Effect of Adenosine Deaminase Replacement Therapy on a Child of Adenosine Deaminase Deficiency with Severe Combined Immunodeficiency Disease

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Department of Pediatrics, Tohoku University School of Medicine, Sendai 980 and *Department of Pediatrics, Yamagata University School of Medicine, Yamagata 990-23

TSUCHIYA, S., ARAI, N., KUDO, M., KONNO, T., TADA, K. and YOKOYAMA, S. Effect of Adenosine Deaminase Replacement Therapy on a Child of Adenosine Deaminase Deficiency with Severe Combined Immunodeficiency Disease. Tohoku J. exp. Med., 1979, 128 (3), 251-258 — Enzyme replacement therapy was performed for a 1-year and 5-month old boy with adenosine deaminase deficiency disease, the first case in Japan. Irradiated fresh red blood cells were administered without any clinical improvement, but there was an increase in the peripheral lymphocytes from 300/mm³ to 1849/mm³, of which 88% had T cell marker. B lymphocytes did not bear any classes of surface immunoglobulins. The proliferative responses of these lymphocytes to phytohemagglutinin, concanavalin A, pokeweed mitogen and allogeneic cells were examined. More than two-fold increase in response to these mitogens was observed in lymphocytes after treatment as compared with responsiveness before treatment, but these responses still remained to a much lesser degree than that of lymphocytes from controls.

adenosine deaminase deficiency; severe combined immunodeficiency; enzyme replacement therapy

Since Giblett et al. (1972) described two patients with adenosine deaminase (ADA) deficiency associated with severe combined immunodeficiency disease (SCID), more than a dozen cases have been reported (Meuwissen et al. 1975). Severe impairment of cellular and humoral immunity in the disease results in death unless treated. The treatment of ADA deficiency disease is the same as other types of SCID and only transplantation of the histocompatible bone marrow, fetal liver or thymus is effective (Hong 1975). On the other hand, Polmar et al. (1976) reported the specific treatment for the ADA deficiency disease, enzyme replacement therapy, which resulted in almost complete restoration of immunological defects after the transfusion of frozen irradiated red blood cells (RBCs). But no other successful cases of this enzyme replacement therapy have been reported (Gatti and Jose 1977; Daoud et al. 1978).

Recently we had a patient with ADA deficiency, the first case in Japan, and

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performed an enzyme replacement therapy according to Polmar's method. In this communication, we report the results of the therapy by which an increase in peripheral blood lymphocyte count, but minor functional restoration of the lymphocytes was observed.

**REPORT OF CASE**

The patient was admitted to Yamagata University Hospital at the age of 1 year and 3 month with complaints of severe pulmonary candidasis and then referred to Tohoku University Hospital one month later. Serum immunoglobulins were completely absent including IgG, IgM, IgA and IgE. Skin tests were negative for delayed hypersensitivity reaction against candida, streptokinase-streptodornase (SK/SD) and dinitrochlorobenzene (DNCB). ADA activity in RBCs was below the detectable level and a diagnosis of ADA deficiency with SCID was made. Clinical data in this particular patient will be described in detail elsewhere.*

The patient and his 19 relatives were examined for their HLA. The patient’s HLA was HLA-A9, -B12/HLA-A(10), -B40 and his mother’s HLA-A9, -B12/-, HLA-B40. As far as HLA-A and -B are concerned, the patient and his mother seemed to be histocompatible. However, we hesitated the bone marrow transplantation, because his mother’s lymphocytes responded to irradiated patient’s lymphocytes in one way mixed lymphocyte culture (MLC) (Fig. 3B).

At the 33rd hospital day, 100 ml of irradiated RBCs were transfused for the purpose of enzyme replacement therapy. At that time the patient was afebrile and seemed to have no severe infection but oral candidasis. At the 44th hospital day, he became febrile and tachypneic, and the pulmonary infiltration was revealed by radiologic examinations. Since then the irradiated red blood cell transfusions were carried out subsequently four times once a week. Amphotericine B, aminobenzylpenicillin, gentamycin sulfate, pentamidine and transfer factor were administered for treatment of his pulmonary infection. His clinical course was, however, downhill without effect of such treatment and the patient died at the 89th hospital day, at the age of 1 year and 7 months. Bacteriological examinations at autopsy revealed Pneumocystis carinii pneumonitis and sepsis caused by Pseudomonas aeruginosa.

