Lymphocyte 5'-Nucleotidase Deficiency in Hypogammaglobulinemia: Clinical Characteristics[1]

N. Lawrence Edwards,[2,3] James T. Cassidy, and Irving H. Fox

Human Purine Research Center, Departments of Internal Medicine, Pediatrics, and Biological Chemistry, University of Michigan Medical Center, Ann Arbor, Michigan, 48109

Received August 31, 1979

Twelve patients with congenital agammaglobulinemia, 2 of 10 patients with selective IgA deficiency, and 4 of 15 patients with common variable immunodeficiency have reduced 5'-nucleotidase activity on lymphocyte plasma membranes. In congenital agammaglobulinemia and common variable immunodeficiency, enzyme-deficient patients have an early onset of immune dysfunction, low levels or an absence of circulating B-lymphocytes, and a positive family history of similar immunodeficiency. The enzyme deficiency involves peripheral E-rosette-forming lymphocytes, suggesting an abnormality of T-lymphocytes in these diseases. No systemic disorder of purine nucleotide degradation to uric acid is detectable in the enzyme-deficient patients. No direct relationship between 5'-nucleotidase activity and *in vitro* production of IgG is evident, since inhibition of the enzyme did not decrease IgG synthesis. At present the data do not allow a distinction between two hypotheses to explain the role of 5'-nucleotidase in hypogammaglobulinemia, that is, whether 5'-nucleotidase deficiency causes the immune dysfunction or is simply a marker for an intrinsic lymphocyte abnormality. However, this enzyme deficiency may provide a useful means of classifying disorders of immunoglobulin synthesis.

INTRODUCTION

Purine nucleotide degradation plays an important role in the regulation of the human immunologic system. A deficiency of adenosine deaminase occurs with severe combined immunodeficiency (1, 2) and purine nucleoside phosphorylase deficiency is associated with T-cell dysfunction (3—5) (Fig. 1). Decreased activity of 5'-nucleotidase has recently been observed in patients with hypogammaglobulinemia of adult onset (6, 7) and in young males with congenital agammaglobulinemia (8). Although an etiologic role for the deficiencies of adenosine deaminase and purine nucleoside phosphorylase in immunodeficiency diseases has been suggested by extensive investigations (9, 10), a cause and effect relationship between lymphocyte 5'-nucleotidase deficiency and hypogammaglobulinemia has not been established.

The purpose of this report is to clarify the relationship between lymphocyte 5'-nucleotidase deficiency and hypogammaglobulinemia by defining the clinical disorders observed in the patients with enzyme deficiency. In our study, every
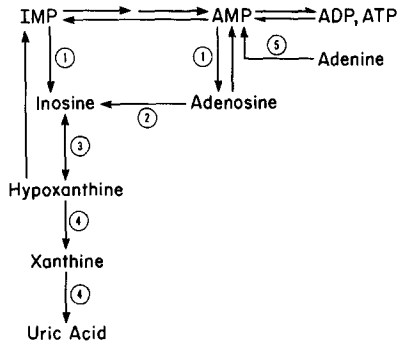
FIG. 1. Purine metabolic pathway. Nucleoside monophosphates (AMP, IMP) are hydrolyzed to their respective nucleosides by 5'-nucleotidase (1). Adenosine deaminase (2) converts adenosine to inosine. Purine nucleoside phosphorylase (3) catalyzes the reversible phosphorolysis of inosine to hypoxanthine, which is then oxidized to xanthine and uric acid by xanthine oxidase (4). Adenine (radiolabeled for our *in vivo* study) is converted to AMP by adenine phosphoribosyltransferase (5). AMP can be converted to ADP and ATP.

patient with congenital agammaglobulinemia, as defined previously (11), has lymphocyte 5'-nucleotidase deficiency. However, patients with other primary immunoglobulin deficiencies also may have this enzyme deficiency. In contrast to adenosine deaminase and purine nucleoside phosphorylase deficiencies, no systemic abnormality of purine nucleotide degradation is observed in subjects with 5'-nucleotidase deficiency. Preliminary experiments *in vitro* showed no direct relationship between 5'-nucleotidase inhibition and the regulation of immunoglobulin synthesis.

