Partial Purine Nucleoside Phosphorylase Deficiency: *STUDIES OF LYMPHOCYTE FUNCTION*

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STUDIES OF LYMPHOCYTE FUNCTION

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ABSTRACT Immune function in two brothers with a deficiency of purine nucleoside phosphorylase was evaluated in vivo and in vitro. Both patients had a history of recurrent infections and profound lymphopenia. Studies of cell-mediated immunity revealed an absence of delayed cutaneous reactivity to a number of antigens, including dinitrochlorobenzene, and significantly reduced lymphocyte proliferative responses to nonspecific mitogens, specific antigen, and allogeneic cells. E-rosetting cells were present but reduced in number (20.0% and 31.5%). Serum immunoglobulin levels, percentages of circulating immunoglobulin- and C3-receptor-bearing B cells, as well as the ability to produce antibody in response to specific antigen in vivo were normal. Moreover, studies of the in vitro induction of specific IgM antibody delineated the presence of T-helper and T-regulator cells. The normal induction of bone marrow precursor T-cell maturation by human thymic epithelium-conditioned medium or thymosin suggested that the initial stages of T-cell generation were intact in these patients. Attempts to reconstitute the in vitro proliferative response with a variety of reagents, including purine nucleoside phosphorylase itself, were unsuccessful. Selective impairment of certain aspects of T-cell function in these patients and a less severe clinical picture than previously described may be explained by the presence of a partial deficiency of nucleoside phosphorylase activity and incomplete block of purine catabolism.

INTRODUCTION

The inherited immune deficiency diseases encompass a heterogeneous group of disorders often presenting with life-threatening infections and abnormalities of thymus-dependent (T)[1] and thymus-independent (B) lymphocyte function. In the last few years, increased attention has been focused on the association of inborn errors of purine metabolism and immunodeficiency. Giblett et al. were the first to document the association of adenosine deaminase (ADA) deficiency and the combined immunodeficiency of T- and B-cell lines (1). This finding, which has been substantiated by several groups (2), has stimulated great interest because it was the first description of an enzyme deficiency leading to a defect of specific immunity. Although the biochemical events which result in immune dysfunction are unclear, these data indicated that abnormalities of purine catabolism may have profound effects on the acquisition and expression of normal immunity.

The importance of pathways of purine degradation in maintaining the integrity of the immune system has been further revealed by the recognition of a second enzyme abnormality, that of purine nucleoside phosphorylase (PNP). Absolute deficiency of this enzyme, which catalyzes the reversible phosphorylytic cleavage of purine nucleosides (other than adenosine) to their respective free purine bases, has also been linked to immune deficiency (3, 4). In contrast to ADA deficiency, PNP deficiency appears to be associated with a selective impairment of T-cell function and apparently normal humoral immunity. We have recently studied two brothers with partial PNP deficiency, a history of recurrent infections, and impaired cell-mediated immunity. In this paper, we describe the results of our

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[1] *Abbreviations used in this paper:* ADA, adenosine deaminase; ADC, antibody-dependent cytotoxicity; B, thymus-independent; CRBC, chicken red blood cells; HTCM, thymus epithelium-conditioned medium; MIC, mitogen-induced cytotoxicity; OA, ovalbumin; PFC, plaque-forming cell; PHA, phytohemagglutinin; PNP, purine nucleoside phosphorylase; SRBC, sheep red blood cells; T, thymus-dependent.

in vivo and in vitro studies of immune function in these patients, and in an accompanying manuscript,[2] we illustrate the metabolic consequences of this disorder which have been evaluated in studies of purine and pyrimidine biosynthesis.

CASE SUMMARIES

Case 1. D. B. was a 9-yr-old male at the time of study. He was well until 6 yr of age when, following a severe bout of chicken pox, he developed recurrent bacterial infections of both upper and lower respiratory tracts and otitis media. At the age of 8, he developed recurrent episodes of pain and swelling of hands and feet. Full immunization, including live virus administration (polio, rubella, measles), was carried out without incident, and growth and development were essentially normal. Cytomegalovirus has been isolated from his urine. On physical examination he was on the 25th percentile for weight and the 10th to 20th percentile for height. He demonstrated markedly carious teeth, scarring from varicella, purulent nasal discharge, and scarring of the right tympanic membrane. Lymphoid tissue was palpable throughout and tonsils were present. Radiological investigations revealed bronchial wall thickening, opaque paranasal sinuses, and a sclerotic right mastoid. Studies of granulocyte function, including bacterial killing and chemotaxis, and complement activity (C3, C4, total hemolytic) were normal.

