Purine metabolic enzymes in lymphocytes. III. Effects of immunosuppressants on adenosine deaminase and purine nucleoside phosphorylase activities.

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SUMMARY
Mice were treated with a single injection of 6-mercaptopurine riboside (6MP-R), predonine or cyclophosphamide (CY), and the effects of these immunosuppressants on blastogenic responses to phytohemagglutinin P (PHA-P) or bacterial lipopolysaccharide (LPS) and on adenosine deaminase (ADA) or purine nucleoside phosphorylase (PNP) activities were studied with spleen lymphocytes. The retardation of blastogenic responses to both PHA-P and LPS were associated with the retardation of both intracellular ADA and PNP activities in 6MP-R-treated mouse spleen lymphocytes. Both PHA and LPS responses were suppressed in lymphocytes from mice treated with predonine, whereas no suppression of both ADA and PNP activities was observed. In mice treated with CY, LPS response of spleen lymphocytes was markedly suppressed but PHA response was almost the same with that of normal lymphocytes. PNP activity was retarded in lymphocytes of CY-treated mice, while ADA activity of them was rather enhanced. These results
suggest that the suppression with 6MP-R may be corresponding to the inherited immunodeficiency with enzyme deficiency, and that the suppression with predonine may be corresponding to immunodeficiency which is not associated with enzyme deficiency.

INTRODUCTION

There have been many reports on adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) deficiency associated with combined immunodeficiency (2, 3, 9, 10, 13, 16). The enzyme deficiency has been found in about one third to one half of patients with autosomal recessive combined immunodeficiency (4, 10). These observations suggest that these purine metabolic enzymes, ADA and PNP, are closely related to the immune responsiveness.

We reported previously the high ADA and PNP activities in B lymphocytes, and high ADA and low PNP activities in T lymphocytes collected from normal mouse spleen (6). We also reported the increase in ADA and PNP activities in T-cell responses and the increase in PNP activity in B-cell responses (7).

In a present paper, we deal with the effects of immunosuppressants such as cyclophosphamide, predonine and 6-mercaptopurine riboside on ADA and PNP activities in spleen lymphocytes and on in vitro blastogenic responses to phytohemagglutinin P and bacterial lipopolysaccharide.

MATERIALS AND METHODS
Animals. Both sexes of ICR/JCL mice were raised by the Central Animal Laboratory of Gunma University and used at age of 8-12 weeks old.

Immunosuppressant treatment. Cyclophosphamide (CY),
6-mercaptopurine riboside (6MP-R) and predonine were used as immunosuppressants. These immunosuppressants were dissolved in 0.85% NaCl solution at appropriate concentrations. Mice were subcutaneously treated with a single injection of immunosuppressant 4 days prior to the sacrification.

Preparation of crude enzyme extract. Lymphocytes were collected from spleens of mice and crude enzyme extract was prepared from the lymphocytes by the method described previously (6).

Determination of enzyme activities. ADA and PNP activities in the crude enzyme extract were determined and the enzyme units in $10^7$ of lymphocytes were calculated as described previously (6). The stimulation index (SI) was calculated as follows;

$$SI = \frac{\text{Units of enzyme activity in experimental group}}{\text{Units of enzyme activity in control group}}$$

In vitro stimulation of lymphocytes with phytohemagglutinin P or bacterial lipopolysaccharide. Lymphocytes were collected from spleens of mice and suspended in culture medium at a concentration of $1 \times 10^6$ cells/ml. One tenth milliliter of the cell suspension was placed in a well of a microculture plate and 0.01 ml of phytohemagglutinin P (PHA-P, final 20 μg/ml) or lipopolysaccharide of Escherichia coli (LPS, final 50 μg/ml) was added. The cells were cultured at 37°C for 3 days and labelled with 0.05 μCi of methyl[3H]-thymidine (2 Ci/mM, New England Nuclear Corp., Boston, Mass., USA) for the last 24 hours. The cells were harvested after 3 days' incubation on a glass filter and dpm in the cells was counted with a scintillation counter. Stimulation index (SI) was determined as follows;
SI = \frac{\text{dpm in stimulated group - background dpm}}{\text{dpm in nonstimulated group - background dpm}}.

PHA-P and LPS were purchased from Difco Laboratories, Detroit, Mich., U.S.A.

Culture medium. Lymphocytes were cultured in RPMI 1640 medium (Nissui Co., Ltd., Tokyo) supplemented with 10% fetal calf serum (Microbiological Associates, Bethesda, Md., USA), and 50 μg/ml of streptomycin and 50 units/ml of penicillin G.

RESULTS

Effect of 6MP-R on blastogenic responses and the enzyme activities.

ICR mice were subcutaneously treated with a single injection of 100, 50 or 25 μg per mouse of 6MP-R. The spleen lymphocytes were collected from mice 4 days after 6MP-R treatment. The results are presented in Fig. 1. As shown in Fig. 1a, both responses to PHA-P and LPS were markedly retarded in spleen lymphocytes of 6MP-R-treated mice. As shown in Fig. 1b, both ADA and PNP activities were also retarded in lymphocytes of mice treated with 6MP-R. No or a little decrease in lymphocytes number was observed in a spleen of each mouse by 6MP-R treatment. These results suggest that both T and B lymphocyte responses were inhibited by 6MP-R through the inhibition of intracellular purine metabolism in both T and B lymphocytes.