**MATERIALS AND METHODS**

**Red blood cell transfusion.** Fresh type-A RBCs from normal adult donors were prepared after removal of white blood cells (WBCs) at Miyagi Red Cross Blood Center. After irradiation with 5000 rads, 100 ml of the RBC suspension in sterile saline equivalent to 100 ml of peripheral blood were transfused.

**Immunological examination.** Heparinized blood was drawn in sterile tubes and peripheral blood lymphocytes were isolated by Ficoll-Conray gradient centrifugation. After washing 3 times with Hanks’ solution, lymphocytes were resuspended in RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum and kanamycin. Lymphocyte blastogenesis induced by mitogens was observed in microculture plate containing $10^5$ cells/0.2 ml/well. The final concentrations of mitogens added were as follows: phytohemagglutinin P (PHA-P), 1:2,000 dilution of stock solution (DIFCO, Detroit, Michigan), concanavalin A (ConA), 5 μg/ml (Miles, Elkhart, Indiana) and pokeweed mitogen (PWM), 1:100 dilution of stock solution (GIBCO, Grand Island, New York). The microcultures were incubated at 37°C for 72 hr in an atmosphere of 5% CO₂ in air, pulsed with 0.8 μCi of methyl-³H-thymidine (20 Ci/m mole, New England Nuclear, Boston) for the last 4 hr and harvested by a multiple automated sample harvester. In two experiments, calf intestine ADA preparation (Type I, 10 mg/ml, 220 units/mg protein, Sigma, St. Louis) was added to the assay system.

* Yokoyama et al. Submitted for publication.
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One way MLC (Tan et al. 1976), rosette formation for thymus derived (T) and bone marrow derived (B) lymphocytes (Tachibana and Ishikawa 1973) and membrane immunofluorescence for surface immunoglobulin bearing cells (Kumagai et al. 1975) were kindly performed by Dr. T. Nishihira, the Second Department of Surgery, Tohoku University School of Medicine, Dr. N. Watanabe, Department of Immunology, the Research Institute for Tuberculosis, Leprosy and Cancer, Tohoku University, and Dr. T. Abo, Department of Microbiology, Tohoku University School of Dentistry, respectively.

ADA activity in RBCs. After removal of plasma and WBCs, the RBCs were washed 3 times with saline, and then lyzed by freezing and thawing in dry ice-acetone. The cell lysates were centrifuged at 3,000 rpm for 20 min and the supernatants were used for enzyme assay. ADA activity in the RBC lysates was measured by linked enzyme assay using 0.3 μmole adenosine (Wako, Tokyo) as substrate, endogenous nucleoside phosphorylase and exogenous 10 μl of xanthine oxidase (Boehlenger, Mannheim, 10 mg/ml) (Hopkinson et al. 1969). Increased uric acid was measured at 293 mμ in a UVIDEC-405X recording spectrophotometer (Japan Spectroscopic Co., Ltd, Tokyo).

RESULTS

Effect of exogenous ADA on lymphocyte blastogenesis induced by mitogens

The effect of addition of calf intestine ADA on lymphocyte blastogenesis was examined according to Polmar’s experiment (Polmar et al. 1975). Two experiments were independently carried out on lymphocyte preparations obtained at different dates. Since the patient’s lymphocyte count was below 300/mm³ during the period when the experiments were done, only one well was set up for each mitogen. No effect of exogenous ADA on the stimulation index for lymphocyte blastogenesis against PHA, ConA and PWM was observed, as shown in Table 1.

<table>
<thead>
<tr>
<th>Cells</th>
<th>ADA</th>
<th>PHA</th>
<th>ConA</th>
<th>PWM</th>
<th>Unstimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>141,045±36,335c)</td>
<td>97,788±39,392</td>
<td>42,054±11,487</td>
<td>1,510±1,135</td>
</tr>
<tr>
<td>Patient’s</td>
<td>4,800±1,785</td>
<td>9,506±4,618</td>
<td>7,257±1,835</td>
<td>1,442±749</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.5u</td>
<td>178,119±3,466</td>
<td>124,301±12,910</td>
<td>49,688±3,221</td>
<td>1,538±571</td>
</tr>
<tr>
<td>Patient’s</td>
<td>2.5u</td>
<td>10,000</td>
<td>6,454</td>
<td>5,025</td>
<td>2,456</td>
</tr>
<tr>
<td>Control</td>
<td>2.5u</td>
<td>176,115±18,494</td>
<td>191,448±4,531</td>
<td>54,760±2,437</td>
<td>2,394±1,322</td>
</tr>
<tr>
<td>Patient’s</td>
<td>2.5u</td>
<td>4,658</td>
<td>6,196</td>
<td>4,848</td>
<td>494</td>
</tr>
</tbody>
</table>