Case 2. M. B. was a 10½-yr-old male at the time of study. He has suffered from recurrent ear, sinus, and pulmonary infections since the first months of life. He was fully immunized without incident, including rubella, polio, measles, and small-pox vaccines, by 1½ yr of age. At the age of 4 yr he had an episode of uncomplicated chicken pox. Since the age of 7, he has had repeated hospitalizations for recurrent pulmonary infections and progressive pulmonary insufficiency. At this time, funduscopic examination revealed bilateral chorioretinitis, and cytomegalovirus was and continues to be isolated from his urine. A lung biopsy revealed bronchiectasis, chronic inflammatory cells, and no cytomegalovirus. History of growth and development revealed slow linear growth with delayed milestones. On admission he was less than the 3d percentile for height and at the 10th percentile for weight. He had a chronic purulent nasal discharge, chronic otitis media, and pulmonary rales bilaterally. Bilateral retinal lesions were visualized. Lymphoid tissue was palpable throughout and tonsillar tissue was visualized. Studies of granulocyte function and complement activity were also normal.

[2] Edwards, N. L., E. W. Gelfand, W. D. Biggar, and I. H. Fox. Purine nucleoside phosphorylase deficiency: studies of purine and pyrimidine metabolism. *J. Lab. Clin. Med.* In press.

METHODS

All studies were carried out both on and off a 1,300-calorie, 50-g protein, purine-free diet. Since the results of studies carried out on or off this diet did not differ significantly, all data were pooled.

Cell-mediated immunity. Delayed cutaneous reactivity was assessed after intradermal skin testing with the following antigens: *Candida albicans* (100 protein nitrogen units, Hollister-Stier, Mississauga, Ontario), streptokinase-streptodornase (50 units, Lederle Laboratories, Pearl River, N. Y.), mumps (Connaught Laboratories, Toronto, Ontario), purified protein derivative, Dermatophytin (1/30, Hollister-Stier), and diphtheria/tetanus toxoid (1/10, Connaught Laboratories). Initial sensitization to dinitrochlorobenzene was carried out using a 5% (wt/vol) solution and retesting with up to a 1/100 concentration of the sensitizing dose. Allogeneic (third party) skin grafts were attempted in both patients but were technically impossible to maintain.

E-rosette formation and 3-day (lectin) and 6-day (specific antigen) lymphocyte stimulation studies were carried out as previously described (5, 6). Mixed lymphocyte culture reactivities were determined using a modification of the microculture technique of Hartzman et al. (7). For the in vitro studies, peripheral blood mononuclear cells were obtained by Isopaque-Ficoll centrifugation, and all cultures were performed in RPMI 1640 medium containing 10–15% heat-inactivated (56°C × 30 min) AB serum.

Humoral immunity. Serum levels of IgG, IgA, and IgM were estimated by radial immunodiffusion (Hyland Laboratory, Div. of Travenol Laboratories, Inc., Costa Mesa, Calif.) and IgE by radioimmunoassay (Phadebas, Pharmacia Fine Chemicals, Inc.). Tetanus, diphtheria, polio, cytomegalovirus, and toxoplasmosis antibody titers were determined by standard methods. Enumeration of C3-rosetting (EAC) and surface immunoglobulin (sIg)-bearing B lymphocytes were carried out as previously described (8, 9). In vitro induction of specific IgM antibody to the antigens ovalbumin (OA) and sheep red blood cells (SRBC) was evaluated using a microplaque assay system (10). Briefly, peripheral blood mononuclear cells were cultured for 5–6 days in RPMI 1640 containing 10% human serum (IgM-free from patients with congenital agammaglobulinemia). The antigens SRBC or OA were added at the beginning of culture. Flat-bottom Micro-test II plates (Falcon Plastics, Div. of Bioquest, Oxnard, Calif.) were used for the fluid phase micro-plaque assay (10).

Lymphocytotoxins. Complement-dependent cytotoxicity for ^{51}Cr-labeled autologous or allogeneic lymphocytes was assessed in a two-stage assay with patients' serum as the source of antibody and fresh rabbit serum as the source of complement. In the sensitization phase of the assay, cells were incubated with patients' serum for 1 h at 4°C or 37°C. The complement-dependent phase was carried out at 22°C for 2 h or 37°C for 1 h, after which time ^{51}Cr release was measured.

Induction of E-rosettes. Single, small aspirates (less than 1.0 ml) of bone marrow were separated on gradients of Isopaque-Ficoll. Induction of E-rosettes in the mononuclear cell fraction was assessed after incubation with thymus epithelium-conditioned medium (HTCM) or the control, fibroblast conditioned medium (11, 12). Cells were incubated with dilutions of the conditioned media for 2 h at 37°C, washed, and assayed for E-rosettes in the usual manner. Rosettes were counted after an incubation period of 2 h at 4°C. To assess the induction of E-rosettes by thymosin fraction V (kindly provided by Dr. A. Goldstein, Galveston, Tex.), equal volumes of Isopaque-Ficoll-separated bone marrow cells, (2–3 × 10^6/ml), 0.5% SRBC, and dilutions of thymosin were incubated to-

gether at 37°C for 10 min, centrifuged (200 *g*, room temperature, 5 min), and rosettes enumerated after 16 h incubation at 4°C (13). Induction of E-rosettes in peripheral blood cells was carried out in an identical manner.

