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Purine metabolism, immune reconstitution, and abdominal adipose tumor after gene therapy for adenosine deaminase deficiency

To the Editor:

Adenosine deaminase (ADA) is an important enzyme for purine metabolite degradation or salvage into the nucleotide pool. Inherited defects in the function of ADA cause accumulation of adenosine, deoxyadenosine, and total deoxyadenosine nucleotides, resulting in severe T- and B-cell immune deficiency. ADA-deficient patients often present in infancy with increased susceptibility to infections and autoimmunity.¹ Allogeneic bone marrow (BM) transplantation with HLA-matched sibling donors can correct the immune deficiency. Since 2000, gene therapy with the patient's BM cells transduced *ex vivo* with retroviruses carrying a correct ADA gene transcript has been performed in more than 30 patients.² After gene therapy, most ADA-deficient patients had a significant reduction in blood levels of toxic purine metabolites, with progressive immune reconstitution.² Malignant cell transformation has not been reported with gene therapy for ADA deficiency in contrast to other immune deficiency diseases, such as IL-2 receptor γ deficiency and chronic granulomatous disease. Herein we have carefully evaluated purine metabolism and immune reconstitution in a patient with ADA deficiency who had an abdominal tumor 3 years after gene therapy.

The patient presented at 1 month of age with increasing respiratory difficulties, lymphopenia, and reduced T- and B-cell numbers and function (see Table E1 in this article's Online Repository at www.jacionline.org). ADA activity in his red blood cells (RBCs) was less than 1% of normal control values. Sequencing of the patient's ADA gene revealed compound heterozygosis, including a G216R substitution on one allele and 30698_30702del-GAAGA on the other, mutations that were previously described in other ADA-deficient patients. The patient had no HLA-matched related donor and was subsequently enrolled in an experimental ADA gene therapy protocol. At 16 months of age, in May 2007, the patient received nonmyeloablative conditioning with 2 mg/kg busulfan (Busilvex; Pierre Fabre, Castres, France) for 2 days, followed by an infusion of BM-derived CD34⁺ cells transduced with the ADA-containing vector in accordance with a clinical trial protocol, as previously described.²

Three years after gene therapy, the patient had not had any significant infections, his developmental milestones were appropriate for his age, and his height and weight were normal. The ADA activity in his peripheral blood lymphocytes increased to 402 nmol/h/mg protein (normal value, 2100 $\pm$ 320 nmol/h/mg protein). ADA activity in the patients' peripheral RBCs was about 5% of the normal value (4 nmol/min/mL; normal control value, 55-194 nmol/min/mL). Deoxyadenosine in the urine, which was 26.3 mmol/mol creatinine before treatment, had decreased to 4.4 mmol/mol creatinine, although it was still increased (normal value, <0.1 mmol/mol creatinine). Immune evaluations revealed

low numbers of circulating CD3⁺CD4⁺ and CD3⁺CD8⁺ T cells, with *in vitro* responses to mitogens and antigens comparable with normal control values (see Table E1). Analysis of the repertoire of T-cell receptors among CD3⁺ cells was normal; however, a more detailed evaluation demonstrated reduced representation of V β 4 and V β 5.2 families among the CD4⁺ subpopulation, as well as reduced V β 5.3, V β 7.1, and V β 9 representation but expanded V β 5.2 and V β 13.1 representation among the CD8⁺ cells (see Fig E1 in this article's Online Repository at www.jacionline.org). This might be explained, at least in part, by reduced thymic output, as evident by the patient's low T-cell receptor excision circle levels. The patient also had a reduced number of CD19⁺ B cells and required intravenous immunoglobulin replacement. Among peripheral lymphocytes, ADA gene-corrected cells were detected in 61.1%, 73.0%, 44.3%, and 65.6% of CD4⁺, CD8⁺, CD19⁺, and natural killer cells, respectively, indicating a mixed population of transduced, as well as native lymphocytes.

