Letter to the Editor

Delayed-onset adenosine deaminase deficiency: Strategies for an early diagnosis

To the Editor:

Adenosine deaminase (ADA) deficiency is a well-known cause for severe combined immunodeficiency (SCID). However, hypomorphic mutations in the *ADA* gene can lead to a different immunodeficiency with variable phenotype including signs of immune dysregulation.[1,2] Early diagnosis and therapeutic management often remain a challenge. We report on 2 ADA-deficient patients with delayed-onset (P1) and late-onset[1] phenotype (P2) illustrating these difficulties and describe how newborn screening could have facilitated early diagnosis in one of these patients.

The clinical and laboratory findings of our patients are summarized in Table I. Chronic lymphopenia and elevated IgE levels were key findings in routine laboratory investigations. Further analysis of lymphocyte subsets identified a $T^{(+)}B^{low}NK^{low}$ phenotype. The T-cell receptor repertoire was diverse.

The presence of autoimmune phenomena (Table I) in the context of panlymphopenia prompted us to determine ADA levels[3] that were undetectable in dried blood spot punches. Correspondingly, total adenine deoxyribonucleotides levels were elevated. Subsequent sequencing of the *ADA* gene revealed compound heterozygous mutations in P1 and P2. Both patients carried the same intron 8 splice donor site mutation (c.780+1G>A), while the second mutation was c.637G>T (predicting a novel Val213Phe substitution in exon 7) in P1 and c.396dupA (p.Val133SerfsX38) in P2. Expression of a cDNA encoding the V213F mutation in an *Escherichia coli* system yielded about 1% of wild-type ADA activity, classifying it as a "group III allele," which is in line with missense mutations found in previous late-onset ADA-deficient patients.[1]

While P1 was lost to follow-up after diagnosis, we initiated enzyme replacement therapy (ERT) in P2. He achieved immune reconstitution within 4 weeks as determined by the normalization of cell numbers, T-cell proliferation, and pneumococcal vaccine responses. A marked drop in ADA activity 3 months later (Fig 1) was due to the presence of neutralizing antibodies. An increase in the polyethylene glycol–modified ADA dose resulted in sustained normalization of ADA activity and continuous systemic detoxification. The chronic cough of P2 cleared within weeks after the initiation of ERT, and former radiological signs of interstitial alveolitis resolved completely as found in a recent computed tomography scan. Improvement in pulmonary symptoms correlated with the level of detoxification rather than with the later occurring immune reconstitution, in line with previous reported observations.[4]

The clinical course of our patients and other individuals summarized in Felgentreff et al[2] clearly demonstrate that patients with delayed-onset and late-onset ADA deficiency suffer significantly from the damaging consequences of their disease. Those diagnosed as adults showed severe chronic lung disease and other serious health problems and have not survived long after diagnosis.[5,6] While ERT is a generally accepted treatment in ADA-SCID to stabilize patients prior to stem cell transplantation, its use in patients with a later onset of the disease is less well documented.[7] Although initial immune reconstitution can be observed in most patients receiving ERT, several develop neutralizing antibodies against the bovine-derived ADA, which can completely reverse immune recovery.[8] Fortunately, in P2 and other patients, it has been possible to overcome this problem by increasing the dose.[9] In 2 adult sisters,[6] immune recovery was minimal under ERT and one of them died because of chronic lung disease. However, more favorable results have been reported in other patients.[7] The data from P2 are encouraging, also regarding the lung disease observed in some ADA-deficient patients.[4] We believe that ERT also has an important role in patients diagnosed beyond infancy, mainly to prevent the development of comorbidities that increase the risk for stem cell transplantation.