Antibody-dependent (ADC) and mitogen-induced (MIC) cytotoxicity. These studies were carried out using peripheral blood mononuclear cells as effector cells and ^{51}Cr-labeled chicken red blood cells (CRBC), Chang liver cells, or M4 (human melanoma cell line) cells as targets (14). In ADC studies, rabbit anti-target cell antibodies were used to sensitize the target cells. In MIC, phytohemagglutinin (PHA-P, 5 μg, Difco Laboratories, Detroit, Mich.) was added to mixtures of effector cells and unsensitized target cells. All studies were carried out for 4 h at 37°C using 5×10^4 target cells and different effector cell:target cell ratios. The data were expressed as specific ^{51}Cr release which represents the differences between experimental and control tubes (14).

Reagents. Inosine, hypoxanthine, uridine, and nucleoside phosphorylase were obtained from Sigma Chemical Co., St. Louis, Mo. Stock solutions were made up in phosphate-buffered saline (pH 7.2) and passed through a Millipore filter before use in culture. OA was also obtained from Sigma Chemical Co. In the plaque assay, OA was coupled to indicator red cells using chromic chloride (10).

RESULTS

The results of some of the studies of cell-mediated immunity are shown in Table I. Both patients were severely lymphopenic, with absolute lymphocyte counts often between 200 and 300/mm^3. The percentages of mononuclear cells forming E-rosettes were significantly lower than normal, and when expressed in terms of absolute numbers, were profoundly depressed. However, they were significantly higher than those reported in the first patient with a complete deficiency of PNP (3) and are similar to the results described in one other patient who demonstrated approximately 25% E-rosetting cells at 15 mo of age (4). In addition, D. B. and M. B. were unable to manifest delayed cutaneous reactivity to a battery of antigens and failed to acquire sensitivity to dinitrochlorobenzene.

TABLE I
Evaluation of Cell-Mediated Immunity

	D. B.	M. B.	Normal
Absolute lymphocyte count	<750/mm^3	<750/mm^3	>1,200/mm^3
% E-rosettes*	20.0±4.7	31.5±5.7	54.5±6.1
Delayed cutaneous reactivity			
C. albicans	Neg	Neg	
Streptokinase-streptodornase	Neg	Neg	
Mumps	Neg	Neg	
Dermatophytin	Neg	Neg	
Diphtheria/tetanus	Neg	Neg	
Dinitrochlorobenzene	Neg	Neg	

* Expressed as a percentage of Isopaque-Ficoll-separated mononuclear cells. Results indicate the mean±1 SD of at least eight separate determinations.

Despite the presence of cells with surface marker characteristics of T-lymphocytes, the in vitro proliferative responses to the nonspecific mitogens phytohemagglutinin (PHA), concanavalin A, and pokeweed mitogen (Table II); specific antigens (Table II); and allogeneic cells (Table III) were markedly impaired. Indeed, even when concentrations of M. B.'s cells were adjusted to provide normal numbers of E-rosetting cells, no improvement of the proliferative response was observed. This impairment of T-lymphocyte proliferation is similar to the findings in other PNP-deficient patients (3, 4). No lymphocytotoxic antibodies were detected in either patient's serum, nor did their serum inhibit the proliferative response of normal cells. In addition, the stimulating capacity of the PNP-deficient cells in mixed leukocyte culture was normal. Peripheral blood cells, obtained from the mother who was heterozygous for PNP deficiency, were normal in terms of their capacity to form E-rosettes and their response to nonspecific mitogens and allogeneic cells.

A summary of studies of humoral immunity is given in Table IV. As shown here, circulating immunoglobulin levels and several antibody determinations, including the responses to reimmunization, were essentially normal. D. B.'s response to killed polio immunization was less than that usually observed at this age. Salivary IgA was normal. There was no evidence of restricted homogeneity or monoclonal components to any of the immunoglobulin classes. They appeared to have a normal percentage of circulating B lymphocytes, although in the face of the lymphopenia, absolute numbers of circulating B lymphocytes were less than normal. Normal numbers of plasma cells were observed in the bone marrow specimens. The proportion of monocytes in the mononuclear cell preparations, estimated by EA_{IgG}-rosettes (8) and latex ingestion, was normal and ranged from 15% to 25%.

