Adenosine deaminase (ADA) deficiency and its biochemical consequences cause severe combined immunodeficiency (SCID). Treatment strategies, designed to correct the biochemical abnormalities, include transplantation of matched bone marrow or haploidentical bone marrow stem cells, repeated partial exchange transfusions with frozen irradiated human red blood cells (RBC), or weekly injection of polyethylene glycol-modified bovine ADA (PEG-ADA). To evaluate the effect of these therapeutic options, we studied in vitro T-cell function and in vivo antibody responses to the T-cell-dependent neoantigen, bacteriophage \( \phi X174 \), in 10 children with ADA-deficient SCID. In untreated patients, T-cell function was severely depressed, and only minute amounts of antibacteriophage antibody were produced. Transplantation of bone marrow from a matched sibling (one patient) or a phenotypically matched parent (one patient) resulted in a stable graft, normal T-cell function, and substantial but subnormal antibody titers to bacteriophage, with reduced memory and impaired switch from IgM to IgG. Patients receiving T-cell-depleted haploidentical bone mar-
row stem cells had markedly depressed antibody responses for as long as 3 years posttransplantation, despite rapidly improving T-cell function that became normal in two of four patients. Two methods of enzyme replacement were explored. During treatment with human RBC transfusions, antibody responses to bacteriophage were as severely depressed as in untreated ADA-deficient patients. Treatment with weekly injections of PEG-ADA resulted in normalization of T-cell numbers in all four patients, normal or near-normal T-cell function in two, and mildly but variably improved T-cell function in the other two patients. Quantitatively and qualitatively normal antibody responses to bacteriophage were observed in three of four patients. Assessment of antibody responses to immunization with bacteriophage \( \phi X174 \) is a useful method to monitor humoral immune function in treated ADA-deficient patients and can be used to estimate when intravenous immunoglobulin (IVIG) prophylaxis may be safely discontinued.

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**Antibody Responses to Bacteriophage \( \phi X174 \) in Patients With Adenosine Deaminase Deficiency**


GENETICALLY DETERMINED deficiency of the enzyme adenosine deaminase (ADA) is a cause of the clinical syndrome of severe combined immunodeficiency (SCID) with its associated recurrent infections and failure to thrive. If untreated, the condition is usually fatal during infancy.\(^1\) In the absence of ADA, one of its substrates, deoxyadenosine, is preferentially phosphorylated, resulting in accumulation of deoxyadenosine triphosphate (dATP)\(^3\) and inactivation of S-adenosylhomocysteine hydrolase.\(^4\) Because elevated concentrations of dATP interfere with DNA synthesis,\(^5\) lymphocyte proliferation and differentiation are severely impaired. Several therapeutic strategies have been proposed to correct the biochemical consequences of ADA deficiency. Transplantation of bone marrow from a histocompatible donor, resulting in most instances in complete or partial engraftment of lymphocytes and excellent immunologic reconstitution, is considered the treatment of choice.\(^6\) More recently, transplantation of T-cell-depleted haploidentical bone marrow stem cells from a parent has been performed in patients who lack a matched sibling donor, resulting in chimerism and immune reconstitution in some patients with ADA-deficient SCID,\(^6\) but not in others.\(^8\)-\(^10\) As is the case with most SCID patients, ADA-deficient bone marrow recipients are generally not conditioned by cytoreduction before bone marrow transplantation. Repeated partial exchange transfusions with frozen irradiated human red blood cells (RBC), first proposed by Polmar et al,\(^11\) have been given in an attempt to replace the missing enzyme. However, most ADA-deficient SCID patients treated in this fashion have shown little clinical or immunologic improvement.\(^9\)\(^12\)\(^-\)\(^16\) By contrast, as shown recently, weekly injections of polyethylene glycol-modified ADA (PEG-ADA) are effective not only in reducing toxic levels of deoxyadenosine nucleotides, but also in improving cellular immune function.\(^9,17\)\(^-\)\(^19\)

To evaluate the effect of these therapeutic options on humoral immunity, we studied antibody responses to bacteriophage \( \phi X174 \) and concomitantly assessed T-cell function in 10 children with ADA deficiency who were treated with marrow transplantation, with human RBC transfusions, or with weekly injections of PEG-modified bovine ADA. We selected bacteriophage \( \phi X174 \), since the antibody response to this antigen is a complex process requiring antigen-processing and -presenting cells, antigen-specific T-helper cells, and antigen-specific B lymphocytes capable of switching from IgM to IgG synthesis.\(^20\) Furthermore, since nonimmunized individuals do not have antiphage antibody, the
use of blood products, including intravenous immunoglobulin (IVIG), does not interfere with data interpretation.