MATERIALS AND METHODS

Materials

The commercial sources of the reagents used in this study were as follows: adenosine, inosine, AMP, ATP, fructose, uricase, xanthine oxidase, and purine nucleoside phosphorylase, Sigma Chemical Company, St. Louis, Missouri; bovine serum albumin (fraction V, grade B), adenine, hypoxanthine, and xanthine, Calbiochem, San Diego, California; [8-^{14}C]hypoxanthine (50 mCi/mmol), [8-^{14}C]inosine (60 mCi/mmol), [8-^{14}C]AMP (50 mCi/mmol), and carrier-free [^{125}I]-iodine, Amersham Company, Arlington Heights, Illinois; [8-^{14}C]adenine (52 mCi/mmol), New England Nuclear Corp., Boston, Massachusetts; Ficoll–Paque, Pharmacia Fine Chemicals, Piscataway, New Jersey; sheep erythrocytes, Microbiological Associates, Walkersville, Maryland; pokeweed mitogen and RPMI-1640 with Hepes buffer, Grand Island Biological Company, Grand Island, New York; fetal calf serum, Reheis Chemical Company, Chicago, Illinois; carbonyl iron, BDH Chemicals Ltd., Dorset, England; adenosine-α, β-methylene diphosphate (AOPCP) and rabbit anti-human IgG antiserum, Miles Laboratory, Elkhart, Indiana; fluorescein-conjugated goat F(ab')$_2$ anti-human immunoglobulins and anti-μ-chain antisera, Cappel Laboratories, Cochranville, Pennsylvania; and in-line Cathivex filter units (0.22 and 0.45 μM) for the intravenous infusion of [8-^{14}C]adenine and D-fructose, Millipore Corp., Bedford, Massachusetts.

Patient Population

The immunodeficiency subjects in this study are followed by our Pediatric Immunology Service or by Dr. Kenneth Rich, Childrens Hospital, Chicago. Twelve patients with a previous diagnosis of congenital agammaglobulinemia were subdivided into three groups (Table 1). One child fit the classic definition of X-linked agammaglobulinemia with lateral affected maternal male relatives as proposed by the WHO Conference on classification of primary immuno-deficiencies (12). A second group of seven patients had at least one male sibling affected with infantile-onset agammaglobulinemia. A third group of four males satisfied all the other criteria for classic X-linked agammaglobulinemia except that they had no known relative with similar illness. Other patients with primary immunoglobulin deficiencies were classified as common variable immunodeficiency (CVID) or selective IgA deficiency (SIgAD) according to proposed definitions (11, 12). Control subjects for lymphocyte 5′-nucleotidase assay were healthy adult volunteers or age-matched nonimmunodeficient children seen at The University of Michigan Pediatric Clinic.

TABLE 1

CLINICAL DESCRIPTION OF 5′-NUCLEOTIDASE-DEFICIENT SUBJECTS

Patient	5′-Nucleotidase (nmol/hr/10^6 cells)	Sex	Age of onset of symptoms (months)	Immunoglobulins (mg/dl)			B-Cells %	Clinical diagnosis[a]
				G	A	M		
GS	3.7	M	8	60	0	10	1	CA$_\gamma$ II
JR	4.0	M	6	130[b]	0	50	0	CA$_\gamma$ III
CA	4.8	M	9	200[b]	0	0	0	CA$_\gamma$ II
PD	5.2	M	?[c]	260[b]	0	200	2	CA$_\gamma$ III
NA	5.5	M	18	150[b]	0	9	2	CA$_\gamma$ II
BT	5.6	M	6	20	0	0	0	CA$_\gamma$ I
SW	6.1	M	6	0	0	0	0	CA$_\gamma$ III
SM	6.3	M	24	200[b]	0	0	0	CA$_\gamma$ II
RM	6.5	M	36	220[b]	0	0	1	CA$_\gamma$ II
KV	6.5	M	9	450[b]	0	15	3	CA$_\gamma$ III
AH	6.9	M	4	20	0	0	0	CA$_\gamma$ II
RB	7.2	M	6	140	0	30	6[d]	CVID
MH	7.7	M	3	110[b]	0	0	1	CA$_\gamma$ II
BL	8.6	M	32	77	0	0	3[d]	CVID
DS	9.8	M	16	2200	0	192	6	SIgAD
AH	10.4	F	6	65	7	10	7[d]	CVID
DB	10.4	M	360	3135	0	135	11	SIgAD
CR	11.3	M	3	320[b]	30	70	8[d]	CVID

[a] CA$_\gamma$—congenital agammaglobulinemia (I—classic X-linked; II—familial male affected without lateral transmission; III—infantile onset with no known affected relatives); CVID—common variable immunodeficiency; SIgAD—selective IgA deficiency.

[b] Postimmunoglobulin therapy.

[c] Patient PD was adopted at age 6 years, at which time he already had severe recurrent sinopulmonary disease.