Both patients demonstrated their ability to generate a specific plaque-forming cell (PFC) response after in vitro sensitization with the antigens SRBC and OA (Fig. 1). We have previously established that this response is T cell dependent and that the antigen dose kinetics are in part determined by the relative numbers of T lymphocytes and B lymphocytes and the

TABLE II
*Lymphocyte Proliferation in PNP Deficiency**

	D. B.	M. B.	Normal
3-day culture			
Unstim	328±62	304±155	668±469
PHA	1,344±526	1,365±830	35,714±10,902
Concanavalin A	2,183±529	2,984±700	18,945±3,386
Pokeweed mitogen	1,400±250	1,140±146	13,885±1,029
6-day culture			
Unstim	322±136	309±51	198±29
PHA	2,759±534	4,915±224	37,823±2,484
Streptokinase-streptodornase	152±40	300±62	13,185±677
C. albicans	116±50	188±60	10,626±727
Dermatophytin	ND	409±97	4,254±747
Purified protein derivative	ND	377±24	12,960±1,122

Unstim, unstimulated; ND, not determined.
* 2.5×10^5 mononuclear cells were cultured in 0.5 ml of RPMI 1640 + 10% AB serum for 3 days in the presence of PHA-P (10 μg/ml), concanavalin A (10 μg/ml), and pokeweed mitogen (1/100); and for 6 days in the presence of PHA, streptokinase-streptodornase (40 μg/ml), *C. albicans* (10 PNU/ml), Dermatophytin (10 PNU/ml), and purified protein derivative (4 μg/ml). Results of triplicate cultures are expressed as mean counts per minute [^{3}H]thymidine incorporation (±1 SD). Several concentrations of mitogen and antigen were tested, and only optimal responses are shown.

presence of T-helper and T-regulator cells[3] (10, 15, 16). Antigen dose kinetics are illustrated in Fig. 1. At optimal antigen concentrations, their PFC response was not significantly different from normal. For both SRBC and OA, the optimal antigen concentrations were lower than in the normal controls, as may have been predicted from the numbers of identifiable T cells and B cells added per culture (10, 16). On the basis of numbers of PFC generated and the presence of an antigen dose-response curve, we would conclude that these patients with partial PNP deficiency have both T-helper and T-regulator cell function.

We next attempted to determine whether the lymphopenia and failure to manifest normal T-cell immunity could be due to a failure of T-cell differentiation or an unresponsiveness to T-cell-inducing factors. HTCM has been shown to be capable of inducing a population of precursor T cells in bone marrow to bind to SRBC (i.e., form E-rosettes) (11, 12). As shown in Table V, Isopaque-Ficoll-separated bone marrow cells from both patients could be induced to form E-rosettes after incubation with HTCM in a dose-dependent fashion. Similar results were observed using thymosin fraction V (Table V), which has also been shown to act on a precursor T-cell subpopulation (13). The optimal concentrations of both HTCM and thymosin appeared to be higher for patient cells than for normals. In contrast to the studies using bone marrow cells, post-Ficoll patient peripheral blood mononuclear cells (and peripheral blood mononuclear cells from normal subjects) did not respond to either HTCM or thymosin, suggesting the absence of circulating T-precursor cells in the face of the significant T-cell lymphopenia. Initial enrichment of precursor cells in peripheral blood mononuclear cells, i.e., by gradient centrifugation (12), may be required for their detection, but insufficient numbers of cells were available.

Earlier investigations of the mutant enzyme activity in these patients revealed a 3.5-fold increase in PNP activity in their red blood cells with higher inosine con-

TABLE III
*Mixed Leukocyte Culture Response in PNP Deficiency**

	Am	Bm	Cm	Dm
A	56±9	65±14	684±86	304±40
B	56±8	78±35	863±89	483±161
C	1,847±99	2,219±266	63±2	2,386±97

* 5×10^4 responder cells (A, B, or C) were incubated together with 1×10^5 mitomycin C-treated stimulator cells (Am, Bm, Cm, Dm) for 7 days. Results of one experiment (mean cpm ±1 SD [^{3}H]thymidine incorporation) carried out in triplicate are shown. A, patient D. B.; B, patient M. B.; C, normal; D, pool of four normal donors.

[3] The term "T-regulator cell" is used here to denote the function of suppression or modulation of the magnitude of the immune response exerted by T lymphocytes or a specialized subset of T lymphocytes.