Routine abdominal ultrasound 3 years after gene therapy revealed a previously undetected large abdominal tumor, with the patient asymptomatic. Magnetic resonance imaging showed a well-defined right-sided 4.1 $\times$ 7.0 $\times$ 7.3-cm tumor within the mesentery and below the liver (Fig 1). The mesenteric tumor and an attached segment of the small bowel were removed. The tumor was well circumscribed, thinly encapsulated, and confined to the mesentery (Fig 1). The cut surface was lobulated, soft, and yellow, with a fatty appearance. Microscopic examination revealed a tumor composed of mature adipocytes with slight variation in cell size and no cytologic atypia or immature adipocytes, which is consistent with a benign lipoma (Fig 1). The tumor was nearly devoid of lymphocytes but did contain small capillaries. Cytogenetic analysis by means of G-banding showed a balanced inverted insertion of chromosome region 8q11.23-8q24 from one chromosome 8 into the long arm of one chromosome 19 at 19q13.13 (Fig 1). The rearrangement was seen in all 30 adipocytes examined. The translocation was not present in the patient's BM, lymphoid cells, or skin cells obtained before and after gene therapy. Quantitative PCR analysis for the presence of the viral construct used for gene therapy was detected in 3.4% of the tumor cell DNA, likely representing ADA gene-transduced peripheral blood cells in the tumor blood vessels.

Adipocyte tumors include a spectrum of abnormally proliferating mesenchymal cells, ranging from benign lipomas and lipoblastomas to malignant liposarcomas. Lipoblastomas are often present in childhood as a rapidly growing tumor in the extremities or trunk. Abdominal lipoblastomas comprise less than 10% of all cases and are usually localized to the retroperitoneum, whereas rare cases have been reported to arise in the mesentery. Lipoblastomas are characterized cytogenetically by rearrangement of chromosome 8q11-13, which has been identified in the vast majority of cases, similar to the patient described here.^{3,4} The observation that the tumor was of nonhematopoietic origin and that DNA from tumor cells did not contain viral gene sequences with the exception of a minor contaminant argued against a role for gene therapy-related insertional mutagenesis in this tumor formation. Another potential predisposing factor for the tumor was the use of busulfan, which has been associated with secondary malignancies; however, a link between this drug and lipid tumors has never been reported.

Large lipid tumors have not been described previously in ADA-deficient patients; however, these patients tend to have hematologic malignancies.⁵ Moreover, ADA-deficient patients also were reported to acquire genetic abnormalities, including