[d] Although CVID patients generally have normal numbers of B-cells, a lack of surface Ig-bearing lymphocytes in both early and late onset CVID has been reported in as many as 15–20% of the patients (e.g., Preud'homme et al., J. Immunol. 114, 481, 1975).

Studies of Purine Nucleotide Degradation

Five patients with congenital agammaglobulinemia, three patients with common variable immunodeficiency, and eight gouty adult patients were hospitalized in the Clinical Research Center at The University of Michigan. Patients were given a purine-free, 70 g protein, weight-maintenance diet. No medications which altered uric acid production or excretion were given. On the fourth hospital day, 15 μCi [8-^{14}C]adenine in 3 μmol adenine was administered intravenously through 0.22-μm Cathivex filters to label the intracellular adenine nucleotide pool (total administered dose was 23.2 $\times$ 10^6 cpm in our liquid scintillation spectrometer). Baseline and postinfusion urines were assayed for creatinine content and radioactivity. On the seventh hospital day, intravenous fructose (0.5 g/kg) was given over 10 min as a provocative test to cause adenine nucleotide pool degradation (13).

Ecto-5'-Nucleotidase Assay

Lymphocyte separation was carried out on blood freshly collected in heparin-coated tubes (14). Lymphocyte 5'-nucleotidase was measured at pH 7.5 by a radiochemical method (8). To test for an interfering effect of adenosine kinase, an inhibitor of nucleoside transport, 10 μM dipyradamole, or an inhibitor of adenosine kinase, 100 μM 4-amino-5-iodo-7-β-D-ribofuranosyl-7,14-pyrrole (2,3-d) pyrimidine, was added to our assay system (15, 16). The addition of either of these compounds did not increase nucleoside formation. These data suggest that adenosine kinase does not significantly influence our 5'-nucleotidase assay.

Substrate competition assays were performed for lymphocytes from normal and 5'-nucleotidase-deficient subjects using 25 μM [8-^{14}C]AMP and 1 mM purine or pyrimidine nucleoside monophosphates. Substrate competition for lymphocyte 5'-nucleotidase was estimated by the inhibition of enzyme activity.

Confluent monolayers of cultured diploid fibroblasts were assayed for 5'-nucleotidase in 35 $\times$ 10-mm culture plates. After growth in Eagle's minimum essential medium with 10% fetal calf serum, monolayers were washed three times with 5 ml of 150 mM NaCl in 10 mM Tris–HCl, pH 7.5. Activity of 5'-nucleotidase was assayed with a 4-ml reaction mixture containing 50 mM Tris–HCl, pH 7.5, 4 mM MgCl$_2$, 150 mM NaCl, and 0.2 mM AMP at 37°C for 7.5 min. One hundred microliters of supernatant was removed at time 0 and 7.5 min and assayed for inorganic phosphate content (17). Fibroblasts of each monolayer were counted following trypsinization. This assay was linear with cell number and time to 20 min.

Cell Preparation

Separation of gradient-purified mononuclear cells into rosette-forming and non-rosette-forming populations was carried out using sheep erythrocytes (18). The fresh mononuclear cells, suspended to 2 $\times$ 10^6 cells/ml in Hanks' balanced salt solution without calcium and magnesium, were mixed with equal volumes of 0.5% washed sheep erythrocytes and 15% heat-inactivated, absorbed fetal calf serum in siliconized glass tubes and centrifuged at 200*g* for 5 min. The mononuclear cell–sheep erythrocyte suspension was incubated for 10 hr at 4°C. The cells were then gently resuspended by rotation and placed on a Ficoll–Paque gradient

for separation into rosette-forming and non-rosette-forming cells. Monocyte depletion of either the mixed peripheral mononuclear cells or the non-rosette-forming fraction was performed using carbonyl iron (19). Monocyte contamination of mononuclear cell preparations was determined by a nonspecific esterase stain (20).

In Vitro Immunoglobulin Synthesis

To study the differentiation of lymphocytes to immunoglobulin secreting plasma cells, peripheral blood lymphocytes were adjusted to a concentration of 1×10^6 cells/ml in RPMI-1640 with Hepes buffer supplemented with penicillin, streptomycin, glutamine, and heat-inactivated fetal calf serum. Two-milliliter aliquots were dispensed into loosely capped 10×75-mm plastic tubes to which were added 100 μl of pokeweed mitogen and 0 to 100 μM AOPCP. All cultures were prepared in duplicate and incubated for 7 days at 37°C in humidified chambers containing 5% carbon dioxide in air. At the end of the incubation period, the culture tubes were spun and the supernatant fluids were aspirated and assayed for IgG concentration. The IgG secreted into the medium was measured by a tube-binding radioimmunoassay with rabbit anti-human IgG antiserum and ^{125}I-labeled human IgG (21).