TABLE IV
Humoral Immunity in PNP Deficiency

	D. B.	M. B.	Normal
Immunoglobulins			
IgG, *mg/100 ml*	1,250	1,900	500–1,440
IgA, *mg/100 ml*	120	280	50–240
IgM, *mg/100 ml*	140	120	40–250
IgE, *ng/ml*	163	153	40–200
Antibody determinations			
Isohemagglutinins (anti-B)	1:64	1:64	1:32
Schick test	Neg	Neg	Neg
Polio	Type 1 2 3	1 2 3	
Pre	1:32 1:16 1:16	1:16 1:16 1:16	
Post	1:32 1:32 1:32	>1:64 >1:64 >1:64	1:64
Tetanus			
Pre, *U/ml*	>0.01 <0.1	>0.01 <0.1	
Post, *U/ml*	>0.1 <1.0	>0.1 <1.0	10-fold rise in titre
Diphtheria			
Pre, *U/ml*	>1.0 <10	>0.01 <0.1	
Post, *U/ml*	>1.0 <10	>0.1 <1.0	
Cytomegalovirus	1:64	1:128	
Toxoplasmosis	1:512	1:1,024	
B-lymphocytes*			
Surface IgM, %	9	10	5–12
Surface IgA, %	1	1	0–4
Surface IgG, %	1	2	0–4
EAC rosettes, %	22	18	10–25

* Expressed as a percentage of peripheral blood mononuclear cells. Results in D. B. and M. B. represent the means of three separate experiments; the range for normals in our laboratory is also given.

centrations (17). We therefore studied the effect of the addition of inosine on PHA-induced lymphocyte proliferation. At concentrations of inosine which did not affect thymidine transport and have been shown to result in the activation of PNP (0.2–2 mM) (17), reproducible enhancement of only the normal PHA response was observed with a dose-dependent inhibition of PNP-deficient cell responses (Fig. 2). Concentrations of inosine greater than 3 mM (which may inhibit thymidine incorporation at the level of a common nucleoside transport system) were partially inhibitory to normal cells when activation was assessed by ^{14}C-amino acid incorporation.

Since the absence of PNP was associated with a block in the catabolism of inosine to hypoxanthine and impaired nucleic acid synthesis,[2] we attempted to circumvent the defect in vitro by adding a variety of reagents to the incubation mixtures and assessing PHA-induced proliferation (Table VI). The addition of both uridine or hypoxanthine, alone or in combination, failed to improve the PHA response of PNP-deficient cells. Higher concentrations of uridine (10^{-4} M) inhibited [^{3}H]thymidine incorporation of both patient and control cells and likely represents a competitive block of thymidine transport rather than true inhibition of activation or proliferation. The addition of hypoxanthine to cultures of normal cells consistently increased the PHA response. The addition of PNP, at concentrations shown to result in the conversion of inosine to hypoxanthine, did not improve the proliferative response. At higher concentrations, PNP regularly inhibited the PHA-induced response of both normal and patient cells. This may be due to the presence of a toxic substance in the commercial preparation, because it was not dialyzed before use but diluted several-hundredfold in medium. Because red blood cells are rich in PNP,[2] similar experiments were carried out in the presence of human red blood cells. As seen in Table VI, the presence of human red cells similarly did not influence the proliferative response.

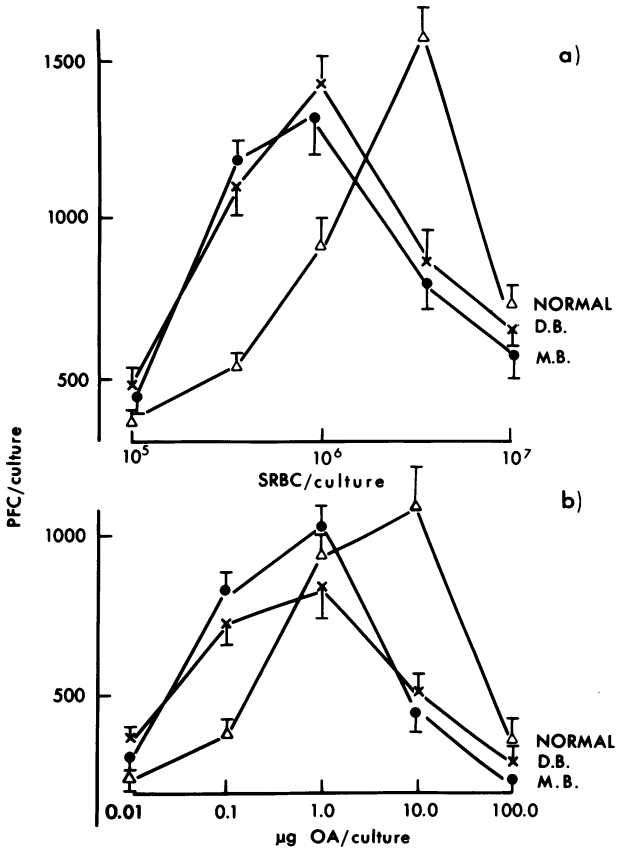

FIGURE 1 Plaque forming cell response in PNP deficiency. 3×10^6 peripheral blood mononuclear cells were cultured for 5 days with varying concentrations of SRBC or OA. Antigen dose kinetics are illustrated here and are expressed as PFC per culture (±1 SD). For both D. B. and M. B., the optimal concentration of SRBC was 10^6 cells and 1.0 μg of OA; in the normal, the optimal concentrations were at least threefold higher. Less than 100 PFC were seen in cultures incubated in the absence of antigen.