MATERIALS AND METHODS

Subjects

The clinical and laboratory data of all 10 subjects are summarized in Table 1.

Bone Marrow Transplantation

Patient 1. The case of this now 14-year-old boy who developed persistent respiratory infections at 5 weeks and bilateral pneumonia and pseudomonas septicemia at 3 months of age has been reported previously. Growth and development were severely delayed. ADA activity was undetectable in RBC, peripheral blood lymphocytes (PBL), and cultured fibroblasts and metabolic consequences characteristic for ADA deficiency were present. At the age of 6 months, and without preceding cytoreduction, he received an unfractionated bone marrow transplant (5 x 10^8 nucleated cells/kg body weight) from his HLA-identical sister who had normal RBC-ADA activity. Methotrexate was administered prophylactically and no significant graft-versus-host disease (GVHD) was observed. Following transplantation, symptoms of chronic infections disappeared and the infant began to thrive. At the age of 5 years, he developed severe varicella infection requiring hospitalization. Since that time, no other problems with infections have been reported.

Patient 2. The older sister of this 4 1/2-year-old boy died of infections at four months of age. His parents are first cousins. He was found to be lymphopenic at birth. ADA deficiency was confirmed at birth by demonstrating complete absence of lymphocytes and of RBC-ADA activity. Methotrexate was administered prophylactically and no significant graft-versus-host disease (GVHD) was observed. Following transplantation, symptoms of chronic infections disappeared and the infant began to thrive. At the age of 5 years, he developed severe varicella infection requiring hospitalization. Since that time, no other problems with infections have been reported.

Patient 3. This 3-year-old girl was found to have an absent thymus on a chest x-ray taken as a newborn. At 6 months of age, she developed oral thrush and interstitial pneumonia, and was found to be ADA-deficient. At 10 months of age, she received a haploidentical T-cell-depleted bone marrow graft from her mother. Pretransplant cytoreduction was performed with busulfan and cyclophosphamide. Following transplantation, she improved and showed normal growth and development. No further infections were reported. Serum IgG increased to normal levels 4 months after transplantation.

Patient 4. This patient, reported as patient 13 in reference 8, and RP in reference 16, is now a 7 1/2-year-old boy who developed recurrent severe infections and failure to thrive in infancy. RBC-ADA activity was low and metabolic changes characteristic for ADA deficiency were present. He and his younger brother (patient 5) were found to have a deletion of a portion of the ADA structural gene (at the 3' end of exon 1). At the age of 2 months, he received a single T-cell-depleted haploidentical stem cell graft from his mother, without preceding cytoreduction or GVHD prophylaxis. He improved rapidly, assumed a normal growth pattern, and later experienced varicella infection without complications.

Patient 5. The younger brother of patient 4, patient 5 is now 5 years old. ADA deficiency was diagnosed by amniocentesis and confirmed at birth by demonstrating complete absence of lymphocytes and of RBC-ADA activity. He received a haploidentical T-cell-depleted bone marrow stem cell transplant (1.2 x 10^6 nucleated cells/kg body weight) from his mother at 10 days of age, without preceding cytoreduction or GVHD prophylaxis. He has been clinically well, showing normal growth and development, and subsequently had uncomplicated varicella.

Patient 6. The older sister of this 4-year-old boy died during infancy of infectious complications after cardiac catheterization. The boy became symptomatic at age 2 months, when he developed chronic diarrhea, recurrent otitis media and pneumonia, thrush, and failure to thrive. When studied at the age of 2 months, RBC-ADA activity was absent and he had the metabolic abnormalities characteristic for ADA deficiency. At 4 months of age, he received a T-cell-depleted haploidentical bone marrow transplant from his mother, without cytoreduction or GVHD prophylaxis. He shows normal growth and development and is generally healthy, but requires monthly IVIG infusions.

Enzyme Replacement

Patient 7. Previously reported as patient 12 in reference 8, patient 1 in reference 9, and LB in reference 16, patient 7 is now a 9 1/2-year-old girl. She presented with recurrent infections and failure to thrive at 1 month of age and was diagnosed with ADA-deficient SCID at the age of 7 months. Metabolic changes typical for ADA deficiency were present. At the age of 8 months and again at 19 months, she received T-cell-depleted haploidenti-

---

Table 1. Patient Ages and Laboratory Findings at Time of Diagnosis of ADA Deficiency

