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Immunology

Animal model for immune dysfunction associated with adenosine deaminase deficiency

(2′-deoxycoformycin/combined immunodeficiency/purine interconversions/lymphocytotoxicity)

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ABSTRACT **An *in vivo* murine model for immunodeficiency of both B and T cells is produced by continuous intraperitoneal infusion of 2′-deoxycoformycin (DCF), a specific tightly binding inhibitor of adenosine deaminase (ADase; adenosine aminohydrolase, EC 3.5.4.4). After DCF infusion, ADase of thymus, spleen, and lymph nodes was inhibited to varying degrees ranging from 57% to 100%. Immunodeficiency under these conditions was indicated by: (*i*) a striking decrease in lymphocyte response to the T-cell mitogens concanavalin A and phytohemagglutinin and the B-cell mitogen *Escherichia coli* lipopolysaccharide; (*ii*) an impairment of delayed hypersensitivity measured by the footpad reaction; (*iii*) a decrease in antibody production measured in both *in vivo* and *in vitro* plaque-forming cell assays; (*iv*) a significant prolongation of mouse skin allograft survival after transplantation into the C57BL/6J (*H-2*^{b}) strain of skin from BALB/c (*H-2*^{d}) mice; and (*v*) a marked lymphopenia. Histological examination indicated lymphoid degeneration in the thymus, lymph nodes, and spleen with no alterations in other tissues including bone marrow, kidney, lung, gastrointestinal tract, and liver except for the occurrence of hepatitis. A decrease in the number of Thy-1-positive cells in both spleen and lymph nodes further supported the fact of cytotoxicity of DCF to T cells. Anorexia and weight loss were observed within 5 days of continuous DCF infusion at 0.4 mg/kg body weight per day. These data indicate that this method provides an experimental model for future studies on the biochemical mechanisms responsible for the genetically determined severe combined immunodeficiency disease in man.**

The metabolism of the naturally occurring purine nucleosides adenosine and 2′-deoxyadenosine is of special importance to proper lymphocyte function and differentiation. This relationship is particularly emphasized by reports of children with severe combined immunodeficiency disease (SCID) who also have an inherited deficiency of the enzyme adenosine deaminase (ADase; adenosine aminohydrolase, EC 3.5.4.4) in all tissues (1, 2). SCID is characterized by defects in both humoral and cellular immune functions, which result in death due to infection unless bone marrow is transplanted from a histocompatible donor. A causal relationship between the enzyme defect and the syndrome is supported by the facts that (*i*) ADase deficiency and SCID are individually rare occurrences, which renders it unlikely that their appearance together is fortuitous; (*ii*) cytostatic and cytotoxic effects of low concentrations of adenosine and 2′-deoxyadenosine on lymphoid cells in culture generally are potentiated by ADase inhibitors (3–5); and (*iii*) an inherited deficiency of another enzyme in the pathway of adenosine metabolism, purine nucleoside phosphorylase, is also associated with immunodeficiency (6). Chromosomal mapping studies argue strongly against a genetic deletion affecting ADase and immune response genes (7, 8).

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Considerable controversy exists concerning the biochemical mechanism that causes an ADase deficiency to be specifically lymphocytotoxic. Proposals include inhibition of ribonucleoside diphosphate reductase (a critical enzyme in DNA biosynthesis) by increased levels of 2′-deoxyATP (9, 10), pyrimidine starvation produced by increased adenine nucleotides (4), and increased levels of cyclic 3′,5′-AMP, a potent antiproliferative agent (11). An animal model for immunodeficiency associated with ADase deficiency would be of great value for distinguishing among these possibilities.

2′-Deoxycoformycin (DCF), a microbial fermentation product (12), is an extremely tightly binding stoichiometric inhibitor of ADase (13). Histopathological examination of mice receiving a single injection of DCF has indicated a toxicity highly specific to the lymphoid system (14), and immunosuppressive activity of DCF in a tumor test system has been reported (15). Previous studies in our laboratory (16) have demonstrated that continuous low-dosage infusion of DCF into mice produces a high degree of inhibition of ADase, the extent of which is dependent on the mouse strain and the presence of a tumor. This method of administration is particularly advantageous because of a tissue-dependent recovery of ADase that occurs in response to single injections (17–20).