Other Assays

Erythrocytes were obtained by spinning heparinized blood at 1000*g* for 5 min at 4°C. The plasma was removed and frozen at −20°C. The erythrocytes were washed twice with cold 150 m*M* sodium chloride and frozen at −70°C for subsequent enzyme assay.

Adenosine deaminase, purine nucleoside phosphorylase, and adenine and hypoxanthine–guanine phosphoribosyltransferases were assayed using radiochemical methods (22–24). Serum urate concentration and urinary uric acid, oxypurines, and inosine were quantitated by enzymatic spectrophotometric methods (25–27). The inosine assay measured both inosine and 2'-deoxyinosine. Creatinine was measured by a modification of the technique of Folin and Wu (28). Protein was estimated by the method of Lowry *et al.* (29), with crystalline bovine serum albumin as a standard.

RESULTS

Enzyme Deficiency and Clinical Features

Plasma membrane 5'-nucleotidase values are listed in Fig. 2. Normal subjects showed a wide range of lymphocyte 5'-nucleotidase activity, from 12.3 to 41.1 nmol/hr/10^6 lymphocytes (mean value of 21.1). Enzyme levels were relatively constant for a given individual on multiple analyses. All 12 patients with congenital agammaglobulinemia showed a reduction in lymphocyte 5'-nucleotidase activity, with values ranging from 3.7 to 7.7 nanomol/hr/10^6 lymphocytes. Four of 15 patients with common variable immunodeficiency and 2 of 10 with selective IgA deficiency also had reduced 5'-nucleotidase levels. All patients had normal values for erythrocyte adenosine deaminase and purine nucleoside phosphorylase. Lymphocyte 5'-nucleotidase was measured in six mothers of eight patients with con-
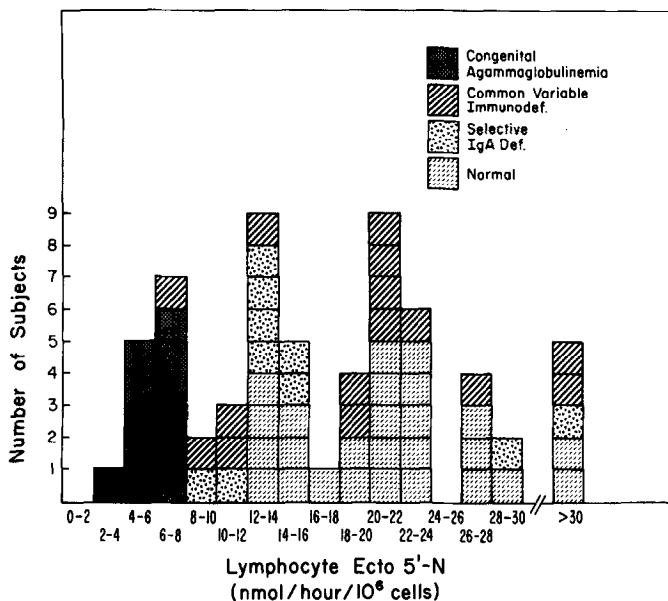
FIG. 2. Distribution of lymphocyte 5'-nucleotidase (5'-N) in primary hypogammaglobulinemia. Low enzyme activity is present in 12 of 12 patients with congenital agammaglobulinemia, 4 of 15 with common variable hypogammaglobulinemia, and 2 of 10 patients with selective IgA deficiency.

genital agammaglobulinemia. They had a mean value of 20.2 (range 13.0–26.8) nanomol/hr/10^6 cells, compared to the mean value for 12 normal females of 25.8 (range 14.5–40.2).

The clinical and immunologic features of these patients are listed in Tables 1 and 2. No single clinical variable used in classification uniquely correlated with lymphocyte 5'-nucleotidase deficiency. In general, patients diagnosed as either congenital agammaglobulinemia or common variable immunodeficiency with low 5'-nucleotidase levels had lower percentages of circulating B-cells and an earlier onset of immune deficiency. No difference in the clinical or immunologic features was evident for the patients with selective IgA deficiency having normal or deficient lymphocyte 5'-nucleotidase.