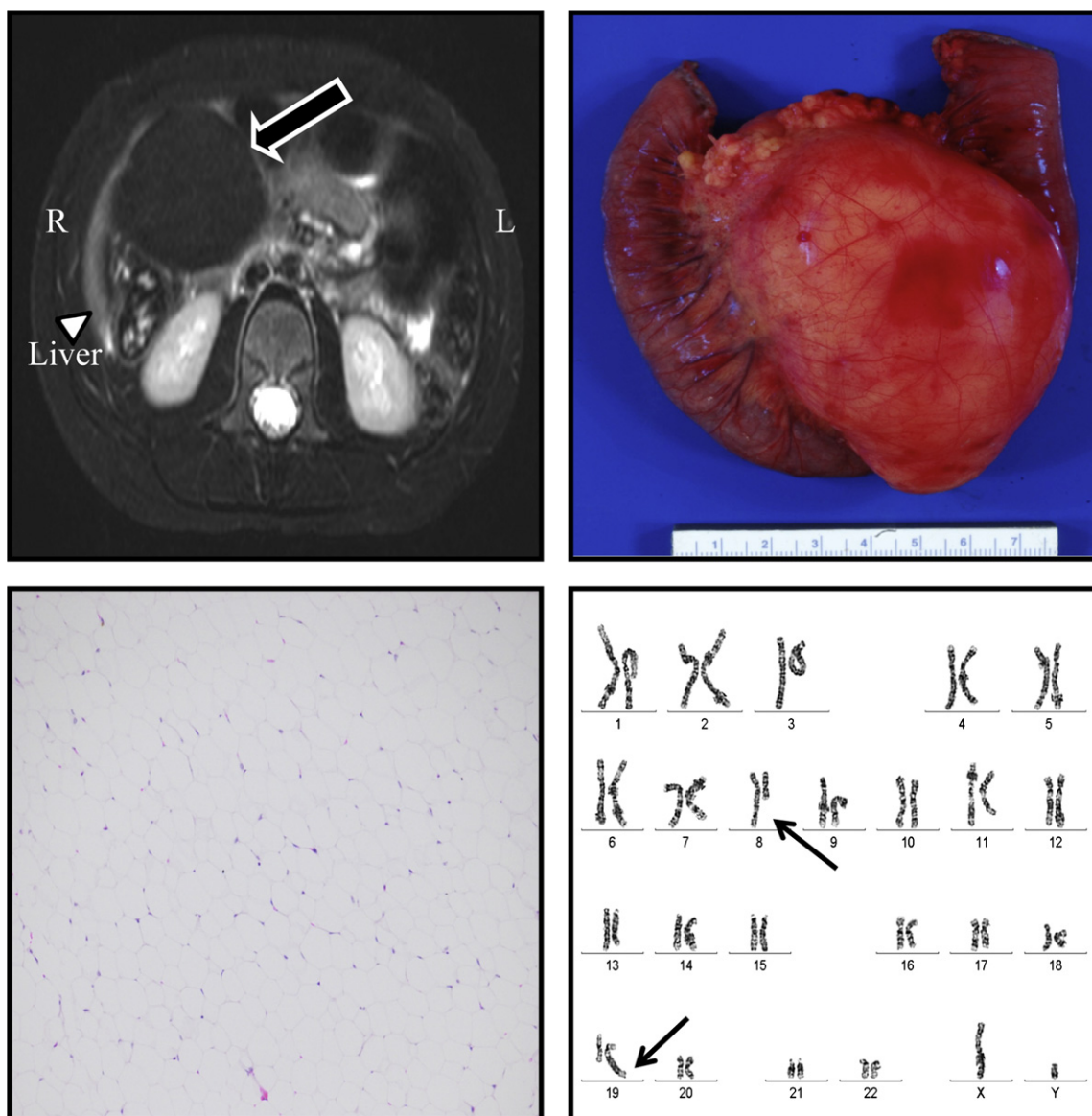


FIG 1. Analysis of the abdominal tumor. Magnetic resonance imaging (upper left panel) demonstrated a large right-sided abdominal tumor (arrow) near the liver (arrowhead). The tumor had a diffuse, homogeneous high signal on T1 and T2 sequences isointense to the subcutaneous fat, with no evidence of cystic changes or calcification. Gross inspection of the tumor revealed a well-circumscribed, thinly encapsulated tumor confined to the mesentery (upper right panel). Hematoxylin and eosin staining of the resected tumor demonstrated mature adipocytes with no cytologic atypia or mitoses (lower left panel). G-banding analysis of the resected tumor showed balanced inverted insertion of 8q11.23-q24 into chromosome 19 at 19q13.13, which is typical of lipoblastoma (lower right panel).

trisomy 8, as well as 17q22 to 22q13 translocations, which have been linked to extensive dermatofibrosarcoma protuberans.⁶ The increased frequency of tumors in patients with ADA deficiency might be related to nucleotide imbalances, which impair DNA recombination in ADA-deficient lymphocytes⁷ and might contribute to the increased irradiation sensitivity of ADA-deficient fibroblasts.⁸ Indeed, our patient had increased concentrations of deoxyadenosine in the urine and total deoxyadenosine nucleotides in RBCs, indicating continued abnormal purine homeostasis similar to that found in ADA-deficient patients who had dermatofibrosarcoma protuberans and lymphoma after BM transplantation or PEG-ADA replacement therapy.^{5,6}

Alternatively, incomplete immune reconstitution, as suggested by the reduced thymic output, skewed T-cell repertoire, low numbers of circulating B cells, and requirement of intravenous immunoglobulin, might have led to inappropriate immune surveillance and tumor formation. Similar immune dysregulation has been linked to onset of autoimmunity and increased risk of cancer development.⁹ In addition, because similar metabolic and immune abnormalities, including skewed T-cell repertoire and reduced T-cell receptor excision circle levels, are frequently observed in ADA-deficient patients after BM transplantation and enzyme replacement therapy,⁵ they too need to be followed closely for development of tumors.

In conclusion, gene therapy has thus far efficiently protected our patient from significant infections and supported normal growth and development. However, the degree of correction of purine metabolism and immune reconstitution might either predispose to or inadequately protect against noninfectious morbidities.