We report here evidence that continuous infusion of DCF into mice results in an immunodeficiency of both B and T cells. These data demonstrate that inhibition of ADase without the addition of adenosine or its analogs is sufficient to produce severe immune dysfunction.

MATERIALS AND METHODS

Materials. C57BL/6J (*H-2*^{b}) and BALB/c (*H-2*^{d}) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). DCF was a generous gift from the National Cancer Institute through the assistance of J. Douros. Phytohemagglutinin P and *Escherichia coli* lipopolysaccharides were purchased from Difco, and concanavalin A was from Calbiochem (San Diego, CA). RPMI-1640 medium, Dulbecco's phosphate-buffered saline (P_i/NaCl), Hanks' balanced salt solution, and minimal essential medium were obtained from GIBCO. Fetal calf serum was purchased from Microbiological Associates (Walkersville, MD), and anti-Thy I.2 antiserum was from Litton Bionetics (Bethesda, MD). Bovine serum albumin was obtained from Sigma. Terramycin soluble powder was supplied by Pfizer (New York).

Abbreviations: ADase, adenosine deaminase; SCID, severe combined immunodeficiency disease; DCF, 2′-deoxycoformycin, (*R*)-[3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol]; SRBC, sheep erythrocytes; P_i/NaCl phosphate-buffered saline.

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Adenosine was purchased from Schwarz/Mann (Orangeburg, NY). [8-^{14}C]Adenosine (water/ethanol solution; 54.6 Ci/mol; 1 Ci = 3.7 × 10^{10} becquerels) and [*methyl*-^{3}H]thymidine (aqueous solution; 60 Ci/mol) were supplied by New England Nuclear (Boston, MA). Thin-layer cellulose plates were obtained from Eastman Kodak (Rochester, NY). Histopathological evaluation was performed in the Department of Pathology at Memorial Hospital in collaboration with Stephen S. Sternberg.

Drug Infusion. Male mice (18–23 g) maintained on Purina rat chow and tap water supplemented with terramycin were infused intraperitoneally at a constant rate as described (16). DCF dissolved in 0.9% NaCl was infused at 0.4 mg/kg body weight per day for 5 days or 0.16 mg/kg body weight per day when a longer treatment was required in studies of delayed hypersensitivity, *in vivo* antibody formation, and skin graft rejection. A decrease in dose was required in the latter tests because survival times at the higher doses were 6–8 days after start of the infusion. Control mice simultaneously infused with 0.9% NaCl were routinely included.

Cell Preparation. Animals were sacrificed by decapitation, and lymphoid tissues (spleen, thymus, and lymph nodes) were excised aseptically, minced, and gently passed through a fine stainless steel sieve into P_i/NaCl. Cell aggregates were disrupted by aspiration with a Pasteur pipette and the remaining cell clumps and debris were removed by passage through the sieve. Cells were washed three times with P_i/NaCl at 4°C. Cell viability was 85–90% by the trypan blue exclusion test. Erythrocytes were separated from heparinized blood by centrifugation at 5000 × *g* followed by three washes with P_i/NaCl at 4°C.

Mitogen Response. Mitogen-induced blastogenesis was determined by incorporation of [^{3}H]thymidine into DNA. Mononuclear cells were cultured in Falcon 3040 microtiter plates at a density of 5 × 10^{6} cells per ml in 0.2 ml of RPMI-1640 medium supplemented with 5% fetal calf serum and penicillin (100 units/ml) for 72 hr at 37°C in a humidified atmosphere of 5% carbon dioxide in air. The mitogens concanavalin A, phytohemagglutinin, and *E. coli* lipopolysaccharides were present at optimal concentrations of 1.25, 12.5, and 50.0 μg/ml, respectively. [^{3}H]Thymidine (1 μCi) was present during the last 4 hr of the incubation. After the cells were collected with a microharvester, they were washed several times with distilled water. The labeled DNA was precipitated with 5% trichloroacetic acid and assayed by liquid scintillation.

