

Heterogeneity of phenotype in two siblings with adenosine deaminase deficiency

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Adenosine deaminase (ADA) deficiency is the cause of about one third of the autosomal recessively inherited cases of severe combined immunodeficiency. Disease severity in ADA deficiency is variable, presumably related in part to heterogeneity in the genotypes causing the disease. We now report on two children in a single family with ADA deficiency who presented with distinct clinical courses. One child presented with severe immunodeficiency and recurrent infections that led to the diagnosis of severe combined immunodeficiency and ADA deficiency at 7 months of age. The older child, who was diagnosed at 3 years of age (after the diagnosis of the younger child), did not have a history of serious or opportunistic infections. Although she was lymphopenic, immune responsiveness was intact, in terms of antibody production, delayed-type hypersensitivity, and in vitro T-cell function. The difference in clinical course in these two siblings is an important observation and demonstrates that the phenotypic expression of ADA deficiency can vary within a family, even in a situation in which protective isolation from infectious pathogens was not a factor in causing the milder course. These observations indicate that expression of disease severity in ADA deficiency may depend to a significant degree on environmental factors and/or on heterogeneity at other genetic loci, which may regulate or modify the expression of the ADA gene or the activity of its product. Furthermore, these observations highlight the importance of recognizing patients with ADA deficiency who present with less severe disease and support the impression that functional immunity in patients with the "late-onset" form of ADA deficiency can deteriorate over time. In such patients unexplained lymphopenia may be a useful early marker for ADA deficiency even before the onset of severe infections. (J ALLERGY CLIN IMMUNOL 1994;93:543-50.)

Key words: Adenosine deaminase deficiency, severe combined immunodeficiency

Adenosine deaminase (ADA) deficiency is responsible for about one third of the autosomal recessively inherited cases of severe combined immunodeficiency (SCID).^{1, 2} In the majority of patients the onset of clinical symptoms occurs

Abbreviations used

ADA:	Adenosine deaminase
IVIG:	Intravenous immunoglobulin
PEG-ADA:	Polyethylene glycol-coupled adenosine deaminase
SCID:	Severe combined immunodeficiency

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within the first 6 months of life, with recurrent bacterial and viral infections, diarrhea, and failure to thrive. In other patients the onset of clinical disease occurs later, occasionally after the second year of life.^{3, 4} In a third group of about a dozen healthy individuals, identified in screening programs as having a so-called "partial-form" of ADA deficiency, normal immune function is present.^{2, 5} Although these children lack ADA activity in red cells, they have appreciable (normal) activity in their nucleated cells and do not manifest the characteristic erythrocyte metabolic

RG anti-phi-X174 Titers Prior to PEG-ADA Therapy

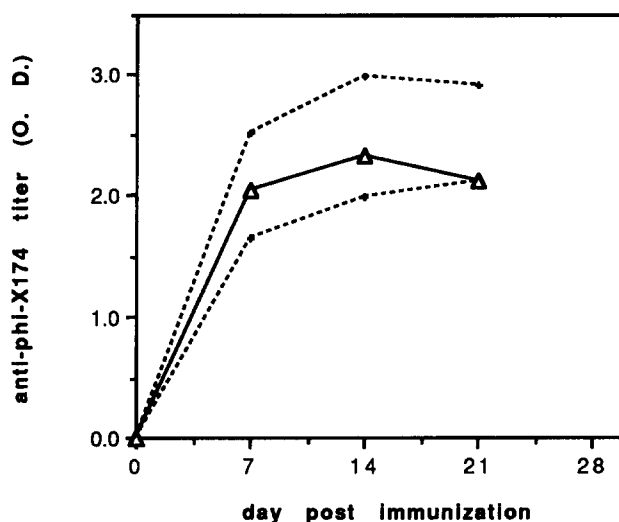


FIG. 1. The primary antibody responses to bacteriophage ϕ X174 were normal in R. G. before PEG-ADA therapy. Serum anti- ϕ X174 titers after primary immunization are represented by the solid line, expressed as optical density (O.D.) in an ELISA. Dotted lines represent 2 standard deviations above and below the mean of the response of normal individuals.

abnormalities found in patients with ADA deficiency who have immunodeficiency. Disease severity in individuals with ADA deficiency is thus variable, possibly related to heterogeneity in the genotypes causing the disease.