Effect of predonine on blastogenic responses and the enzyme activities.

Mice were subcutaneously treated with a single injection of 25, 12.5 or 6.3 μg per mouse of predonine 4 days prior to the sacrifice. Blastogenic responses and enzyme activities were
determined with spleen lymphocytes of predonine-treated mice and compared with those of normal mice. As shown in Fig. 2a, both PHA and LPS responses were declined in spleen lymphocytes collected from predonine-treated mice. However, as shown in Fig. 2b, ADA and PNP activities in lymphocytes collected from predonine-treated mice were almost the same with those in lymphocytes from normal mice. The number of lymphocytes collectable from each spleen was decreased by the treatment with predonine. These results suggest that predonine is cytotoxic to both T and B lymphocytes but does not inhibit intracellular purine metabolism.

Effect of cyclophosphamide on blastogenic responses and enzyme activities.

Mice were subcutaneously treated with a single injection of 2.5, 1.3 or 0.6 mg of cyclophosphamide (CY). Blastogenic responses and enzyme activities of spleen lymphocytes were determined 4 days after CY treatment. As shown in Fig. 3a, LPS response was markedly retarded in spleen lymphocytes of CY-treated mice, whereas no inhibition of PHA response was observed in CY-treated mice. Fig. 3b represents the results of enzyme activities in CY-treated mouse spleen lymphocytes. PNP activity decreased in lymphocytes of CY-treated mice but ADA activity rather increased. The number of spleen lymphocytes collectable from each mouse spleen decreased by about one half of normal mouse. These results suggest that CY is more toxic to B lymphocytes than T lymphocytes and CY rather stimulates T lymphocytes.
DISCUSSION

ADA and PNP are thought to be closely related to the immune responses because many workers have been found ADA or PNP deficiency associated with immunodeficiency (2, 3, 9, 10, 13, 16). We reported in a previous paper that both ADA and PNP activities distributed in both T and B lymphocytes; high ADA and PNP activities in B lymphocytes, and high ADA and low PNP activities in T lymphocytes of mouse spleen (6). We reported also that the enhancement of both ADA and PNP activities might be associated with the development of T-cell responses and that the enhancement of PNP activity with B-cell responses (7).

ADA deficiency was found in about one third to one half cases of patients with autosomally recessive combined immunodeficiency (4, 10). It has been reported that purine analogs such as azathioprine or 6-mercaptopurine had low cytotoxicity against both T and B lymphocytes but had immunosuppressive activity through intracellular events on metabolism (5, 11). Our results with 6MP-R agree with these reports and suggest that inherited immunodeficiency with the enzyme deficiency may resemble to the secondary immunodeficiency with purine analogs.

Corticosteroid has cytotoxic activity to whole lymphocytes except some steroid-resistant lymphocytes and decreases the number of both T and B lymphocytes in a whole body (1, 15). The decrease in the number of T and B lymphocytes is a main reason of immunosuppression with corticosteroid. Our present study, however, suggest that PHA and LPS responses of survived lymphocytes were also suppressed with predonine treatment, although the intracellular ADA
and PNP activities were almost same with those of normal lymphocytes. We think three possibilities for the reason of this discrepancy between enzyme activity and blastogenic responsiveness; 1) steroid-resistant cells are cells which can not response to PHA and LPS, 2) the enhancement of enzyme activities necessary to induce blastogenic response are suppressed by intracellular events with steroid, and 3) PHA or LPS can not get into the cells of which membrane is modulated with steroid. About two third to one half cases of patients with combined immunodeficiency have normal ADA and PNP activities. Our results suggest that the secondary immunodeficiency with corticosteroid may be resemble to the inherited immunodeficiency which is not associated with enzyme deficiency.

In Cy-treated mice, we observed in a present study the slight increase of ADA activity and the decrease of PNP activity in spleen lymphocytes, suggesting that CY treatment induce proportional increase of T-cells which have high ADA and low PNP activities, and that the survived cells are not influenced on intracellular enzyme activity and PHA response. These results agree with other many worker's results about the effects of CY on lymphocyte populations and responsiveness (8, 12, 14). They reported the selective depletion of B-cells and proportional increase of T-cells in lymphoid tissues of CY-treated animals.

REFERENCES


Fig. 1. Effect of 6MP-R on blastogenic response and enzyme activity. Mice were subcutaneously treated with a single injection of 6MP-R. Spleen lymphocytes were collected 4 days after 6MP-R treatment. Blastogenic response to PHA-P (la,●) or LPS (la,○), and ADA (lb,●) or PNP (lb,○) activity were determined.

Fig. 2. Effect of predonine on blastogenic response and enzyme activity. Mice were subcutaneously treated with a single injection of predonine. Spleen lymphocytes were collected 4 days after predonine treatment. Response to PHA-P (2a,●) or LPS (2a,○), and ADA (2b,●) or PNP (2b,○) activity were determined.
Fig. 3. Effect of CY on blastogenic response and enzyme activity.
Mice were treated subcutaneously with a single injection of CY.
Spleen lymphocytes were collected 4 days after CY treatment. Response
to PHA-P (3a,•) or LPS (3a,O), and ADA (3b,O) or PNP (3b,●) activity
were determined.