Delayed Hypersensitivity. C57BL/6J mice were sensitized by injection of 10^{7} sheep erythrocytes (SRBC) suspended in 0.9% NaCl into the right footpad on the second day of a 7-day infusion of DCF. The mice were challenged 4 days later with 10^{8} SRBC red blood cells in 0.05 ml of 0.9% NaCl injected into the left footpad. Footpad thickness was measured before challenge and 24 hr later. The percentage increase over the initial thickness represents a measure of the delayed hypersensitivity reaction (19).

Cytotoxicity. Splenocytes (2.5 × 10^{5}) depleted of erythrocytes were incubated with 0.05 ml of anti-Thy 1.2 serum for 30 min at 4°C. A control was incubated under the same conditions in the absence of the antiserum. The cells were washed once with P_i/NaCl, resuspended in 0.1 ml of a 1:20 dilution of rabbit complement, and incubated for an additional 30 min at 37°C. Viability was determined by trypan blue exclusion.

Plaque-Forming Cell (PFC) Assay. For the *in vitro* PFC assay a modification of the procedure of Mishell and Dutton (20) was used. Splenocytes (5 × 10^{6}) were cultured with 5 × 10^{6} SRBC in 2 ml of RPMI-1640 medium supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (0.1 mg/ml), and mercaptoethanol (0.05 mM) in a carbon dioxide incubator for 5 days. Harvested cells were washed once with minimal essential medium. The direct anti-SRBC PFC assay was performed essentially as described by Jerne and Nordin (21). For the *in vivo* PFC assay, 4 × 10^{8} SRBC in 0.2 ml of P_i/NaCl were injected intraperitoneally on the second day of a continuous infusion of DCF (0.16 mg/kg body weight per day) or 0.9% NaCl. The animals were sacrificed 5 days later, the spleens were removed aseptically, and the PFC assay was performed.

Skin Grafting. Split-thickness skin grafts obtained from the back of a BALB/c (*H-2^{d}*) male mouse were scraped free of all fat and blood vessels and placed in sterile Hanks' balanced salt solution. The skin was cut into small squares and placed on the right flanks of C57BL/6J (*H-2^{b}*) mice (under anesthesia) from which only the epidermal layer had been removed. Bandages were removed 8 days later. The signs of rejection were determined as described by Billingham (22).

ADase Activity. Cells suspended in P_i/NaCl were lysed by six cycles of freezing and thawing; erythrocytes were hemolyzed with 3 vol of 5 mM imidazole·HCl (pH 7.0). Supernatants were obtained by centrifugation at 2000 × *g* for 10 min. Enzyme activity was determined by a radioactive assay using [8-^{14}C]adenosine as described (23). One unit of activity is defined as the amount of enzyme that deaminates 1 μmol of substrate per min under the specified steady-state conditions. For determination of specific activity, protein was quantified by the method of Lowry *et al.* (24) with bovine serum albumin as standard.

RESULTS

ADase Levels. The effects of DCF infusion on specific ADase activity in lymphoid cells and erythrocytes of C57BL/6J mice are summarized in Table 1. Assays were performed on undialyzed crude lysates of cells and thus should be representative of intracellular enzyme levels. Control ADase values in lymphoid cells ranged over 2 orders of magnitude, from 0.0045 unit/mg of protein for lymph nodes to 0.91 for thymus. Infusion of DCF resulted in significant activity inhibition in each of the tissues examined. The presence of residual ADase after DCF infusion has also been observed in nonlymphoid tissues in previous studies in our laboratory (16). It is notable that the degree of inhibition of ADase for lymphoid tissues generally paralleled the order of specific activity—thymus > spleen > lymph nodes—suggesting a role for the intracellular enzyme concentration in determining the extent of inhibition. *In vitro* experiments, however, demonstrated that the ADase in crude lysates of these cells could be inhibited more than 90% by a molar excess of DCF. Thus, the variation in tissue response well may be related to differences in rates of drug penetration combined with varying rates of cellular proliferation or protein synthesis.