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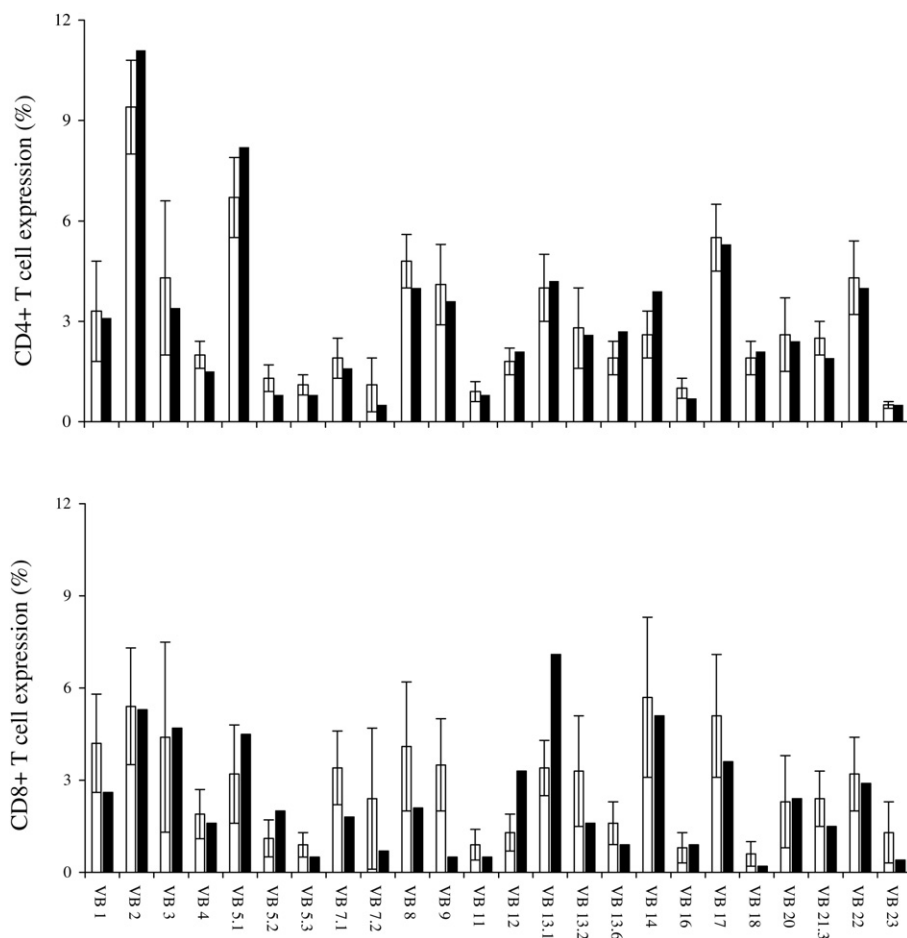


FIG E1. T-cell receptor diversity. Relative expression of the various V β families among CD4⁺ (upper panel) and CD8⁺ (lower panel) T cells in the patient 3 years after gene therapy (solid bars) or normal age-matched control subjects (open bars $\pm$ SD), as determined by means of fluorescence-activated cell sorting analysis with the IOTest Beta Mark Kit (Beckman Coulter, Mississauga, Ontario, Canada).

TABLE E1. The patient's immune evaluations before and after gene therapy*

	At diagnosis	Age-matched healthy control subjects	Three y after gene therapy	Age-matched healthy control subjects
White blood cells ($\times 10^9/L$)	3.5	5-15	5.9	5-12
Neutrophils ($\times 10^9/L$)	2.9	1.5-8.5	3.6	1.5-8.5
Lymphocytes ($\times 10^9/L$)	0.18	4-10.5	1.2	1.5-8.0
CD3 ⁺ cells ($\times 10^9/L$ [%])	0.03 (20)	2.5-5.5 [†] (53-84)	0.86 (87)	1.4-3.7 [†] (56-75)
CD4 ⁺ cells ($\times 10^9/L$ [% lymphocytes])	0.02 (13)	1.6-4.0 [†] (35-64)	0.56 (46)	0.7-2.2 [†] (28-47)
CD8 ⁺ cells ($\times 10^9/L$ [% lymphocytes])	0.01 (3)	0.56-1.7 [†] (12-28)	0.31 (25)	0.49-1.3 [†] (16-30)
CD19 ⁺ cells ($\times 10^9/L$ [% lymphocytes])	0.01 (7)	0.3-2.0 [†] (6-32)	0.11 (9)	0.39-1.4 [†] (14-33)
CD16/CD56 ⁺ cells ($\times 10^9/L$ [% lymphocytes])	0.03 (20)	0.17-1.1 [†] (4-18)	0.05 (5)	0.13-0.7 [†] (4-17)
SI to anti-CD3	NA	NA	500	413
SI to PHA	1	366	603	667
SI to herpes simplex virus	NA	NA	18	30
T-cell receptor excision circles/0.5 μg	NA	NA	47	>350

NA, Not available; SI, stimulation index.

*Immunologic evaluations were performed at The Hospital for Sick Children, Toronto, Ontario, Canada, and were beyond the 3-year follow-up investigations required by the clinical trial.

[†]Tenth and 90th percentiles in healthy age-matched control subjects.^{E1}