We report on two siblings with very dissimilar clinical courses. The younger child presented first, at 7 months of age, with severe failure to thrive, *Pseudomonas aeruginosa* sepsis, and later with *Pneumocystis carinii* pneumonia, prompting the diagnosis of SCID with ADA deficiency. Subsequently, her 3-year-old sister was also found to have ADA deficiency but with intact immune responsiveness. Unlike individuals with the "partial-form" of ADA deficiency, this child, like her severely affected younger sister, had markedly reduced levels of ADA activity in blood mononuclear cells and erythrocytes and had erythrocyte metabolic abnormalities that are associated with immunodeficiency.

The degree of disparity in the clinical course of these siblings is unprecedented and suggests that environmental factors or other genetic loci may strongly influence the phenotypic expression of SCID associated with ADA deficiency.

METHODS

T-cell subsets were identified by flow cytometry with commercially available monoclonal antibodies. Lymphocyte proliferation studies were performed in microtiter plates with optimal doses of mitogens. ADA,

S-adenosylhomocysteine hydrolase, and total deoxyadenosine nucleotides were determined as described previously.⁶

Immunization with bacteriophage ϕ X174 was performed as previously described⁷ in a manner approved by the Committee on Human Subjects in Medical Research at Stanford University. Antibody titers against bacteriophage ϕ X174 were determined by ELISA.⁸

Case presentation

Proband (E. G.). E. G. was the 3.4 kg full-term product of an uncomplicated pregnancy to nonconsanguineous parents. She was well until 4 to 5 months of age when she began to have episodes of recurrent otitis media and upper respiratory infections. Her height and weight at 4 months of age were both below the 3rd percentile. At 6 and 7 months of age, she was hospitalized for treatment of pneumonia, otitis media, and diarrhea. She was treated with cefuroxime sodium during the first admission, and with ceftriaxone sodium during the second, with some improvement in symptoms. Cultures of blood and urine were negative, but serum transaminases were mildly elevated. Screens for hepatitis A and B were negative. At 9 months of age, she was again hospitalized with fever, pneumonitis, diarrhea, and weight loss; and she again improved when antibiotics were administered. One week after discharge, she was readmitted with fever. A blood culture was positive for *Pseudomonas aeruginosa*, and she was treated with ceftazidime. Because of hypoxia and cough, she was referred for bronchoscopy, which revealed alveolar washings that were positive on silver

TABLE I. Immunologic evaluation before treatment with PEG-ADA

Test	Data on E. G.	Data on R. G.	Normal values
WBC	6.6 (80S/2B/12L) 4 mono, 2 eos	2.9 (65S/OB/19L) 10 mono, 4 eos	5-10 × 10 ³ /μl
Hgb/Hct	10.2/31	13.2/39.6	10-13 gm/dl/30%-41%
ESR	67	ND	5-15 mm/hr
IgG	1603*	530	500-1200 mg/dl
IgM	892	142	42-239 mg/dl
IgA	22	60	14-108 mg/dl
IgG1	634*	442	190-830 mg/dl
IgG2	34	105	30-240 mg/dl
IgG3	13	37	9-130 mg/dl
IgG4	0	7	3-120 mg/dl
Tetanus titer	<0.01†	<0.10 >6.20 (after immunization)‡	>0.10 IU/ml = protective
Diphtheria titer	<0.01†	0.05 >2.50 (after immunization)‡	>0.01 IU/ml = protective
Varicella titer	ND	1.5	>0.8 (index)
Rubella	ND	6.6	>0.8 (index)
anti-A§	1:8	1:16	Variable
anti-B	<1:2	<1:2	Variable

WBC, White blood cell count; S, segmented neutrophils; B, basophils; L, lymphocytes; *mono*, monocytes; *eos*, eosinophils; *Hgb/Hct*, hemoglobin/hematocrit; *ESR*, erythrocyte sedimentation rate; *ND*, not done.