Table 1. Effect of DCF infusion on ADase activity in C57BL/6J mouse lymphoid organs and erythrocytes

Tissue	ADase, units × 10^{3}/mg protein: Saline-infused	DCF-infused	% depression*
Thymus	191.0	0.7	>99
Spleen	62.0	11.1	82
Lymph nodes	7.0	2.6	63
Erythrocytes	2.6	0.05	98

Mice were infused for 5 days with 0.9% NaCl or DCF (0.4 mg/kg body weight per day). Each value is the mean of duplicate determinations which generally agreed to within 5%, on cells pooled from three animals.

* Percentage of original activity that was inhibited by DCF treatment.

Table 2. Mitogen-induced transformation of lymphocytes from DCF-infused C57BL mice

	[^{3}H]Thymidine incorporation, cpm*					Stimulation index†		
	Background‡	Con A	PHA	Background§	LPS	Con A	PHA	LPS
Saline-infused:								
Spleen	2574 ± 458	51,099 ± 1,494	16,147 ± 5,013	1671 ± 100	17,809 ± 5,058	19.9	6.3	10.7
Lymph nodes	1040 ± 236	52,869 ± 1,503	86,452 ± 1,963	ND	ND	50.8	83.1	ND
Thymus	1021 ± 206	23,216 ± 1,281	ND	ND	ND	22.7	ND	ND
DCF-infused:								
Spleen	963 ± 154	4,493 ± 958	3,202 ± 713	1061 ± 32	2,089 ± 210	4.7	4.0	2.0
Lymph nodes	1121 ± 103	1,378 ± 461	2,853 ± 402	ND	ND	1.2	2.5	ND
Thymus	478 ± 35	3,532 ± 388	ND	ND	ND	7.4	ND	ND

Mitogen stimulation was determined by incorporation of [^{3}H]thymidine into DNA at optimal mitogen concentrations. "Saline-infused" and "DCF-infused" designate treatment with 0.9% NaCl and DCF (0.4 mg/kg body weight per day), respectively, for 5 days. ND, not done; Con A, concanavalin A; PHA, phytohemagglutinin; LPS, *E. coli* lipopolysaccharide.

* Mean ± SD of triplicate determinations on tissues pooled from three mice.

† Stimulation index is defined as [^{3}H]thymidine incorporation into DNA in the presence of mitogen divided by the incorporation with no mitogen present.

‡ Background for both Con A and PHA stimulation.

§ Background for LPS stimulation.

Erythrocyte ADase consistently was inhibited >90% after a 5-day infusion (Table 1). This result, which has also been noted after a single injection of DCF (18) and in *in vitro* experiments with human erythrocytes (25), is reasonable in view of the lack of protein synthesis in these cells and their relatively long lifespans.

Effects on Immune Function. Lymphocyte blastogenesis induced by mitogens is generally considered to reflect the function of immunocompetent cells. We therefore examined the effect of DCF infusion on the stimulation of lymphocytes by the T-cell mitogens concanavalin A and phytohemagglutinin and the B-cell mitogen *E. coli* lipopolysaccharide. The response to the T-cell mitogens was markedly depressed in each tissue (Table 2). The most dramatic effect was observed in the lymph nodes, in which the large stimulation of control cells was virtually abolished after DCF treatment. The decrease in response to lipopolysaccharide indicated an impairment in B-cell function as well. It is notable that in most cases a low level of stimulation by mitogens was still observed after DCF infusion. Whether this result reflects a subpopulation of cells that contain the residual ADase (Table 1) is yet to be established. Because control tissues were obtained from animals infused with physiological saline for 5 days, these effects can be attributed to DCF and not to the stress associated with the infusion. It is also notable that background values, representing incorporation of radioactive thymidine in the absence of mitogen, were also generally depressed after DCF infusion in all tissues except lymph nodes (Table 2).

Additional evidence for suppression of T-cell function is the effect of DCF infusion on delayed hypersensitivity reactions (Table 3). Sensitized mice infused with physiological saline had a 47% increase in footpad thickness 24 hr after intradermal challenge with SRBC. In contrast, mice infused with DCF showed essentially no change in footpad thickness after the challenge. Because the footpad size could be measured with a precision of ±0.1 mm, this difference is highly significant ($P < 0.001$) and clearly supports a striking decrease in T-cell effector function.