The origin of the effector cells mediating ADC or MIC remains controversial. We have suggested that monocytes/macrophages are the most active effector cells for CRBC target cells in the presence of either anti-target cell antibody or phytohemagglutinin (14). For nucleated, nonerythroid, antibody-coated targets such as Chang liver cells or M4 melanoma cells, the effector cell has been identified as a non-Ig-bearing, non-E-rosetting, Fc receptor-bearing mononuclear cell (14, 18, 19). As shown in Fig. 3, the response of both D. B. and M. B. cells in ADC assays against antibody-coated CRBC, Chang, and M4, and MIC against CRBC were essentially normal, although the response of patient D. B. was somewhat lower in all assays.

DISCUSSION

Inborn metabolic errors of purine degradation have now been associated with several distinct immune

TABLE V
Identification of T-Precursor Cells in PNP Deficiency

	% E-rosettes*		
	D. B.	M. B.	Normal
PBS	0.3	0.8	3.9
HFCM (1/3)‡	0.2	1.4	3.4
HTCM			
(1/3)	7.3	4.1	4.6
(1/10)	3.7	2.4	8.0
(1/30)	1.7	1.7	5.4
Thymosin			
300 μg/ml§	4.0	0.4	5.0
100 μg/ml	3.5	3.0	8.9
30 μg/ml	3.9	1.7	7.9
10 μg/ml	1.0	0.8	6.1

HFCM, human fibroblast-conditioned medium; HTCM, human thymus-conditioned medium; PBS, phosphate-buffered saline in which thymosin was diluted.

* E-rosettes expressed as a percentage of more than 1,000 nucleated marrow cells counted. Data are from one experiment and represent the means from two independent observers. Results in the normal are similar to previously published reports (11, 21).

‡ Numbers in parentheses indicate final concentrations used.

§ Refers to the final concentration of thymosin in the incubation mixture.

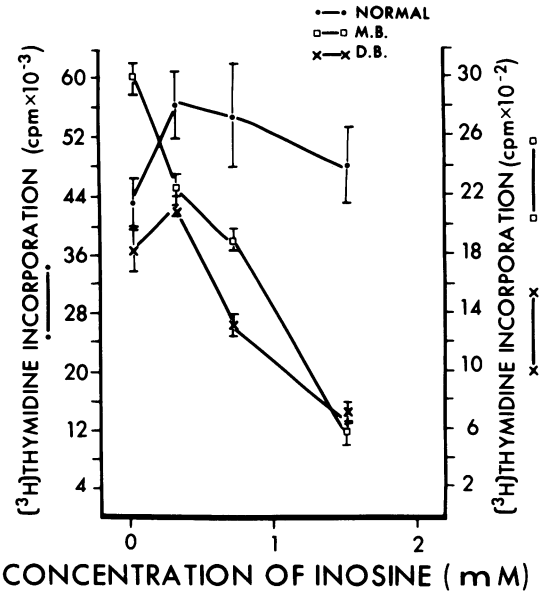

FIGURE 2 Effect of inosine on PHA-induced lymphocyte proliferation. 2.5×10^5 peripheral blood mononuclear cells were incubated with different concentrations of inosine and an optimal concentration of PHA-P (10 μg/ml) for 72 h in 0.5 ml of RPMI 1640 and 10% AB serum. The scales of the vertical axis are different for patients and normal. Results of one such experiment carried out in triplicate are illustrated here.

deficiency syndromes (3–5). In the two brothers reported here with a newly described syndrome of partial PNP-deficiency, we have documented a selective impairment of T-cell function in the presence of apparently normal B-cell function. Both clinically and functionally, they differ in several ways from previously reported patients with a complete deficiency of PNP activity (4, 5). Their clinical courses have been somewhat milder than other described patients, although M. B. now requires frequent hospitalization for recurrent pulmonary infections. Earlier they tolerated live virus immunization, and their major problem with virus at the present time remains the chronic excretion of cytomegalovirus in the urine. It is possible that further deterioration, both clinically and functionally, will take place with time, as suggested by Stoop et al. (4).

Metabolic studies in these boys indicated a functional block of purine catabolism at PNP in vivo and were complemented by evidence for an incomplete block of purine catabolism in studies using affected patient red cells and fibroblasts.[2] Kinetic studies with the mutant enzyme from these two patients have demonstrated a ten-fold increase in the Michaelis constant for inosine and guanosine (17), and this, in part at least, may account for the partial deficiency observed. This incomplete block may also account for the milder clinical symptomatology and less severe laboratory evidence of immune dysfunction. The impairment of most aspects of T-cell function was profound with marked lymphopenia and only a low level residual lymphocyte proliferative response to nonspecific mitogens and allogeneic cells and no response to specific antigens. Since T-lymphocytes normally account for approximately 80% of the circulating lymphocyte pool, represent the responsive cells in the proliferative assays, and are essential in the afferent or recognition limb in delayed hypersensitivity reactions, these findings are in keeping with significant impairment of the generation and (or) circulation of functional T cells. Obvious explanations for T-cell lymphopenia and impaired function such as the presence of lymphocytotoxins or serum inhibitors were ruled out.