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of birth</td>
<td>2/77</td>
<td>7/87</td>
<td>12/88</td>
<td>9/84</td>
<td>12/86</td>
<td>6/89</td>
<td>7/82</td>
<td>11/76</td>
<td>7/81</td>
<td>11/88</td>
</tr>
<tr>
<td>Age at time of diagnosis (mo)</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>3</td>
<td>At birth</td>
<td>2</td>
<td>7</td>
<td>4</td>
<td>72</td>
<td>1</td>
</tr>
<tr>
<td>RBC-ADA activity, nmol/h/mg proteins (normal &gt; 20)</td>
<td>0</td>
<td>0.31</td>
<td>0.17</td>
<td>0.4*</td>
<td>0.06</td>
<td>0.04</td>
<td>0.2</td>
<td>0</td>
<td>0.33</td>
<td>0.06</td>
</tr>
<tr>
<td>dAXPt, nmol/mL RBC (normal &lt; 2)</td>
<td>510</td>
<td>1,158</td>
<td>654</td>
<td>300</td>
<td>1,060</td>
<td>1,839</td>
<td>1,240</td>
<td>1,100</td>
<td>200</td>
<td>874</td>
</tr>
<tr>
<td>% dAXP, of total adenine nucleotides (normal &lt; 0.5%)</td>
<td>34%</td>
<td>61%</td>
<td>56%</td>
<td>29%</td>
<td>51%</td>
<td>58%</td>
<td>64%</td>
<td>50%</td>
<td>16%</td>
<td>73%</td>
</tr>
<tr>
<td>Lymphocytes/μL</td>
<td>350</td>
<td>132</td>
<td>144</td>
<td>71</td>
<td>0</td>
<td>96</td>
<td>&lt;100-500</td>
<td>125-925</td>
<td>144</td>
<td>1</td>
</tr>
<tr>
<td>T cells/μL</td>
<td>10</td>
<td>1</td>
<td>36</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>&lt;20-100</td>
<td>&lt;20-100</td>
<td>176-920</td>
<td>190</td>
</tr>
<tr>
<td>Mitogen-induced proliferation*</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>4%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>2%</td>
<td>&lt;10%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Serum IgG (mg/dL)</td>
<td>122</td>
<td>220</td>
<td>303</td>
<td>243</td>
<td>540</td>
<td>140</td>
<td>146</td>
<td>92</td>
<td>844</td>
<td>360</td>
</tr>
<tr>
<td>IgM (mg/dL)</td>
<td>0</td>
<td>28</td>
<td>0</td>
<td>7</td>
<td>111</td>
<td>30</td>
<td>79</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA (mg/dL)</td>
<td>0</td>
<td>74</td>
<td>10</td>
<td>7</td>
<td>2.4</td>
<td>0</td>
<td>16</td>
<td>9</td>
<td>111</td>
<td>7</td>
</tr>
</tbody>
</table>

*Following a blood transfusion.
†Total adenine deoxyribonucleotides.
‡Expressed as percent of controls.

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eral bone marrow stem cell transplants (9.2 × 10^9 and 3.2 × 10^9 nucleated cells/kg body weight, respectively) from her father, without prior cytoreduction or GVHD prophylaxis, along with periodic irradiated human RBC transfusions. Following each transplant, a period of transiently improved T-cell function was observed. Despite continued treatment with irradiated RBC transfusions, her failure to thrive persisted, her immune functions declined to pretransplant baseline, and recurrent pulmonary infections remained a major problem, resulting in chronic pulmonary disease. She was too sick to participate in normal activities, including attending school. In April 1986, she was started on PEG-ADA injections, prepared from bovine intestinal ADA (Enzon, South Plainfield, NJ). Her maintenance dose of PEG-ADA, injected intramuscularly once a week, has been 20 U/kg weight. Following initiation of PEG-ADA therapy, the number of acute infections decreased markedly, the symptoms of chronic infections improved, and she began to gain weight and to attend school.

Patient 8. Previously reported as patient 2 in reference 9, and as T.D. in reference 14, patient 8 is a 15-year-old girl, who presented at 3 months of age with severe infections and failure to thrive. RBC-ADA activity was absent, and metabolites characteristic for ADA deficiency were demonstrated in serum and urine. Following diagnosis, treatment with irradiated human RBC infusions was initiated. Six unsuccessful attempts were made at transplantation with cultured thymic epithelial cells. Recurrent pulmonary infections remained a major problem, resulting in severe chronic lung disease that prevented her from attending school. In August 1986, PEG-ADA (20 U/kg weekly) treatment was initiated. She started to gain weight and, for the first time, was able to attend school. Although her chronic lung disease and pulmonary function tests have improved, she still depends on oxygen supplementation and receives prophylactic immunoglobulin infusions to protect her from acute viral infections.