The length of survival of skin allografts across major histocompatibility barriers was also used to document depression of cell-mediated immunity (Table 4). Split-thickness skin from the backs of BALB/c ($H\text{-}2^d$) mice was grafted to the right flanks of C57BL ($H\text{-}2^b$) mice and the survival time was monitored (22). Because the survival time of grafts to control animals was ≈9.2 days, it was not feasible to use DCF infusion at 0.4 mg/kg body weight per day during the entire period of skin graft challenge. We therefore administered DCF by single intraperitoneal injection in a dosage of 0.1 mg/kg body weight either daily or three times a week. Prolongation of the time of rejection over control animals was observed in the DCF-treated group, representing increases of 30.4% and 19.6% for the mice injected daily and three times a week, respectively. These increases were statistically significant ($P < 0.025$).

For further prolongation of survival time, a modified infusion procedure was developed that consisted of repetitive cycles of a 5-day infusion of DCF (0.08 mg/kg body weight per day) followed by 4–6 days of infusion of 5% dextrose. One animal to which this protocol was applied had a 25-day graft survival time, representing a prolongation of 150%. This survival time is about double that observed with any other animal from either the DCF or control groups. These data imply that, for optimal

Table 3. Effect of DCF infusion on delayed hypersensitivity reaction in C57/BL mice

Infusion	Footpad thickness, mm* Day 6	Day 7	% increase
Saline	1.7 ± 0.1	2.5 ± 0.1	47
DCF	1.8 ± 0.1	1.8 ± 0.1	0†

Delayed hypersensitivity was measured by the increase in footpad size 24 hr after injection of SRBC into previously sensitized mice. SRBC injection into unimmunized mice did not cause footpad swelling. Footpad size was measured on the sixth and seventh days of infusion. DCF was infused at a rate of 0.16 mg/kg body weight per day.

* Mean ± SD of determinations on three mice.

† For the difference after DCF infusion, $P < 0.001$.

Table 4. Effect of DCF administration on skin allograft survival

Treatment	Survival, days*	% prolongation of survival
Control	9.2 ± 0.8	—
Single DCF injection		
Three times/week	11.0 ± 0.8	19.6
Daily	12.0 ± 1.9	30.4

DCF was administered to C57BL/6J ($H\text{-}2^b$) mice by single intraperitoneal injection of 2.5 μg in 0.2 ml of 0.9% NaCl (0.1 mg of DCF per kg body weight) either three times a week at equal intervals or on a daily basis.

* Mean ± SD of five animals. For increases in prolongation time after DCF, $P < 0.025$.

Table 5. Effect of DCF infusion on antibody formation from mouse C57BL/6J spleen lymphocytes

Method of sensitization	Plaque-forming cells, no.*	
	Saline-infused	DCF-infused
In vitro†	1,608 ± 216	368 ± 143
In vivo‡	23,039 ± 14,507	2167 ± 1244

Spleen cells were sensitized either *in vitro* by preincubation with SRBC or *in vivo* by direct intraperitoneal injection of SRBC. Antibody production was determined by counting the number of plaque-forming cells. Plaques formed by control splenocytes preincubated in the absence of SRBC have been subtracted from the data. The rate of DCF infusion was 0.40 and 0.16 mg/kg body weight per day for the *in vitro* and *in vivo* procedures, respectively.

* The decreases in mean values after DCF infusion were statistically significant ($P < 0.05$ and $P < 0.1$ for *in vitro* and *in vivo* data, respectively).

† Mean ± SD of five determinations on cells pooled from three animals, expressed as number of plaques per 1×10^7 splenocytes.

‡ Mean ± SD of three determinations on individual spleens, expressed as number of plaques per spleen.

immunosuppression, DCF should be infused continuously at low levels until signs of toxicity are apparent. The results from both methods of administration support the view that DCF suppresses cell-mediated immune responses.

The effect of DCF infusion on antibody production was characterized by using both *in vivo* and *in vitro* plaque-forming assays (21). The longer period of infusion required for the *in vivo* procedure again necessitated lowering the dose of DCF from 0.4 to 0.16 mg/kg body weight per day. In both cases the number of antibody-producing cells was significantly depressed (Table 5). The depression noted in the *in vivo* assay, in which the data are expressed as the number of plaques per spleen, is consistent with the loss of spleen weight after DCF treatment (see below). However, the degree of reduction of antibody formation in both assays is greater than can be accounted for by a reduction in the number of B cells. Whether these results reflect a direct interference of DCF with antibody synthesis and/or antigen processing, a suppression of T helper cell function, or both remains to be determined.