*Total IgG and IgG subclasses were determined on separate occasions before the start of IVIG.

†E. G. was immunized on three occasions with tetanus/diphtheria vaccine, and titers were drawn 10 days after the last booster.

‡R. G. had initially negative titers to tetanus and diphtheria, and these increased after immunization before the start of therapy with PEG-ADA and IVIG.

§Both children are blood type O+.

stain for *Pneumocystis carinii*. In vitro T cell responses to phytohemagglutinin and concanavalin A were absent, and erythrocyte ADA activity was barely detectable (Fig. 1), establishing the diagnosis of SCID with ADA deficiency. (Human immunodeficiency titers were negative in both child and mother.) Treatment with intravenous immunoglobulin (IVIG), administered every 4 weeks, was begun, and because of the absence of a normal histocompatible sibling, she was treated with twice weekly administration of polyethylene glycol-modified ADA (PEG-ADA) (30 U/kg).⁶ Immune function gradually increased, accompanied by clinical improvement and substantial weight gain.

Older sister of proband (R. G.). R. G. was the 3.9 kg full-term product of an uncomplicated pregnancy. She had *Varicella zoster* infection (chicken pox) at 6 months of age, which resolved without complication. At 18 months of age, she had a urinary tract infection, which responded to treatment with trimethoprim-sulfamethoxazole. Several weeks later, neutropenia with anti-neutrophil antibodies was noted. The neutropenia was thought to be secondary to a trimethoprim-sulfamethoxazole reaction, and it resolved spontaneously over several months. At 22 months, recurrent episodes of otitis media began. These responded to oral administration of antibiotics, but because of their frequency, ventilating (pressure equalization) tubes were placed at

30 months. Improvement followed, and she continued to gain weight along the 90th percentile. At 39 months of age, she remained in good health. At that time, however, because ADA deficiency was diagnosed in her younger sibling, R. G. was evaluated and found to be ADA-deficient. Although absolute lymphocyte counts were low, lymphocyte function (production of antigen-specific antibody, delayed-type hypersensitivity, and in vitro proliferation) was normal (see Results section). However, over the next several months, the lymphocyte count declined further, and persistent sinusitis developed, with chronic cough and decreased physical activity. R. G. began receiving IVIG at 44 months of age, and PEG-ADA at 45 months of age.

RESULTS

The two siblings with ADA deficiency who are described here had distinct clinical courses and laboratory findings. The younger child, E. G., was diagnosed as having SCID at 7 months of age, with a history of recurrent respiratory infections, including *Pneumocystis carinii* pneumonia, diarrhea, and failure to thrive. She had normal immunoglobulin levels but no detectable antibody titers to tetanus or diphtheria toxoids after immunization and no detectable T-cell function

TABLE II. T-cell studies before PEG/ADA treatment

Antigen	Data on E. G.	Data on R. G.	Normal values
CD3	370*	420*	781-2,392 cells/mm ³
CD4	60	230	447-1750 cells/mm ³
CD8	190	160	413-1260 cells/mm ³
B (CD20+)	10	20	100-500 cells/mm ³
Delayed-type hypersensitivity			
skin tests			
Candida	—	—	
Tetanus	—	1.2 cm induration	
In vitro proliferation			
PHA	2,727 cpm†	34,211 and 47,860 cpm‡	29,300-185,000
Con A	1,293 cpm	30,147 and 45,897 cpm‡	14,900-170,200
PWM	ND	28,242 and 60,827 cpm‡	13,500-45,200
Tetanus toxoid	ND	33,807 cpm	11,500-85,300

PHA, Phytohemagglutinin; Con A, concanavalin A; PWM, pokeweed mitogen; ND, not done.

*Repeat lymphocyte subsets counts performed 6 months after initiation of PEG-ADA therapy were as follows. E. G.: CD3 = 1982; CD4 = 857; CD8 = 777. R. G.: CD3 = 460; CD4 = 420; CD8 = 130.

†Repeat proliferation studies performed 2 months after initiation of PEG-ADA therapy: PHA, 40,387 cpm; Con A, 33,510 cpm; PWM, 28,664.