Patient 9. This patient is now a 10½-year-old girl who, during her first 3 years of life, had mild upper respiratory tract infections, Subsequently, she developed recurrent episodes of fever, sinusitis, chronic bronchitis, pneumonia, pneumococcal bacteremia, and septic arthritis of the left hip. The diagnosis of ADA deficiency was made at the age of 5 years, when she was noted to have persistent lymphopenia, eosinophilia, and depressed responses to mitogens in vitro. Although serum immunoglobulin levels were normal, she failed to produce antibodies to polysaccharides. ADA activity was less than 1% of normal in RBC extracts and 1% of normal in white blood cell extracts. She also had the typical metabolic consequences of ADA deficiency, including elevated adenine deoxyribonucleotides in RBC-lysates and decreased S-adenosylhomocysteine hydrolase activity. In April 1987, she was started on PEG-ADA therapy. Her weekly dose of PEG-ADA has been 20 U/kg intramuscularly. Following the initiation of PEG-ADA therapy, the number of infections decreased, immunoglobulin infusions could be discontinued, and she remained free of chronic disease.

Patient 10. This patient, now 3 years old, developed severe respiratory distress 3 days after birth, when he was found to be lymphopenic. The diagnosis of SCID due to ADA deficiency was confirmed at 4 weeks of age. A chest x-ray showed chondro-osseous dysplasia, and biochemical changes characteristic for ADA deficiency were present. He is the offspring of Amish parents, descendants from two large families closely related by intermarriage. Analysis of the gene defect revealed a point mutation, resulting in a Gly→Arg substitution at codon 216.22 At 6 weeks of age, he was begun on PEG-ADA therapy, initially 30 U/kg/wk, then 30 U/kg twice weekly. His clinical course improved, but his immunologic function remained abnormal. He is still receiving monthly infusions of IVIG.

### Methods

PEG-ADA (Enzon) was administered intramuscularly at a dose of 20 U/kg once weekly, except in patient 10, who received 30 U/kg twice weekly.

### Biochemical Analysis

**RBC-ADA activity and levels of total adenine nucleotides and adenine-deoxy-ribonucleotides (dAXP) in RBC were determined as previously described.**

**Immunologic Assessment**

Standard methods established in laboratories available to the participating investigators were used to measure absolute numbers of T cells (CD2+ or CD3+ cells), CD4+ and CD8+ lymphocytes, in vitro mitogen- (phytohemagglutinin, concanavalin A, and pokeweed mitogen) and antigen- (tetanus, diphtheria, candida, streptokinase) induced proliferation, and serum immunoglobulin concentrations.

Bacteriophage φX-174 was grown, harvested, and purified as previously described.20,24-26 Each lot was adjusted to a final concentration of 1 × 10^11 plaque-forming units (PFU)/mL, aliquoted, tested for sterility and lack of pyrogens, and stored at −70°C until thawed for use. After obtaining informed consent, phage was administered intravenously at a dose of 2 × 10^9 PFU/kg body weight. The dose was chosen to achieve a serum concentration of approximately 5 × 10^10 PFU/mL 15 minutes after primary immunization. This was repeated 6 weeks later by a second injection of phage at the same dose. Most patients received more than two phage immunizations at intervals indicated in Table 2. Blood samples for antibody titers were collected immediately before immunization and, in most instances, at 1, 2, and 4 weeks after each immunization. Serum was stored at −20°C until analyzed. Antibody activity was determined by a neutralizing antibody assay and expressed as the first-order rate constant (Kd), or phage inactivation as described by a standard formula.24,25 Susceptibility of phage-neutralizing antibody to 2-mercaptoethanol (2-ME) was determined by the method of Grubb and Swahn26; neutralizing antibody resistant to 2-ME was considered to be IgG. Each serum sample was also assayed for phage-specific IgM or IgG antibodies by the enzyme-linked immunoabsorbent assay (ELISA) technique as described previously.20

### RESULTS

**Effect of Treatment on the Clinical Course of ADA Deficiency**

Following bone marrow transplantation, growth and development normalized and patients became largely asymptomatic; eventually, all but one were able to live without prophylactic IVIG therapy.

Patients 7 and 8 received RBC transfusions during early childhood without significant clinical improvement. They continued to have recurrent sinopulmonary infections and failure to thrive, and were unable to participate in normal activities. After they were started on PEG-ADA injections, both children began to gain weight and were able to attend public school. Because of chronic lung disease, patient 8 continues to receive IVIG therapy to prevent acute pulmonary infections. Two additional patients were started on PEG-ADA. One, patient 9, became completely asymptomatic; the other, patient 10, showed only limited improvement in immune function during PEG-ADA therapy and,
although clinically improved, still requires immunoglobulin infusions.