Toxicity and Histopathology. Mean (±SD) mouse weights changed from 20.1 ± 0.9 g to 15.2 ± 1.0 g after a 5-day infusion of DCF, a decrease of ≈25%; negligible weight change was observed in control animals infused with 0.9% NaCl. A noticeable loss of appetite was observed in the DCF-infused group. Mean splenic weights for a 20-g mouse changed from 0.102 ± 0.011 to 0.036 ± 0.004, a decrease of about 65%. In comparison, a 28% decrease in splenic weight and a 35% decrease in mean mouse weight have been reported after a single high-dose injection of DCF (14). The weight of the thymus was also decreased dramatically after DCF infusion; however, its small size precluded accurate weight determination. The weights of other tissues were not significantly affected by DCF infusion. Morphological evidence of hepatitis was also observed in the group treated with DCF.

Table 6. Analysis of peripheral blood elements

	Normal	DCF-infused
Leukocytes, no. $\times 10^{-3}/mm^3$	12.1 ± 2.5	6.7 ± 0.6
Differential count, %		
Neutrophils	15.9 ± 2.8	79.7 ± 1.2
Monocytes	2.0 ± 1.0	6.7 ± 3.1
Lymphocytes	83.0 ± 2.5	13.7 ± 3.1
Erythrocytes, no. $\times 10^{-6}/mm^3$	8.2 ± 1.1	9.7 ± 0.5
Hemoglobin, g/dl	13.8 ± 0.8	17.5 ± 2.4
Hemotocrit, %	42.8 ± 6.4	52.2 ± 6.6
Mean corpuscular volume, μm^3	52.5 ± 0.7	53.7 ± 1.5
Mean corpuscular hemoglobin:		
Amount, pg	16.9 ± 1.4	16.9 ± 0.4
Concentration, g/dl	32.9 ± 3.8	33.1 ± 1.3

Heparinized blood was obtained by orbital bleeding. Results are shown as mean ± SD of determinations from three C57BL/6J mice. Normal values were similar to those obtained from blood of saline-infused animals.

Table 7. Cytotoxicity of DCF infusion in C57BL/6J mouse T lymphocytes

	Cytotoxic index (Thy-1$^+$ cells)*	
	Saline-infused	DCF-infused
Spleen	30 ± 5	20 ± 4
Lymph nodes	70 ± 7	46 ± 4

Saline- and DCF-infused mice were treated with 0.9% NaCl and DCF (0.4 mg/kg body weight per day), respectively. Further details are described in *Materials and Methods*. Results are shown as mean ± SD of six determinations of cells pooled from three animals.

* Cytotoxic index = [(% viable cells with complement alone − % viable cells with complement plus anti-Thy 1.2 serum) ÷ % viable cells with complement alone] × 100.

The effect of DCF infusion on blood elements is summarized in Table 6. The most striking effect was a marked lymphopenia. The differential lymphocyte count decreased from 83.0% to 13.7%, and the leukocyte count decreased from 12.1×10^3 to 6.7×10^3 cells per mm^3. In contrast, erythropoiesis was completely normal in the DCF-infused group, and there was no evidence of anemia of any kind.

Histopathological examination after DCF infusion further supported a specific lymphocytotoxicity. Thymus, lymph nodes, and spleen were characterized by marked, diffuse lymphoid degeneration and necrosis. Toxicity to both B and T cells was apparent in the spleen. Other tissues examined (bone marrow, lung, liver, kidney, and gastrointestinal tract) appeared to be normal except for hepatitis. In addition, preliminary experiments indicate that DCF infusion does not affect the number of spleen colony-forming units produced from bone marrow stem cells.