‡Studies done on two separate occasions.

TABLE III. ADA activity in cells from siblings and parents

Source of cells	ADA activity	
	In erythrocytes (nmol/hr/mg)	In mononuclear cells (nmol/hr/mg)
E. G.	0.62	9.2 and 41.4
R. G.	0.21	57.4
Mother	19.1	792.0
Father	14.1	749.7
Normal	52.3 ± 38.8	1197 ± 516

Normal values are expressed as means ± SD.

(Tables I and II). In contrast, her 3.3-year-old sister, R. G., had a much more benign clinical course, characterized notably by an uncomplicated chicken pox infection at 6 months of age. When first evaluated at 39 to 44 months of age (before treatment with PEG-ADA was begun), R. G. had normal levels of serum immunoglobulin and normal titers to *Varicella zoster*. She had normal titers to tetanus and diphtheria after immunization and a normal primary antibody response to the neoantigen bacteriophage ϕ X174 (Fig. 1). In addition, R. G. had a normal in vivo delayed-type hypersensitivity skin test response to tetanus/diphtheria after immunization and normal in vitro proliferative responses to phytohe-

magglutinin, pokeweed mitogen, concanavalin A, and tetanus toxoid (Table II). However, moderate lymphopenia was noted, which was present for several years before the diagnosis of ADA deficiency, when her white blood cell count varied from 1.5 to 4.9 (mean 2.49/mm³ on four determinations), with 8% to 57% lymphocytes (mean, 25%).

Despite the different clinical courses of these two sisters, levels of ADA in the red cells and blood mononuclear cells of the two children were similar: 0.5% to 1% of normal in red cells and about 5% of normal in mononuclear cells (Table III). Both parents had heterozygote levels of cellular ADA activity. Consistent with the deficiency of ADA, impaired catabolism of deoxyadenosine was evident in the two children. The activity of S-adenosylhomocysteine hydrolase in erythrocytes was decreased to about 10% of normal in both patients (0.37 for E. G. and 0.43 for R. G.; normal = 4.2 ± 1.9 nmol/hr/mg), and the level of total deoxyadenosine nucleotides in their erythrocytes was elevated by about 100 times (269 for E. G. and 175 for R. G.; normal = ≤ 2 nmol/ml packed red blood cells).

Response to therapy

Treatment with PEG-ADA resulted in sustained correction of erythrocyte metabolic abnormalities in both patients (Fig. 2). Immune func-

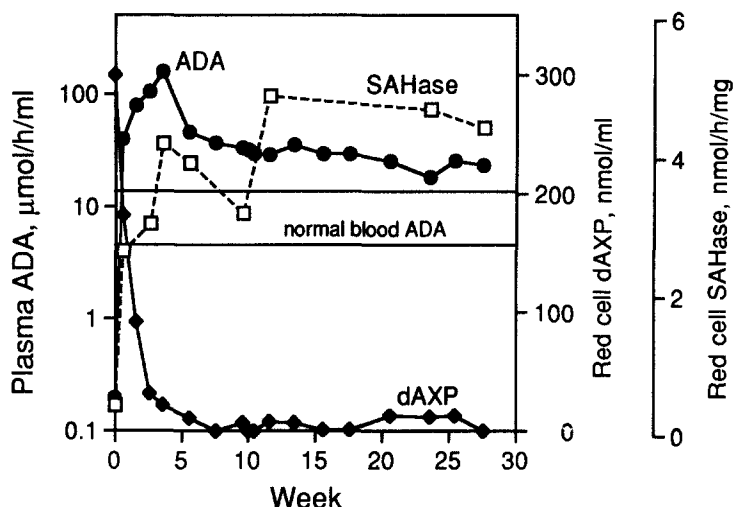


FIG. 2. Levels of deoxyadenosine nucleotides (*dAXP*) (◆—◆) in erythrocytes of E. G. were elevated by about 100 times above normal before PEG-ADA therapy. These levels dropped dramatically after initiation of PEG-ADA therapy at week 0. S-adenosylhomocysteine hydrolase (*SAHase*) (□---□) activity in erythrocytes of E. G. was decreased to about 10% of normal before PEG-ADA therapy, and these improved with PEG-ADA therapy. Plasma ADA activity (●—●) was present after initiation of PEG-ADA therapy, at 30 U/kg twice weekly. The dose was decreased after week 5 to 30 U/kg once weekly. Similar data were obtained with blood samples from R. G.