Effect of Treatment on Metabolic Abnormalities

Following bone marrow transplantation, patients not receiving pretreatment cytoreduction (patients 1, 2, 4, 5, 6) demonstrated persistent mixed chimerism. Only lymphocytes (in patient 1, only T lymphocytes) were of donor origin and ADA-positive; neutrophils and RBC remained ADA-negative and were of host origin. Unexpectedly, when retested 10 years after marrow transplantation, RBC-ADA activity of patient 1 was approximately 25% of normal, suggesting the presence of donor RBC. Patient 3, one of six transplanted patients, was given full cytoreduction before receiving T-cell-depleted bone marrow; she was the only marrow recipient whose entire hematopoietic system was of donor origin. dAXP of RBC decreased substantially in all transplanted patients (Table 3). However, the level of dAXP remained moderately elevated (44 to 212 nmol/mL RBC) in those patients whose engraftment was limited to lymphoid cells, while in patient 3, whose donor-derived erythrocytes express ADA, there was nearly complete disappearance of dAXP in RBC (11 nmol/mL RBC).

During treatment with irradiated RBC transfusions, patients 7 and 8 showed a significant increase in RBC-ADA activity (due to transfused RBC) and a decrease in total dAXP concentration in RBC. Following PEG-ADA therapy (patients 7 to 10), plasma ADA activity increased to above the normal level of total blood (erythrocyte) ADA activity. dAXP in erythrocytes decreased to near-normal levels (3 to 8 nmol/mL RBC) and S-adenosylhomocysteine hydrolase became normal,9,17 indicating effective elimination of deoxy-adenosine by the injected enzyme. Patient 10, who had a limited response to PEG-ADA therapy in terms of immune reconstitution, nevertheless showed biochemical changes similar to the other three PEG-ADA–treated patients, including low dAXP levels.

Effect of Treatment on Immune Function

Patients who had received a successful marrow transplant or were treated with PEG-ADA showed improved although not always normal T-cell function (Table 3). Three of the T-cell–depleted haploidentical marrow graft recipients were found to have persistent lymphopenia, and two of the PEG-ADA–treated patients had reduced numbers of CD4+ and increased CD8+ lymphocytes. Mitogen-induced lymphocyte proliferation was normal in all marrow recipients except patient 6, who had persistently low (but clearly positive) responses. Mitogen responses normalized in patient 9, but remained inadequate in patient 10 during PEG-ADA therapy. Patients 7 and 8, while on PEG-ADA, responded variably to mitogens: lymphocyte proliferation was vigorous at times and severely depressed on other occasions. Antigen-induced lymphocyte proliferation was normal in five of nine patients studied. Serum IgG levels were normal in some, low in others, and uninterpretable in the four patients who received IVIG therapy. IgA and IgM concentrations returned to normal in three of six bone marrow recipients and in all four PEG-ADA–treated patients.

Treatment with irradiated human RBC transfusions had little effect on the severe lymphopenia and impaired mitogen responses of patients 7 and 8 (Table 3). Serum immunoglobulin levels remained depressed. A transient increase in specific antibody titers to diphtheria/tetanus immunization was observed in patient 7, who, while being

Table 2. Immunization With Bacteriophage +X174, Dates and Relationship to Type of Treatment at Time of Immunization

| Patient No. | Pre BMT | Post BMT Post BMT Post BMT Post BMT Post BMT Post BMT Post BMT Post BMT Post BMT | During | During | During | During |
|-------------|---------|---|---|---|---|---|---|---|---|---|---|---|
| 1           | 8/77*   | 11/87* | 9/89† | 11/84† | 12/86† | 6/89† | 3/83# | 2/84# |       |       |       |       |
| 2           | 7/77 (1')| 3/89 (1')| 6/91 (1')| 5/86 (1')| 3/87 (1')| 1/90 (1')| hRBC-T | hRBC-T | PEG-ADA | PEG-ADA |       |       |
| 3           | 11/77 (2')| 4/91 (3')| 6/86 (3')| 12/90 (3')| 6/87 (1')| 3/79 (3')| PEG-ADA |       |       |       |       |       |
| 4           | 1/78 (3')| 3/87 (4')| 1/88 (2')| 3/79 (3')| PEG-ADA |       |       |       |       |       |       |       |
| 5           | 3/80 (4')| 4/87 (2')| 7/91 (3')| 4/87 (2')|       |       |       |       |       |       |       |       |
| 6           | 3/87 (5')|       |       |       |       |       |       |       |       |       |       |       |