As further evidence for lymphocytotoxicity, there was a significant decrease in Thy-1-positive cells in both tissues after DCF infusion compared to both control and saline-infused mice (Table 7). It is notable, however, that the percentage change was not as dramatic as was observed in tests of lymphocyte function [e.g., responses to mitogens that stimulate T cells (Table 2) or delayed hypersensitivity (Table 3)]. The loss in Thy-1-positive cells is consistent with the diminished spleen weight and evidence of lymphoid degeneration noted histologically.

DISCUSSION

The description in 1972 of three SCID patients with an inherited deficiency of ADase was the first report of an enzyme defect associated with an immunodeficiency disease (1, 2). The experimental model described here, of immunodeficiency following infusion of an inhibitor of ADase, provides further evidence for a causal relationship between the aberration in adenosine metabolism and the immunodeficiency in this syndrome. Although SCID is heterogeneous in its clinical manifestations and, most probably, in the metabolic abnormalities basic to the disease as well, several features of our murine model have also been described in SCID patients. These include: (*i*) defects in both B- and T-cell function, as evidenced by depressions in both B- and T-cell mitogen responses, antibody production, and delayed hypersensitivity; (*ii*) defective T-cell development, as evidenced by severe thymic and splenic hy-

poplasia and by the decreased number of T cells in lymph nodes and spleen; (*iii*) severe lymphopenia; and (*iv*) propensity toward the development of infection. The high specificity and tightness of the interaction of DCF with ADase (13) strongly support the idea that this enzyme is the pharmacological target of DCF.

These results are consistent with previous *in vivo* studies indicating that single injections of ADase inhibitors can suppress certain immune responses (15, 26) and are specifically lymphocytotoxic (14). In the latter study, however, lymphopenia was not produced by DCF treatment and the degree of splenic hypoplasia was significantly less than that observed here. No further observations on changes in thymic weight were reported. The extensive immunosuppression and lymphocytotoxicity noted in our studies may be a consequence of the method of DCF administration—i.e., continuous intraperitoneal infusion over a prolonged period. This conclusion is consistent with previous data from our laboratory implying that ADase in certain tissues may be inducible in response to the presence of an inhibitor (16, 27) as well as with reports of a recovery of ADase level after a single injection of DCF (17, 18). This regeneration of enzyme activity, which may occur by synthesis of new protein or turnover of cells, is aided by the rapid clearance of DCF from the plasma (17, 28).

Of special interest are the studies by Burridge *et al.* (29) who reported that single daily injections of DCF into mice did not affect either thymocyte response to concanavalin A or splenocyte response in mixed lymphocyte cultures. Although ADase in crude lysates was found to be substantially inhibited, a greater amount of residual intracellular ADase may exist *in vivo* under these conditions of administration compared to continuous infusion. Regardless of the mechanism involved, however, a low, nontoxic level of DCF constantly present in the circulation may be needed to achieve maximal immunosuppression.

Several laboratories have reported attempts to simulate immune dysfunction *in vitro* by incubation of peripheral blood lymphocytes with ADase inhibitors (3, 30–34). Although there is variability in the data, these studies are in general agreement that concentrations of the inhibitors that depress enzyme activity do not markedly affect the proliferative response to mitogens, whereas high concentrations of adenosine are strongly antiproliferative. The combination of adenosine and an ADase inhibitor in these systems, however, was shown to act synergistically in inhibiting mitogen-induced blastogenesis. The murine model described here differs significantly in that administration of an ADase inhibitor alone is sufficient to produce a profound immunosuppression. This experimental system seems to represent an accurate model of human SCID associated with ADase deficiency and should provide a means for analyzing the biochemical relationship between the ADase deficiency and the immunodeficiency with which it is associated.