tion improved markedly in E. G. within 2 months after treatment with PEG-ADA was started. Thus proliferative responses of mononuclear cells from E. G. when stimulated with phytohemagglutinin, concanavalin A, and pokeweed mitogen were in the normal range 2 months after the start of treatment with PEG-ADA (Table II, footnote). In addition, a primary antibody response to bacteriophage ϕ X174 was normal in E. G. 11 months after the start of treatment with PEG-ADA (Fig. 3), and a tetanus titer after immunization was protective (>1.9 IU/ml) 10 weeks after her last dose of IVIG and 18 months after PEG-ADA therapy began. Clinically, E. G. improved immensely, with considerable weight gain (now in the 25th percentile for weight and the 10th percentile for height) and with a significantly lower frequency of (now only minor) infections. R. G. has also done well. She began school at the age of 5 years and has attended regularly. However, her immune function has not changed dramatically from the pretreatment level (which was remarkably preserved compared with that of her sister); she continues to show low but normal lymphocyte counts (absolute lymphocyte count = $994/\text{mm}^3$, CD4+ lymphocyte count = $530/\text{mm}^3$ [normal CD4+ lymphocyte value = 447 to $1750/\text{mm}^3$]).

DISCUSSION

The disease severity of patients with ADA deficiency is variable. About 80% to 85% of patients present within weeks to several months after birth with life-threatening infections with absent T- and B-cell function, whereas 10% to 15% present late, after the first year of life but usually before 2 years of age. The latter patients may have normal immunoglobulin levels but limited B-cell function, and they have low or absent T-cell function.^{1, 2} In those who present late it has been assumed (although not clearly demonstrated) that immune function is present early on but that it declines over time because of the progressive toxic effects of deoxyadenosine in lymphocytes.^{3, 4} Because the disease is caused by a growing list of specific mutations within a single ADA gene locus,⁹⁻¹³ it has been postulated that differences in clinical presentations are due in part to heterogeneity in the enzymatic activity of mutant ADA proteins.¹² Because the disease is rare and most patients are compound heterozygotes, the precise relationship between genotype and phenotype has been difficult to evaluate. Families with more than one affected individual provide an opportunity to evaluate the influence of genotype on phenotype.

Clinical heterogeneity within families affected

EG anti-phi-X174 Titers After PEG-ADA Therapy

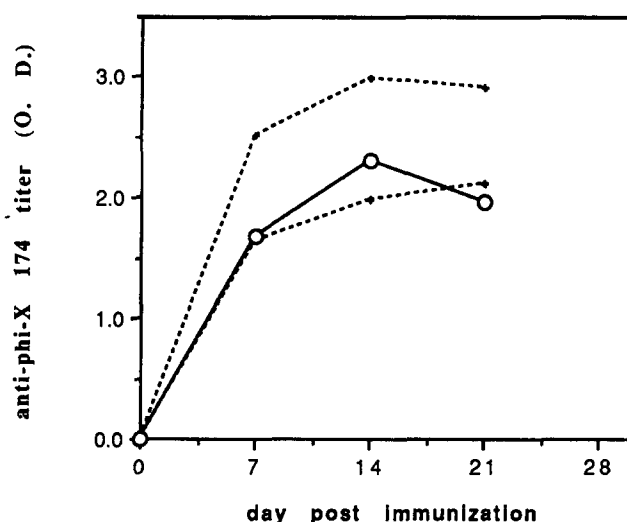


FIG. 3. Antibody responses in E. G. were normal after initiation of PEG-ADA therapy. Serum antibody titers in patient E. G. after primary immunization with bacteriophage ϕ X174 11 months after PEG-ADA therapy was started are represented by the *solid line*, expressed as optical density (O.D.) in an ELISA. *Dotted lines* represent 2 standard deviations above and below the mean of the response of normal individuals.