Abbreviations: BMT, bone marrow transplantation; hRBC, human RBC transfusions.
*HLA-matched bone marrow from a sibling (patient 1), from a parent (patient 2).
†Haploidentical bone marrow from a parent, T-cell–depleted, engraftment.
‡Dates when haploidentical bone marrow stem cell transplants from the father were attempted (without engraftment).
§(1') = Primary, (2') = secondary (etc) immunization with bacteriophage +X174.
Table 3. Immune Status When Immunized With Bacteriophage φX174 (and when most recently studied)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment*</td>
<td>BMT</td>
<td>BMT</td>
<td>BMT</td>
<td>BMT</td>
<td>BMT</td>
<td>hRBC</td>
<td>PEG-ADA</td>
<td>hRBC</td>
<td>PEG-ADA</td>
<td>PEG-ADA</td>
</tr>
<tr>
<td>Lymphocytes/μL (normal ≥ 1,500)</td>
<td>1,100</td>
<td>1,540</td>
<td>2,780</td>
<td>1,400</td>
<td>700</td>
<td>126</td>
<td>&lt;500</td>
<td>290</td>
<td>80</td>
<td>324</td>
</tr>
<tr>
<td>T cells/μL (normal ≥ 900)</td>
<td>816</td>
<td>1,406</td>
<td>2,113</td>
<td>767</td>
<td>210</td>
<td>53</td>
<td>&lt;100</td>
<td>160</td>
<td>16</td>
<td>108</td>
</tr>
<tr>
<td>CD4/CD8 per μL (normal CD4 ≥ 400)</td>
<td>362/396</td>
<td>208/841</td>
<td>473/693</td>
<td>531/248</td>
<td>105/91</td>
<td>30/14</td>
<td>23/21</td>
<td>66/87</td>
<td>ND</td>
<td>78/68</td>
</tr>
<tr>
<td>Mitogen-induced proliferation†</td>
<td>Normal</td>
<td>10%-60% Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>10%-20%</td>
<td>&lt;2%</td>
<td>2%-14%</td>
<td>&lt;5%</td>
<td>30%-50%</td>
</tr>
<tr>
<td>Antigen-induced proliferation</td>
<td>Normal</td>
<td>ND</td>
<td>Normal</td>
<td>ND</td>
<td>Normal in '91</td>
<td>Absent in '91</td>
<td>ND</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>IgG (mg/dL)</td>
<td>1,187</td>
<td>419</td>
<td>1,900</td>
<td>170</td>
<td>155</td>
<td>360#</td>
<td>167#</td>
<td>620</td>
<td>402#</td>
<td>1,079#</td>
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<tr>
<td>IgA (mg/dL)</td>
<td>86</td>
<td>50</td>
<td>213</td>
<td>20</td>
<td>7</td>
<td>28</td>
<td>185</td>
<td>170</td>
<td>64</td>
<td>410</td>
</tr>
<tr>
<td>IgM (mg/dL)</td>
<td>96</td>
<td>57</td>
<td>172</td>
<td>24</td>
<td>12</td>
<td>10</td>
<td>29</td>
<td>190</td>
<td>26</td>
<td>81</td>
</tr>
<tr>
<td>dAXP, nmol/ml RBC (normal ≤ 2)</td>
<td>ND</td>
<td>84</td>
<td>11</td>
<td>44</td>
<td>191</td>
<td>212</td>
<td>ND</td>
<td>12</td>
<td>ND</td>
<td>8</td>
</tr>
</tbody>
</table>

*Treatment with bone marrow transplantation (BMT), human red blood cells (hRBC), or PEG-ADA.
†Expressed as percent of control.
‡While on IVIG therapy.
treated with RBC transfusions, had received two T-cell-depleted bone marrow transplants from her father. Karyotype analysis performed in 1990 showed only cells with two X chromosomes and ADA activity remained less than 1% of normal, confirming that the attempted marrow transplant had failed. Similarly, thymic epithelial cell transplantation attempted in patient 8 had failed to permanently transfer ADA-positive donor cells.

Antibody responses to bacteriophage 4x174 are summarized in Fig 1. Control subjects consist of 22 young adults (10 males and 12 females) and two 13-year-old twin brothers. Each received a primary and a secondary immunization with phage; 13 (five males and eight females) received, in addition, a tertiary, and one male a quaternary injection of phage. Observations made in a group of children (aged 1 to 16 years) who were studied for possible immune deficiency with bacteriophage, and who were ultimately found to be immunologically normal (data not shown), suggest that in normal individuals antibody responses to phage are independent of age and sex. Following primary immunization, the antibody response to phage peaks at 2 weeks and is predominantly of the IgM class. A second immunization results in a brisk antibody increase, which peaks at 1 week and consists of both IgM and IgG. Following third and fourth phage injections, the antibody titers increase further and consist entirely of IgG.20

