss, and bilateral infiltration in the lower zones of both lungs. No history of rash, oral ulcers, photosensitivity, or joint pain was reported. She had a history of favism, nonfebrile seizures since 2 years treated by carbamazepin, and a severe varicella zoster infection at the age of 6 years. On physical examination, she was febrile and tachypnoeic, with fine crackles heard in both lungs. No peripheral lymphadenopathy or hepatosplenomegaly was documented. All routine laboratory results were normal.

High resolution CT (HRCT) of the lungs revealed bilateral hilar and mediastinal lymphadenopathies in conjunction with bilateral diffuse lung parenchymal nodules (fig. 1). CT scan of paranasal sinuses was consistent with pan sinusitis. The patient underwent another lung HRCT 1 month later which showed progression of the abnormalities in both lungs, with parenchymal nodules, consolidations, pleural effusion, and hilar and mediastinal lymphadenopathies. The pulmonary function test showed a severe restrictive pattern. On abdominal CT, mild hepatosplenomegaly and para-aortal lymph nodes were observed.

After worsening of her respiratory condition, bronchoscopy with both bronchoalveolar lavage (BAL) and transbronchial lung biopsy (TBLB) from the nodular lung lesions, and bone marrow aspiration and biopsy were performed. Histopathologic studies of bone marrow

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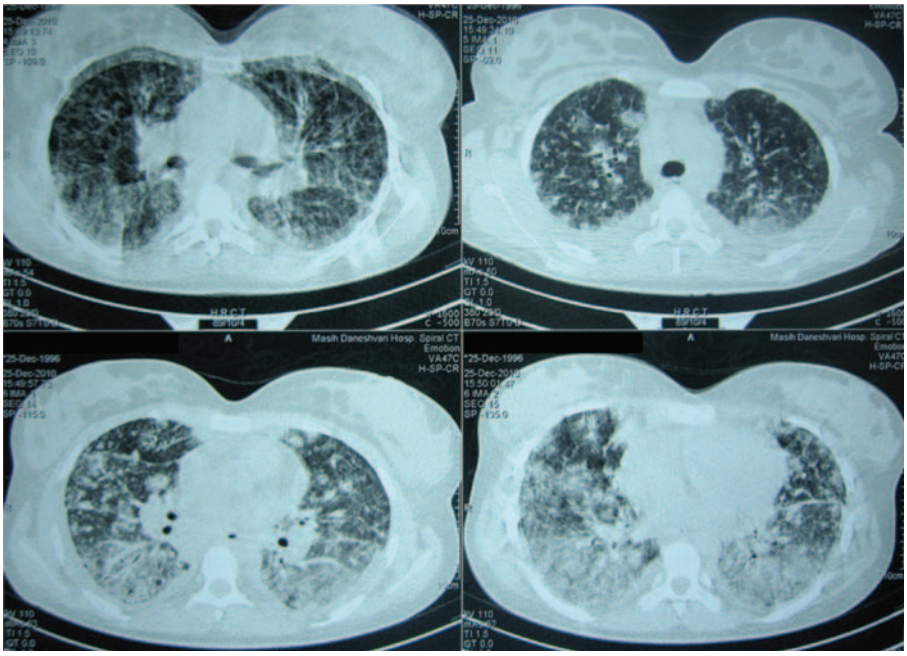

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Fig. 1. HRCT of the lungs shows mediastinal and bilateral adenopathy, diffuse numerous bilateral pulmonary parenchymal nodules and superimposed parenchymal alveolar consolidation and ground glass opacity with air bronchogram in both lower lobes.

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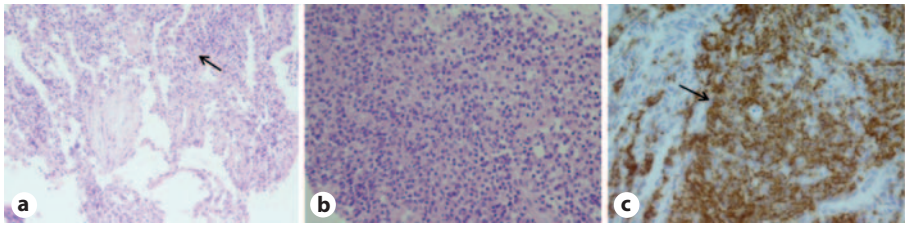