The generation and maturation of T cells is a complex process involving several stages, each of which may be characterized by the acquisition and expression of different arrays of cell surface markers and functions (20). In man, we have postulated that the initial step in T-cell differentiation is dependent on contact between precursor cells and thymic epithelium (5, 21). Subsequent maturation may be directed by thymus-epithelium-derived factors acting within the thymus or peripheral lymphoid tissues (22). On the basis of

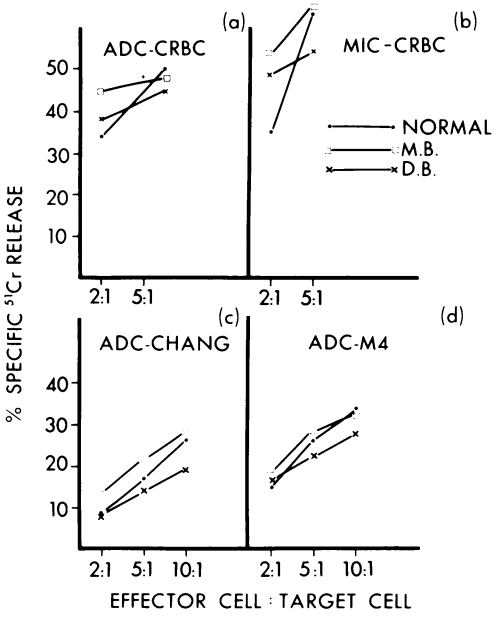

FIGURE 3 Cytotoxic effector cell activity in PNP deficiency. ADC and MIC for three target cells were assayed using varying numbers of peripheral blood mononuclear cells and 5×10^4 targets. The means of three separate experiments are shown for each assay and are expressed as specific ^{51}Cr release (per cent release in experimental tubes [antibody-coated target cells] minus per cent release in control tubes [non-antibody coated target cells]). Control tube lysis for CRBC was less than 2%, for Chang cells 8–13%, and for M4 cells 6–12%.

TABLE VI
*PHA Response: Effect of Various Compounds**

	D. B.	M. B.	Normal
Unstimulated	272±11	140±24	185±26
PHA	889±143	712±156	45,642±1,042
Uridine			
0.1 mM	611±108	451±26	28,427±6,170
0.01 mM	763±52	707±67	43,903±4,896
Hypoxanthine			
1 mM	680±34	548±35	73,430±10,879
0.1 mM	834±204	891±113	65,171±1,182
0.01 mM	728±15	805±40	58,960±1,952
PNP			
0.2 U	432±18	777±45	36,274±440
0.04 U	839±22	634±28	50,049±5,174
10% HRBC			
10 μl	ND	705±217	44,554±3,808
50 μl	ND	815±61	46,033±2,695

* 2.5×10^5 peripheral blood mononuclear cells were cultured for 72 h in 0.5 ml RPMI 1640 containing 10% AB serum. An optimal concentration of PHA (PHA-P, 10 μg/ml) and the reagents were added at the initiation of the cultures. Results from triplicate cultures are expressed as mean counts per minute [^{3}H]thymidine incorporation±1 SD. PNP units were as specified by Sigma. 10% HRBC, 10% hematocrit of human red blood cells; ND, not determined.

studies carried out with thymic epithelial implants in patients with a demonstrated thymic defect (23), we have observed that E-rosette formation is the earliest marker seen after reconstitution, followed by a proliferative response to nonspecific mitogens and allogeneic cells, and subsequently by the appearance of T-helper cells and later T-regulator cell function. In our PNP-deficient patients, significant percentages of E-rosetting cells were observed. In addition, after incubation of bone marrow cells with HTCM or thymosin, there was a dose-dependent induction of E-rosette formation. As discussed above, induction of E-rosette formation by thymic epithelium-derived factors implies that contact-dependent thymic epithelial cell function was intact in these patients (5, 21), and the numbers of E-rosetting cells induced at optimal doses of HTCM (or thymosin) suggest that HTCM-responsive marrow T-precursor cells were also present in normal numbers (11, 12). Thus, the initial stages of T-cell differentiation were intact in these patients. Furthermore, T-helper and T-regulator cell function could be delineated on the basis of the in vivo and in vitro findings: Numerous antibodies were detected with appropriate secondary responses after reimmunization in vivo, and after in vitro sensitization with antigen, both helper and regulator function could be demonstrated by the generation of PFC responses with appropriate time and antigen dose kinetics. Since the antigens used in vivo and in vitro require T cells for the development of antibody (10), functional T cells must have been present, at least at the time of antigen presentation where it appears to be crucial (H-M. Dosch and E. W. Gelfand, unpublished observations).