Patients were immunized multiple times with bacteriophage at various stages of therapy (Table 2). Immune parameters determined at the time of immunization with bacteriophage (or later, as specified by listing the year of the study) are shown in Table 3. Patient 1 received a primary immunization before specific treatment was initiated; he made phage-neutralizing antibody at very low but measurable titers for 2 weeks; no antibody could be demonstrated subsequently (Fig 1A). He received his first posttransplant immunization at 6 weeks (indicated as "primary response" in Fig 1A); his antibody response was approximately 1% of normal and entirely IgM. Subsequent posttransplant immunizations resulted in a persistent increase in titers and switch from IgM to IgG. The fifth immunization was given 10 years after transplantation and elicited a quantitatively near-normal response, but only

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**Fig 1.** Antibody responses to intravenously injected (●) bacteriophage 4x174 were determined in normal controls (geometric mean ± SD indicated by shaded area) and in patients with ADA deficiency receiving various treatment regimens. (A) Patient 1 was immunized before (○) and five times after (●) marrow transplantation from an HLA-matched sibling. Patient 2 (●) received an HLA-matched, MLC-nonreactive marrow transplant from a parent. (B) Patients 3 (●), 4 (○), 5 (●), and 6 (●) were immunized repeatedly (see Table 2 for details) after receiving a T-cell-depleted haploidentical marrow from a parent. Only patient 3 had cytoreduction. (C) Patients 7 (■) and 8 (○) were immunized with phage twice (patient 7) and three times (patient 8) during treatment with RBC transfusions. (D) Patients 7 (■), 8 (○), 9 (●) and 10 (●) were immunized with phage during treatment with PEG-ADA. Percent IgG is indicated in parentheses.
27% of the antibody was of the IgG class. Patient 2 (Fig 1A), who also received a matched bone marrow, showed a similar pattern of recovery over time. The three patients receiving a haploidentical T-cell-depleted bone marrow transplant without cytoreduction (patients 4, 5, 6) showed a much slower recovery of antibody responses to phage (Fig 1B). Patient 4, who was immunized with phage at 6 and at 9 months following bone marrow transplantation, responded with barely measurable antibody titers and failed to switch from IgM to IgG. A tertiary immunization at 20 months posttransplantation was still markedly suppressed, but a fourth immunization given at 30 months induced a significant increase in antibody titer, although the antibody was almost entirely of the IgM isotype. Patients 5 and 6, immunized between 3 and 10 months following marrow transplantation, had markedly depressed antibody responses and impaired switch from IgM to IgG. This abnormal response persisted in patient 6 during tertiary immunization 1 year later. In contrast, patient 3, who received a T-cell-depleted haploidentical marrow after cytoreduction, was able to respond vigorously and, during the secondary response, showed a normal proportion of IgG (37%). During treatment with irradiated human RBC transfusions (patients 7 and 8), antibody responses were severely depressed and without amplification and/or switch from IgM to IgG (Fig 1C). Following PEG-ADA therapy, near-normal primary and normal secondary responses were observed in patients 7, 8, and 9 (Fig 1D), including amplification and switch from IgM to IgG (13% to 45%). Patient 10, whose improvement during PEG-ADA was limited, showed a markedly depressed response and failed to switch to IgG.

DISCUSSION

The profound immune deficiency associated with severe ADA deficiency is the direct result of the biochemical abnormalities caused by the absence of this enzyme. Deoxyadenosine, one of the substrates of ADA, accumulates in ADA-deficient lymphocytes, and in the presence of deoxy-nucleoside kinases is rapidly phosphorylated to dATP, a potent inhibitor of ribonucleotide reductase. As a consequence of this and possibly other mechanisms, DNA synthesis is severely impaired. Because the substrates for ADA in the plasma are in equilibrium with the toxic metabolites trapped inside the cells, enzyme replacement therapy, accomplished by infusing irradiated human RBC or injecting PEG-ADA, as well as transplantation of ADA-positive bone marrow-derived cells, has been shown to reduce the concentration of deoxy-adenosine and dATP within blood cells. Improvement in mitogen- and antigen-induced lymphocyte proliferation has been reported in ADA-deficient SCID patients during PEG-ADA therapy and after bone marrow transplantation. However, humoral immunity has not been analyzed systematically in treated ADA-deficient SCID patients. To assess the extent of immune recovery induced by different treatment modalities, we measured concomitantly a set of immune parameters to quantify T- and B-cell function. To measure in vivo antibody responses, we selected the T-cell–dependent antigen, bacteriophage φX174. If administered intravenously to immunologically competent individuals, phage will elicit typical primary, secondary, and tertiary antibody responses, including the generation of memory cells. Because humans are not naturally exposed to bacteriophage, antibody responses to this antigen can be measured in very young infants with circulating maternal antibodies, and in patients treated with IVIG injections.