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Fig. 2. Hematoxylin and eosin plus CD20 monoclonal immunostaining of lung tissue biopsy. Fragments of distorted alveolar lung parenchyma due to extensive infiltration of small B lymphocytes cells (arrow) are shown (**a**). No lymphoid follicle formation was seen in our specimen. High magnification of tissue biopsy confirmed the aforementioned results (**b**). The infiltrating lymphocytes in lung tissue biopsy were positive for CD20 (arrow) (**c**).

were normal. In lung tissue, fragments of distorted alveolar lung parenchyma due to extensive infiltration of small B lymphocytes (CD20 positive) were observed (fig. 2). The differential count in BAL fluid depicted 34% macrophages (normal >84%), 55% lymphocytes (normal <13%), and 11% neutrophils (normal <3%). Flow cytometric analysis of blood and BAL demonstrated an inverted CD4+/CD8+ ratio (table 1). Due to the prominent interstitial lung involvement and the histopathologic findings, lymphocytic interstitial pneumonia was primarily suggested for the patient.

The PCR results for EBV DNA in sputum, BAL fluid, blood, the lung tissue sample, and bone marrow were highly positive tested twice in two different samples (table 2). There are no reference values for BAL or TBLB and the amount of fluid for the lavage is quite variable. However, considering that the values of quantitative PCR were significantly positive and control samples were all negative for EBV DNA, an EBV-driven process seemed very likely. Moreover, the patient's sputum cultures were negative for all other pathogenic microorganisms.

One week later, the patient deteriorated with high fever, respiratory distress, widespread crackles in the lungs, and hepatosplenomegaly despite treatment with broad spectrum antibiotics, steroids, and acyclovir. Rituximab was initiated based on previous reports [11]; after the second injection the patient was afebrile without any distress, and hepatosplenomegaly subsided. One month after the first rituximab injection, lung HRCT demonstrated a near normal appearance with complete clearance of the parenchymal lesions. EBV-DNA PCR of blood was negative after the third injection (table 2). At present, 6 months after four doses of rituximab, the patient is in complete health status.

To sum up, the presenting features of the patient, the disseminated EBV infection, the EBV-driven B cell proliferation in the lungs, the hypogammaglobulinemia, and the low CD4+ T cell count in peripheral blood (ta-

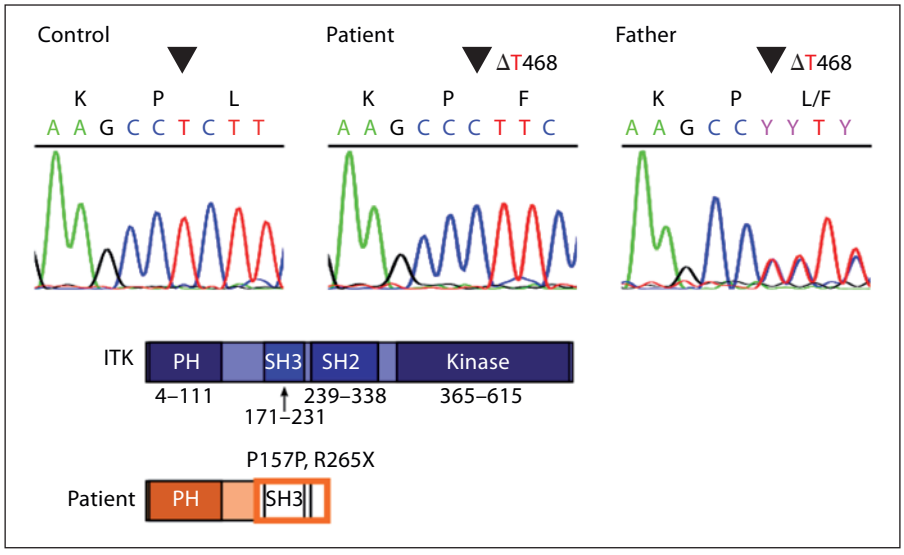

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Fig. 3. Mutated sequence in our patient in comparison to the heterozygous father and a normal control and schemes of the wild-type and mutated ITK protein. The black arrow indicates the position of the single base pair deletion 468delT leading to a frameshift at position 157 and a premature stop codon at position 265 of the ITK protein. The mutation truncates the SH2 and deletes the kinase domain as shown in the schemes of the ITK protein.