a) Mean of 13 control subjects.
b) Mean of three experimental data performed at different date.
c) cpm±s.d.
d) The number in parenthesis indicates stimulation index.

Table 1. Effect of addition of adenosine deaminase in vitro on mitogen induced lymphocyte proliferation
Effect of irradiated fresh RBC transfusion on peripheral blood lymphocyte count

Irradiated fresh RBC transfusions were performed five times at the hospital days, 33, 50, 58, 64 and 69. Lymphocyte count was determined from WBC count and proportion in percentage of lymphocytes which was enumerated by counting more than 500 WBCs on both peroxidase and Wright-Giemsa stained smears of peripheral blood. As shown in Fig. 1, peripheral lymphocytes increased in absolute count from 249/mm³ to 592/mm³ 7 days and up to 1468/mm³ 14 days after the initial RBC transfusion, respectively. The increase in peripheral lymphocyte count reached a maximum (1849/mm³) 8 days after the second RBC transfusion performed at the 50th hospital day. However, a sudden decrease in peripheral lymphocyte count was observed accompanied by deterioration of his pulmonary infection and then no more increase in his peripheral lymphocyte count occurred in spite of subsequent transfusion, though ADA activity in the patient's RBC reached a level within normal limits.

![Fig. 1. Effect of transfusion of irradiated fresh red blood cells on peripheral blood lymphocyte count. \(\downarrow\), irradiated fresh RBC transfusion; \(\cdots\cdot\cdot\), transfusion of HLA-A and -B matched whole blood obtained from the patient's mother.](image)

Surface markers of lymphocytes increased after irradiated fresh RBC transfusion

The subpopulation of lymphocytes increased after the second RBC transfusion was analyzed by rosette formation (Table 2). It was found that 88% of lymphocytes had T cell marker and the absolute number of them increased 13-fold as compared with that before RBC transfusion. On the other hand, B lymphocytes increased
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**Table 2. Lymphocyte surface markers before and after irradiated fresh RBC transfusion**

<table>
<thead>
<tr>
<th></th>
<th>Before RBC transfusion</th>
<th>After RBC transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>4700 /mm²</td>
<td>6950 /mm²</td>
</tr>
<tr>
<td>Lymphocyte count</td>
<td>249</td>
<td>1849</td>
</tr>
<tr>
<td>Peroxidase positive a)</td>
<td>68 %</td>
<td>40 %</td>
</tr>
<tr>
<td>E</td>
<td>15</td>
<td>55</td>
</tr>
<tr>
<td>EA</td>
<td>78</td>
<td>51</td>
</tr>
<tr>
<td>EAC b)</td>
<td>85</td>
<td>52</td>
</tr>
<tr>
<td>EAC mono c)</td>
<td>55</td>
<td>34</td>
</tr>
<tr>
<td>T cell d)</td>
<td>47% (117) x)</td>
<td>88% (1921)</td>
</tr>
<tr>
<td>B cell e)</td>
<td>53 (113)</td>
<td>19 (328)</td>
</tr>
</tbody>
</table>

a) These lymphocytes were obtained after Ficoll-Conray gradient centrifugation.
b) C₃b receptor on the lymphocytes was detected by human complements.
c) C₃d receptor on the lymphocytes was detected by complements obtained from A-SW mice.
d) The percentage of T and B cells were determined from E- and EAC b)-rosette forming cells, respectively.
e) The number in parenthesis indicates absolute count of T and B cells per cubic millimeter.

in number only 3-fold and did not bear any classes of membrane immunoglobulins on their surfaces.