A major difficulty in assessing the effect of therapy is the observation that ADA deficiency is not a homogeneous disease. Most but not all patients with ADA deficiency present during infancy with recurrent life-threatening infections and severely impaired T- and B-cell function. A subgroup of individuals with partial ADA deficiency appears to have few if any clinical symptoms and a normal or only partially impaired immune system. Of the 10 patients studied, one (patient 9) had a milder form and one (patient 10) appeared to have a more severe form than the rest of the patients. However, both patients improved clinically and immunologically, one more than the other, during PEG-ADA therapy.

Patient 1, a boy with severe ADA deficiency, was immunized with phage before treatment. Unlike some patients with SCID and normal ADA activity who demonstrate delayed phage clearance and inability to produce antibody, patient 1 cleared phage within 4 days and produced phage-specific antibody, although at a very low titer and for only 2 weeks, suggesting that some B-cell function was preserved. During treatment with irradiated RBC transfusions, patients 7 and 8 remained lymphopenic and their lymphocytes failed to proliferate in response to mitogens. Antibody responses to phage measured during treatment with RBC transfusions, were severely depressed and similar to the response observed in the untreated patient. This may explain the observation that patients treated with RBC transfusions continued to have chronic infections and required IVIG therapy. In contrast, all bone marrow transplant recipients and those treated with PEG-ADA showed improved immune function, although at different degrees. Analysis of the data suggests that B-cell function recovery was more consistent in PEG-ADA–treated children as compared with bone marrow recipients. ADA-deficient patients, when studied shortly after marrow transplantation, made antibody to phage at very low titers and failed to switch from IgM to IgG, similar to marrow recipients with underlying diseases other than ADA deficiency. After transplantation, repeated immunization with phage over a period of time resulted in progressively improving antibody responses characterized by increased titers and more effective switch to IgG. This improvement was most pronounced in the two patients receiving HLA-matched non–T-cell–depleted bone marrow. However, none of the marrow recipients studied reached the normal range of phage-neutralizing antibody titers, and none switched as completely to IgG (> 90%) as normal controls. Since B lymphocytes are less susceptible than T cells to the toxic effects of deoxy-adenosine and dATP, it is not surprising that B cells of host origin often persist in marrow transplant.
The proportion of host B cells was found in three of four bone marrow recipients, none of whom received pretransplant cytoreduction. Patient 3, who was the only marrow recipient undergoing cytoreduction before transplantation, was found to have complete engraftment of ADA-positive hematopoietic cells of donor origin, including B and T cells. Her antibody responses to phage were brisk, her titers close to normal, and the switch from IgM to IgG significant.

The severely abnormal immune responses observed in patients 7 and 8 during human RBC transfusions improved after therapy was changed to weekly injections of PEG-ADA. In both patients, the response to phage normalized. However, T-cell function varied, being vigorous at times and significantly depressed at other times. Patient 9, who has a less severe immune defect, showed improved T-cell function and a quantitatively and qualitatively normal antibody response to bacteriophage during PEG-ADA therapy. In contrast, patient 10, who had a severe form of ADA deficiency, continued to have persistently low CD4 cell counts and depressed in vitro lymphocyte proliferation to mitogens and specific antigens during treatment with PEG-ADA. His antibody response to phage remained depressed, lacking amplification and switch to IgG. This limited response to PEG-ADA was not due to the presence of an antibody to bovine ADA, nor to incomplete "detoxification," since the metabolic changes secondary to ADA deficiency have improved to near normal, just as in the other patients receiving PEG-ADA. Clinically, all four PEG-ADA-treated patients improved. They began to gain weight and to grow, and two of the four no longer depend on immunoglobulin therapy. Patient 8, who developed severe chronic lung disease before treatment with PEG-ADA, continues to receive IVIG as prophylaxis to prevent further respiratory infection, and patient 10 receives IVIG because of his limited response to PEG-ADA therapy. Thus, normalization of the antibody responses to bacteriophage appears to be an excellent predictor of the patient's B-cell recovery and his/her tolerance of terminating treatment with IVIG.

Immunologists caring for ADA-negative SCID patients now have several options of therapy. If an HLA-matched, ADA-normal sibling is available, the procedure of choice is immediate transplantation of unfractionated bone marrow cells. If an HLA-matched sibling donor is not available, transplantation of a T-cell-depleted haploidentical marrow should be considered. The rapid immunologic recovery of patient 3, confirming the observations by others, suggests that pretransplantation conditioning with myeloablative agents can improve immune reconstitution; this advantage has to be weighed against possible risks related to the use of myeloablative drugs. If marrow transplantation has failed or if the ADA-deficient patient is not considered a suitable candidate for transplantation, PEG-ADA therapy is a safe and effective alternative.

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