by ADA deficiency, however, is often difficult to evaluate because diagnosis of a first child in a family with SCID leads to early diagnosis and treatment of the subsequent affected sibling before the clinical picture becomes full-blown. In the four other families with two children affected with ADA deficiency of which we are aware, in general, siblings have had similar courses in terms of disease severity. In the case of siblings evaluated by Rubinstein et al.,¹⁴ the proband was diagnosed with SCID and ADA deficiency at 22 months of age, whereas his affected brother was diagnosed prenatally, isolated at birth, and started on transfusion therapy at 6 weeks of age. Similarly, in a second pair of siblings, the firstborn child was severely affected clinically and was diagnosed with SCID and ADA deficiency at about 2 months of age¹⁵; the second sibling was diagnosed in utero (unpublished data) and received a bone marrow transplant at a few weeks of age before the clinical picture of SCID could develop. The affected children in both of these families had lymphopenia and severely diminished B- and T-cell function early in life, suggesting a similar immunologic phenotype in each pair of siblings.

In the two other reported families, the older of each of the pairs of siblings died before a defin-

itive diagnosis of ADA deficiency could be made. In the pair reported by Hirschhorn et al. in 1975,¹⁶ both children had similar early clinical presentations with absent immune function. The pair reported by Hong et al.¹⁷ and Giblett et al.¹⁸ differed somewhat from the other pairs in that partial B-cell function was present in both children. The older child died at 13 months of age as a result of disseminated infection, whereas the second child was noted, during the first month of life, to be moderately lymphopenic. Clinical manifestations of recurrent infection did not begin until 24 months but then progressed rapidly to pulmonary failure and death by the age of 30 months. Both children had normal total levels of serum IgG. Neither had detectable delayed-type hypersensitivity, and the second child had severe impairment of her T-cell response to phytohemagglutinin (first child not evaluated). Although the first child presented with pneumonia at 9 months of age, whereas the second remained well until 24 months of age, absence of detectable T-cell function was a common feature in both.

The distinct clinical courses in the two siblings on whom we now report, one with normal immune responsiveness at the time of diagnosis and the other with severe immunodeficiency, repre-

sent an important observation that enhances our understanding of the pathophysiology of ADA deficiency and of the phenotypic expression of the disease. First, the less severe course in one child was not due to protective isolation of the child, because the younger child, who was the proband, was more severely affected. Second, except for several children with the "partial-form" of ADA deficiency, which is associated with neither significant metabolic abnormality nor with immunodeficiency,^{2, 5} there have been no previous reports of normal T-cell functional studies in untreated clinically affected patients with ADA deficiency. Previously reported "late-onset" patients, who were diagnosed with ADA deficiency and SCID at age 5 or 8 years of age, were lymphopenic and had reduced or absent T-cell function when evaluated at presentation.^{3, 4} Although R. G., the older of our patients, had normal immune function when evaluated at 3 years of age, she had been moderately lymphopenic since 1 year of age. Her absolute T-cell counts declined and symptoms of infection increased over a 6-month period of observation after discovery of ADA deficiency. Because her younger sibling had total absence of immune function, we believe that if left untreated, the older child's immune function would have further declined over time to result in severe immunodeficiency. Thus these observations support the impression that functional immunity in patients with ADA deficiency can deteriorate over time from nearly normal to undetectable levels and furthermore suggest that unexplained lymphopenia may be a marker for the "late-onset" form of ADA deficiency even before the onset of severe infections.

Had the two patients described not been siblings, their distinct clinical courses might have suggested that they carried different mutant ADA genes. However, recent studies indicate that both sisters are compound heterozygotes and indeed carry the same two mutant ADA alleles.¹⁹ Thus the contrast in disease severity between the two sisters may have been the result of environmental factors that differed between the two children. Alternatively, because the mutations in our patients involve novel splicing defects, differences in the relative efficiency of messenger RNA splicing may have influenced the clinical presentation, as has been suggested to account for clinical heterogeneity in certain cases of Sandhoff disease (hexosaminidase deficiency).²⁰

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