Since therapeutic success relies on the early initiation of treatment prior to the occurrence of chronic organ damage, we addressed the question whether the diagnosis of late-onset ADA deficiency could be facilitated by using newborn screening methods recently introduced for SCID.[10] We therefore obtained dried blood spot punches from the original Guthrie card from P2. Copy numbers of T-cell receptor excision circles (TRECs) repeatedly proved to be within normal limits at birth, even though lymphopenia (1288/μL) was already documented at age 2 months. ADA deficiency would have therefore been missed in P2 by currently established population-based newborn screenings for SCID, which are based on TREC analysis.[10] Notably, the analysis of dried blood spot punches revealed markedly reduced copy numbers of kappa-deleting recombination excision circles (KRECs)—despite the patient's later documented ability to produce a significant repertoire of specific antibodies. In a screening setting, the phenotype of P2 with normal TRECs and reduced KRECs would have rather predicted a B-cell and not a T-cell deficiency.[11] In addition, we also performed tandem mass spectrometry for the detection of elevated adenosine metabolites[12] and identified that increased levels of desoxiadenosine and adenosine were already present at birth.

Our data indicate that late-onset ADA deficiency may be missed when screening is based on TREC analysis only. In our patient, KREC analysis was more helpful, indicating that decreased KREC copy numbers at birth should also raise suspicion of combined immunodeficiencies. Notably, mass spectrometry showed markedly increased desoxiadenosine and adenosine levels, indicating that this technique may also be useful in newborn screening to identify leaky ADA variants, particularly since spectrometry can be performed at a negligible cost.[12] Prospective analysis in ongoing screening programs will be necessary to prove this hypothesis. It must be considered, however, that screening will also identify patients with partial ADA deficiency,[1] who will not develop symptoms of immunodeficiency and do not require treatment. Determination of lymphocyte counts, total adenine deoxyribonucleotides in red blood cells, and genetic analysis will be helpful in evaluating the individual prognosis, since there is a strong correlation between ADA genotype and both metabolic and clinical phenotype.[1]

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TABLE I. Clinical phenoype and laboratory investigations in P1 and P2

Clinical phenotype	P1	P2	
Ethnicity	Caucasian	Caucasian	
Autoimmunity	AIHA, eczema, asthma, colitis	Asthma	
Intensity of autoimmunnity	Severe (AIHA)	Moderate	
Onset of autoimmunity (y)	6	4	
Infection profile	Sinopulmonary	Otitis, pulmonary	
Intensity of infections	Severe	Moderate	
Onset of infections (y)	3	3	
Radiological findings	Bronchiectasis	Alveolitis	
Treatment	CSA, steroids, SCIG, antibiotics	ERT	
Age at diagnosis (y)	21 (late-onset as defined in Arredondo-Vega et al[1])	7 (delayed-onset as defined in Arredondo-Vega et al[1])	
	P1	**P2**	**Normal range**
Laboratory investigations			
Lymphocytes/μL	<500	900	1000-5300
Eosinophils (% of leucocytes)	24	8	5
T cells			
$CD3^+$/μL	236	880	800-3500
$CD4^+$/μL	110	156	300-1400
$CD8^+$/μL	154	574	200-900
%gamma/delta TCR^+CD3^+	<10	59	<10
%CD45RA of $CD4^+$	9.7	44	48-68
B cells			
$CD19^+$/μL	6	13	100-500
Natural killer cells			
$CD3\text{-}CD16^+CD56^+$/μL	17	20	90-600
Immunoglobulins			
IgG (g/L) (prior SCIG)	10.8	7.8	7-16
IgA (g/L)	0.95	0.41	0.7-4
IgM (g/L)	0.23	0.27	0.4-2.3
IgE (kU/L)	11,200	204	<100
Specific IgG	+Tetanus	+hb, tetanus, hib, measles, mumps	+
	−Measles	−Diptheria, pertussis, rubella,	+
Isohemagglutinins	+	−	+
Autoantibodies	RBCs, neutrophils	PEG-ADA	+
Proliferation			
PHA	Low*	Low/normal after ERT†	
Anti-CD3/CD28	Low*	Low/normal after ERT†	
Tetanus	Low*	ND	
ADA mutation	c.637G>T	c.396dupA	
	(p.Val213Phe)	(p.Val133SerfsX38)	
	c.780+1G>A	c.780+1G>A	
ADA activity (at time of diagnosis)			
In DBSS (μmol/h/mg protein)	0	0	26.4 ± 10
In erythrocytes (IU/L erythrocytes)	<20	111	250-650
RBC nucleotides %dAXP	6.9	7.1	<1
Analysis of newborn DBSS			
TRECs (μL dried blood)[11]	NA	98	>15
KRECs (μL dried blood)[11]	NA	1.3	>10
Desoxyadenosine (μM)[12]	NA	0.7	<0.07
Adenosine (μM)[12]	NA	10	<1.5

Nucleotide numbering according to reference NM_000022.