Table 1. Laboratory findings of the patient

	Patient values	Normal values for her age
WBC ($\times 10^3$)	8.3	4–11
Diff, %		
Neutrophil	78	52–58
Lymphocyte	8	30–38
Monocyte	5	2–6
Band	2	1–5
Platelets ($\times 10^3$)	285	150–450
Hgb, g/dl	11.7	12.3–15.3
CD4/CD8 in peripheral blood, %	0.2	0.9–3.6
CD4/CD8 in BAL fluid, %	0.5	1.1–3.5
CD3 (blood), %	60	59–85
CD4 (blood), %	10	30–60
CD8 (blood), %	45	11–38
CD19 (blood), %	22	6.4–23
CD16+56+ (blood), %	0.2	0.9–3.6
CD3/CD16+56+ (blood), %	2	
IgG, mg/dl	354	700–1,600
IgA, mg/dl	52	70–400
IgM, mg/dl	38	40–230
CMV antibody (IgG), IU/ml	22	Positive: >11
EBV-VCA (IgG) (blood), IU/ml	35	Positive: >20
LDH	843	225–500
Ferritin	1,200	10–50

Table 2. EBV titer in patient samples before and after treatment with rituximab

Sample	Pretreatment viral load, copies/ml	Posttreatment viral load, copies/ml
BAL sample	33,437,252	Not resampled
Lung biopsy	43,716	Not resampled
Bone marrow	8,262,797	Not resampled
Plasma	13,711,687	Undetectable

ble 1) suggested a genetically based immunodeficiency with lymphoproliferation. Considering the female sex of the patient and the consanguinity of the parents, ITK deficiency was suspected. Mutation analysis revealed a homozygous single base pair deletion in exon 5 of the ITK gene (c.468delT) (fig. 3). This is a novel mutation leading to a frameshift and a severely truncated ITK protein.

Discussion

Here we describe a girl with EBV-associated pulmonary B cell infiltration and mediastinal lymphadenopathy attested to a primary immunodeficiency disorder, finally diagnosed with ITK deficiency. This syndrome and its causative mutations were reported very recently by Borkhardt and colleagues [5]. However, due to the exceedingly low number of cases the complete clinical spectrum of the syndrome may not be known at this early stage.

The initial presentation as well as the course of the disease in our ITK-deficient patient shows some dissimilarities to previous reports by Borkhardt and colleagues [6]. First and foremost, in previous reports, ITK-deficient patients mostly presented with generalized lymphadenopa-

thy, hepatosplenomegaly, severe immune dysregulation, and cytopenia. Our patient, however, due to the isolated involvement of the lungs and mediastinal lymph nodes, only had pulmonary symptoms, delaying the diagnosis of a lymphoproliferative disorder several months. Furthermore, ITK deficiency in our patient presented itself at 14 years of age, whereas previously reported ITK-deficient patients presented at earlier ages between 4 and 6 years of age. Moreover, in contrast to previous reports in which patients had atypical EBV-positive B cell proliferations with progression to Hodgkin lymphoma [12] in the majority of cases, this patient demonstrated a polyclonal proliferation of small B cells not suggestive of any malignant lymphoma.

The phenomenal steady response in this patient to the monoclonal anti-CD20 antibody Rituxumab, even though she did not respond to steroids or acyclovir treatment, indicates the efficacy of rituximab as part of a treatment plan for ITK deficiency. The patient remained symptom free after rituximab treatment and the pulmonary involvement and EBV titer faded off, yet stem cell transplantation may be considered as a definite final treatment.

Our patient harbors a homozygous single base pair deletion in exon 5 of the ITK gene (c.468delT), which has not been reported before. This mutation causes a frameshift generating a premature stop codon at position 265 of the 620 residue protein. The resulting truncation of the SH2 and complete deletion of the kinase domain strongly support a loss-of-function mechanism. However, confirmatory functional assays will be needed.

In conclusion, our report expands the clinical and histopathologic picture as well as the genetic variability of ITK deficiency. We therefore support the demand that all patients with EBV-associated lymphoproliferative disorders be analyzed for mutations in ITK. Moreover, genetic studies in patients without defects in ITK, SAP, or XIAP should be encouraged in order to discover further disease-causing mutations in different pathways of TCR and IL-2 signaling, which could manifest by EBV-associated lymphoproliferative disorders.

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