Studies of immunological function in deficiencies of ADA and PNP (1–4) have suggested an etiological relationship between the enzyme defect and the immune disorder. Some of the immunologic investigations were designed to elucidate this relationship by utilizing chemical reagents to reconstitute PHA responsiveness in peripheral blood mononuclear cells in vitro. Toxic effects of adenosine have been proposed as a mechanism for immunodeficiency (24, 25). Although uridine is said to reverse the toxicity of adenosine (23, 25), there was no effect of this compound on lymphocyte proliferation. The addition of hypoxanthine, a product of purine nucleoside phosphorylase, had no effect, indicating that the lack of hypoxanthine (26) is not responsible for the T-cell dysfunction. Incubation of the deficient cells with normal erythrocytes, which contain large quantities of purine nucleoside phosphorylase,[2] or with purified enzyme itself, did not alter the response. Activation of lymphocyte responsiveness with enzyme replacement has been observed in the deficiency of ADA and suggested that the removal of accumulated adenosine was therapeutic (27). Finally, a unique property of the mutant purine nucleoside phosphorylase was the discovery of a K_m abnormality (17), suggesting the possibility that increasing concentrations of inosine could activate lymphocyte function with concomitant activation of the enzyme. Addition of inosine led to a dose-dependent inhibition of PHA-induced proliferation with increased sensitivity of patient cells to inosine as compared to normal cells. This may reflect the existing higher intracellular levels of inosine in these patients (footnote 2, reference 28) and(or) their failure to catabolize the added inosine. Higher concentrations of inosine (greater than 3 mM) were shown to have a small inhibitory effect (10–20% inhibition) on the activation of normal cells by PHA as measured by the incorporation of ^{14}C-amino acids (leucine, threonine, valine) after 48 h of incubation or by [^{3}H]thymidine incorporation after 72 h of incubation. In these experiments, the cells were washed just before the 4-h pulse with isotope precluding a competitive block in isotope uptake by inosine. This apparent inhibition of T-lymphocyte function may occur by a competitive inhibition of ADA by inosine (29). The failure to reverse the defect in vitro has been paralleled by our attempts to restore reactivity in vivo. A program of biweekly red cell transfusions has failed to alter the lymphocyte count, E-rosettes, and PHA response in M. B., although the serum urate increased from 2.5 mg/dl to greater than 6.0 mg/dl, urinary inosine was reduced by 50%, and red cell lysates now contained 40–50% of normal PNP activity (~1,000 nm/h per mg protein). Similarly, there has been no change in patient D. B. despite 12 wk of therapy with oral uridine.

The relatively selective impairment of T-cell function documented here and in other PNP-deficient patients is reminiscent of that observed in DiGeorge's syndrome (30), cartilage-hair hypoplasia (31), and intestinal lymphangiectasia (32). In the latter, fully functional T cells are selectively lost into the gut (33), leading to profound impairment of cell-mediated immunity, lymphopenia, reduced lymphocyte proliferation, and absent delayed cutaneous reactivity. Antibodies are synthesized but are lost into the intestinal lumen resulting in hypogammaglobulinemia. In PNP deficiency, the situation pertaining to the T-cell system may be similar; we have demonstrated that functional T cells are generated and these T cells subserve certain aspects of cell-mediated immunity such as T-helper or T-regulator cell function, accounting for the apparently normal antibody production in vivo in these patients. Since T cells appear to have been generated normally and no antilymphocyte antibodies could be detected, the peripheral lymphopenia and impairment of proliferative responses may reflect a shortened survival of certain populations of PNP-deficient lymphocytes. In the absence of PNP, it appears that T cells possess a unique sensitivity to the accumulation of,

or lack of, some crucial intracellular metabolic product: B cells or other cell types would appear to be less sensitive or have alternative pathways of purine degradation.

In summary, two brothers with markedly reduced PNP activity are described with a selective impairment of T-cell immunity and apparently normal B-cell function. The clinical and laboratory data suggest a less severe picture of immune dysfunction and normal serum urate and urine uric acid. The biochemical, immunological, and genetic data are consistent with a postulated single gene defect at the PNP locus. Although the demonstrated immune defect is likely causally related to the partial deficiency of this purine enzyme, the biochemical basis for this abnormality remains unclear. Further elucidation of the defect will depend upon the measurement of key metabolites and enzyme activities in the affected purine and pyrimidine pathways and the assessment of their influence on different components of the immune system.

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