AIHA, Autoimmune hemolytic anemia; *CSA*, cyclosporine A; *dAXP*, total adenine deoxyribonucleotides; *DBSS*, dried blood spot punches; *hb*, hepatitis B; *hib*, *Haemophilus influenzae*; *NA*, not available; *ND*, not determined; *PEG-ADA*, polyethylene glycol-modified adenosine deaminase; *RBCs*, red blood cells; *SCIG*, subcutaneous immunoglobulin substitution; *TCR*, T-cell receptor.

*Determined by thymidine incorporation assay.

†Determined by carboxyfluorescein diacetate succinimidylester (CFSE) proliferation assay.

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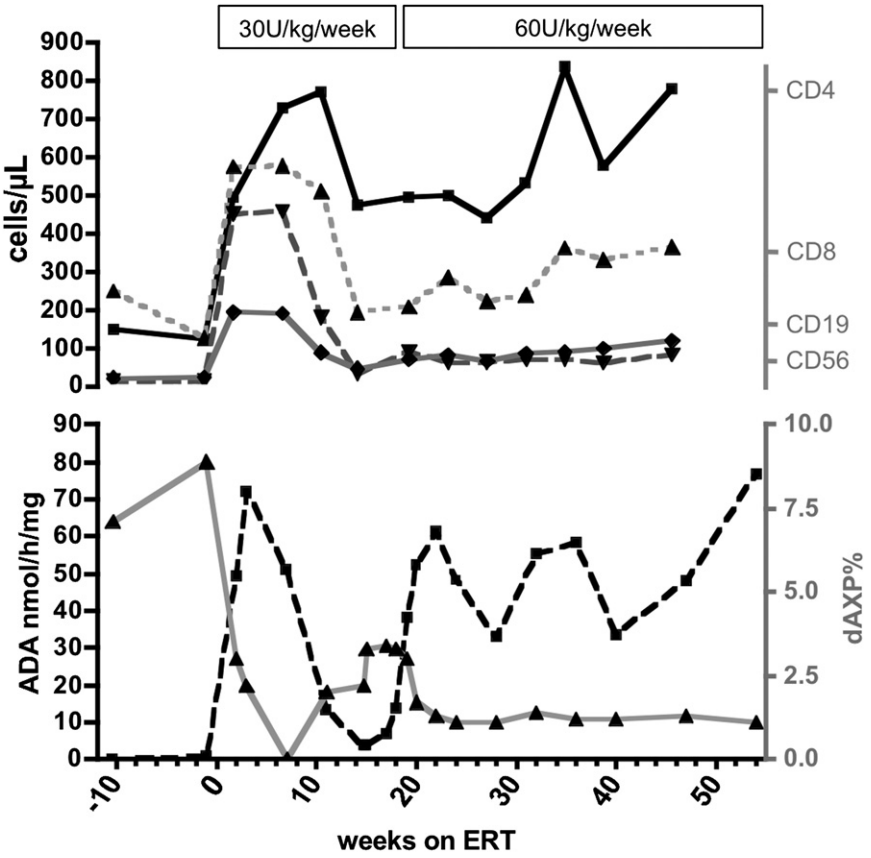

FIG 1. The course of lymphocyte subsets in relation to enzyme activity and metabolites in P2. *Top panel*: the course of lymphocyte subsets determined by flow cytometry before and after the initiation of ERT. *Black line* indicates $CD4^+$ cells, *spotted gray line* $CD8^+$ cells, *gray line* $CD19^+$ cells, and *gray dashed line* $CD16/56^+$ cells. *Bottom panel*: ADA activity *(black dashed line)* and total adenine deoxyribonucleotides (dAXP) levels *(gray line)* as determined by the analysis of dried blood spots from the time point of diagnosis (−10) until 54 weeks after the initiation of ERT with polyethylene glycol–modified adenosine deaminase. "0" indicates the time point when ERT was started. The dose was increased at week 18 as indicated.

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