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1. Dissing, B. & Knudsen, B. (1972) *Lancet* ii, 1316.
2. Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B. & Meuwissen, H. F. (1972) *Lancet* ii, 1067.
3. Snyder, F. F., Mendelsohn, J. & Seegmiller, J. E. (1976) *J. Clin. Invest.* **58**, 654–666.
4. Green, H. & Chan, T. S. (1973) *Science* **182**, 836–837.
5. Ishii, K. & Green, H. (1973) *J. Cell. Sci.* **13**, 429–439.
6. Giblett, E. R., Ammann, A. J., Sandman, R., Wara, D. W. & Diamond, L. K. (1975) *Lancet* i, 1013.
7. Creagen, R. P., Tischfield, J. A., Nichols, E. A. & Ruddle, F. H. (1973) *Lancet* ii, 1449.
8. Pearson, P. L. (1972) *Bull. Eur. Soc. Hum. Genet.* **54**, 8.
9. Cohen, A., Hirschhorn, R., Horowitz, S. D., Rubenstein, A., Polmar, S. H., Hong, R. & Martin, D. W. (1978) *Proc. Natl. Acad. Sci.* **75**, 472–476.
10. Coleman, M. S., Donofrio, J., Hutton, J. J., Hahn, L., Daoud, A., Lampkin, B. & Dyminiski, J. (1978) *J. Biol. Chem.* **253**, 1619–1626.
11. Wolberg, G., Zimmerman, T. P., Hiemstra, K., Winston, M. & Chu, L-C. (1975) *Science* **187**, 957–959.
12. Woo, P. W. K., Dion, H. W., Lang, S. M., Dahl, L. F. & Durham, L. J. (1974) *J. Heterocycl. Chem.* **11**, 641–643.
13. Agarwal, R. P., Spector, T. & Parks, R. E., Jr. (1977) *Biochem. Pharmacol.* **26**, 359–367.
14. Smyth, J. F., Young, R. C. & Young, D. M. (1978) *Cancer Chemother. Pharmacol.* **1**, 49–51.
15. Chassin, M. M., Chirigos, M. A., Johns, D. G. & Adamson, R. H. (1977) *N. Engl. J. Med.* **296**, 1232.
16. Tedde, A., Balis, M. E., Schonberg, R. & Trotta, P. P. (1979) *Cancer Res.* **39**, 3044–3050.
17. Chassin, M. M., Adamson, R. H., Zaharevitz, D. W. & Johns, D. G. (1979) *Biochem. Pharmacol.* **28**, 1849–1855.
18. Agarwal, R. P. (1979) *Cancer Res.* 39, 1425–1427.
19. Lagrange, P. H., Mackaness, G. B. & Miller, T. E. (1974) *J. Exp. Med.* **139**, 528–542.
20. Mishell, R. I. & Dutton, R. W. (1967) *J. Exp. Med.* **126**, 423–442.
21. Jerne, N. K. & Nordin, A. A. (1963) *Science* **140**, 405.
22. Billingham, R. E. (1961) in *Transplantation of Tissues and Cells*, eds. Billingham, R. E. & Silvers, W. R. (Wistar Inst., Philadelphia), pp. 19–23.
23. Trotta, P. P., Peterfreund, R. A., Schonberg, R. & Balis, M. E. (1979) *Biochemistry* **18**, 2953–2959.
24. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
25. Rogler-Brown, T., Agarwal, R. P. & Parks, R. E. Jr. (1978) *Biochem. Pharmacol.* **27**, 2289–2296.
26. Lum, C. T., Sutherland, D. E. R. & Najarian, J. S. (1977) *N. Engl. J. Med.* **296**, 819.
27. Trotta, P. P., Ahland, M. A., Brown, G. F. & Balis, M. E. (1978) *Mol. Pharmacol.* **14**, 199–209.
28. McConnell, W. R., Suling, W. J., Rice, L. S., Shannon, W. M. & Hill, D. L. (1978) *Cancer Treat. Rep.* **62**, 1153–1159.
29. Burridge, P. W., Paetkau, V. & Henderson, J. F. (1977) *J. Immunol.* **119**, 675–678.
30. Fox, I. H., Keystone, E. C., Gladman, D. D., Moore, M. & Cane, D. (1975) *Immunol. Commun.* **4**, 419–427.
31. Ballet, J-J., Insel, R., Merler, E. & Rosen, F. (1976) *J. Exp. Med.* **143**, 1271–1276.
32. Carson, D. A. & Seegmiller, J. E. (1976) *J. Clin. Invest.* **57**, 274–282.
33. Hovi, T., Smyth, J. F., Allison, A. C. & Williams, S. C. (1976) *Clin. Exp. Immunol.* **23**, 395–403.
34. Hirschhorn, R. & Sela, E. (1977) *Cell. Immunol.* **32**, 350–360.