**Effect of irradiated fresh RBC transfusion on in vitro lymphocyte blastogenic responses against mitogens**

Responsiveness of the lymphocytes after RBC transfusion to PHA, ConA and PWM were examined. As shown in Fig. 2, the responses of the lymphocytes to PWM and PHA were increased approximately 2-fold when expressed in terms of stimulation index, though these responses were much lower than those of lymphocytes from controls. In order to examine the effect of RBCs with normal ADA activity on responsiveness of the lymphocytes to the mitogens, the patient’s lymphocytes and RBCs with normal ADA activity were cocultured. There was no effect on responsiveness to PHA and PWM, but the lymphocyte proliferative response to ConA was increased approximately 3-fold.

**In vitro lymphocyte response to allogeneic cells in MLC reaction**

One way MLC was carried out with patient’s peripheral lymphocytes obtained at the 50th hospital day, 17 days after the initial RBC transfusion. As shown in Fig. 3A, the response of the patient’s lymphocytes against his mother’s lymphocytes which seemed to be histocompatible in HLA-A and -B was markedly low, but their response against the lymphocyte from unrelated controls was greater, ranging from 7 to 15 in terms of stimulation index. Maximum response of the mothers’ lymphocytes to the lymphocytes from the two unrelated donors ranged 30 to 40 in stimulation index, but it was 9 to the patient’s lymphocytes (Fig. 3B).
Fig. 2. Effect of irradiated fresh RBC transfusion on in vitro lymphocyte proliferative response to mitogens. *The patient's lymphocytes were cocultured with fresh RBCs of normal ADA activity.

Fig. 3. In vitro lymphocyte response to allogeneic cells in MCL reaction. Responding lymphocytes were obtained from the patient in A and from the mother in B. Stimulating lymphocytes were inactivated by irradiation with a 60Co-source at a dose of 2,000 R. $5 \times 10^6/0.5$ ml of responding lymphocytes and stimulating lymphocyte in each were mixed in $13 \times 90$ mm flat bottom glass tubes and then cultured in an atmosphere of 5% CO$_2$ in air. $\times-\times$, the patient $\times$ control subject 1; $\bullet-\bullet$, the patient $\times$ control subject 2; $\circ-\circ$, the patient $\times$ his mother; $\triangle-\triangle$, the mother $\times$ control subject 1; $\uparrow-\downarrow$, the mother $\times$ control subject 2; $\triangledown-\triangledown$, the mother $\times$ patient.
DISCUSSION

A successful enzyme replacement therapy for ADA deficiency and SCID has been reported by Polmar et al. (1976). However, such response to restore the immunological function has been so far observed in only one child (Seegmiller et al. 1977). We performed the enzyme replacement therapy for a case of ADA deficiency with SCID according to Polmar’s method. Though no clinical improvement occurred, some effects of the treatment on peripheral lymphocytes were observed as follows: 1) an increase in lymphocyte count up to the normal level, 2) an increase of T lymphocytes (88%), 3) more than 2-fold increase in response of lymphocytes to mitogens.

It is of interest that approximately 90% of lymphocytes increased after the enzyme replacement therapy was T lymphocytes without functional restoration. It is likely that there are two steps on the process of immunological restoration of ADA deficiency disease, lymphocyte proliferation and subsequent differentiation. It has been suggested that ADA deficiency in lymphocytes affects not only their proliferation (Ullman et al. 1976; Hershfield et al. 1977), but also their differentiation (Ballet et al. 1976), and the thymus plays a role in this differentiation step (Lewis et al. 1977). In view of these assumptions, it may be stated that RBC transfusion induced lymphocyte proliferation but not subsequent differentiation in our patient.

There are several hypotheses concerning mechanisms involved in rectification of immune function by enzyme replacement therapy in ADA deficiency. Excess toxic metabolites produced by the enzyme defect may be converted to non-toxic ones in transfused erythrocytes (Polmar et al. 1976), or ADA in RBC may be converted to tissue specific isozyme by a tissue converting factor and may reform the ADA deficient state in the cells (Hirschhorn 1975). There are some reports in favor of the former hypothesis (Carson et al. 1977; Simmonds et al. 1978; Cohen et al. 1978).

Though the factors concerning response or non-response of RBC transfusion in ADA deficiency were unknown, failure in restoration of immune function in our patient might be concerned with the age and general condition at the time of the therapy. In view of no risk of graft versus host reaction, transfusion of ADA positive RBCs may be a choice for treatment of ADA deficiency so far as an appropriate histocompatible bone marrow is not available.

References