The existence of circulating or lymphocyte membrane-bound inhibitors of 5'-nucleotidase was investigated with mixing experiments. Plasma from either normal or 5'-nucleotidase-deficient subjects had no inhibitory effect on 5'-nucleotidase activity in assays of either normal or enzyme-deficient lymphocytes. When normal and enzyme-deficient peripheral blood lymphocytes were mixed at a 1 to 1 ratio, the resulting 5'-nucleotidase activity was the expected median value between the normal and the deficient enzyme activity. Therefore, there was no evidence for the existence of an inhibitor.

Additional studies did not provide evidence for a structural alteration of the deficient enzyme. In peripheral blood lymphocytes from normal and enzyme-deficient subjects, 5'-nucleotidase had similar values for pH optimum, degree of inhibition by adenosine-α,β-methylene diphosphate, and Michaelis constant for

TABLE 2
CHARACTERISTICS OF ENZYME-DEFICIENT PATIENTS

Diagnosis	Lymphocyte 5'-nucleotidase (nmol/hr/10⁶ cells)[a]	Family history (number positive)[a]	Age of onset (months)[b]	IgG	IgA (mg/dl)[c]	IgM	B-Cells (%)
Congenital agammaglobulinemia							
5'-N^{-d}	3.7–7.7 (12)[e]	8/12	4–36	0–20	0	0	0–2
CVID[f]							
5'-N^{+d}	12.3–50.7 (10)	0/10	4–192	40–200	0–5	0–340	4–20
5'-N$^-$	7.2–11.3 (4)	1/4	3–36	65–140	0–30	0–30	3–8
SIgAD[g]							
5'-N$^+$	12.3–44.5 (10)	0/10	7–112	1100–2200	0	80–180	6–13
5'-N$^-$	9.8, 10.4 (2)	0/2	16, 360	2200, 3150	0	135, 190	6, 11
Normal values	12.3–41.0 (26)			1030–1140	110–150	70–110	>3

[a] Similar immunodeficiency state in other male family members.
[b] Age when recurrent infections first become obvious when known.
[c] Serum immunoglobulin levels are pretreatment values only.
[d] 5'-N$^-$, 5'-nucleotidase deficient; 5'-N$^+$, normal 5'-nucleotidase activity.
[e] Number of subjects studied in each group.
[f] Common variable immunodeficiency.
[g] Selective IgA deficiency.

5'-AMP (8). The substrate specificity for the purine nucleotides was similar in the normal and enzyme-deficient lymphocytes. Lymphocyte 5'-nucleotidase preferentially degraded substrate ribonucleoside 5'-monophosphates with the following relative activities: AMP = GMP > IMP > CMP > XMP.

An effort was made to distinguish whether the enzyme deficiency in congenital agammaglobulinemia might result simply from the absence of B-cells. Peripheral blood mononuclear cells from 10 normal and 5 agammaglobulinemic subjects were isolated on Ficoll–Paque gradients and then fractionated further into E-rosette-forming and non-E-rosette-forming subpopulations. The E-rosette-forming and non-E-rosette-forming cells had comparable 5'-nucleotidase activities; their values were similar to those found in mixed peripheral lymphocytes from both the normal subjects and the patients with congenital agammaglobulinemia (Table 3, Study A). It is noteworthy that the E-rosette-forming cells had evidence of the enzyme deficiency.

In order to determine whether monocyte contamination of the peripheral blood lymphocytes might account for the enzyme deficiency, monocyte depletion was performed on the peripheral blood mononuclear cells from nine normal subjects and eight patients with congenital agammaglobulinemia using carbonyl iron ingestion (Table 3, Study B). Normal postgradient cells contained 5 to 14% monocytes as determined by nonspecific esterase staining. Postgradient mononuclear cells from subjects with congenital agammaglobulinemia had 10 to 26% monocytes. Monocyte-depleted preparations showed 3 to 7% monocytes for the normal subjects and 4 to 10% for the agammaglobulinemic patients. Although a modest increase in 5'-nucleotidase activity was found, the enzyme deficiency persisted. The alteration of mononuclear cell subpopulations did not account for the observed enzyme deficiency.

TABLE 3
5'-NUCLEOTIDASE ACTIVITY AND LYMPHOCYTE POPULATIONS

	Normal (nmol/hr/10^6 cells)	Congenital agammaglobulinemia (nmol/hr/10^6 cells)
Study A	($N = 10$)	($N = 5$)
Fresh peripheral mononuclear cells	19.3[a] (12.8–27.5)	5.9 (3.3–9.0)
E-rosette-forming cells	22.5 (12.8–34.1)	7.1 (3.3–11.8)
Non-E-rosette-forming cells	28.1 (12.0–43.2)	6.6 (2.8–8.4)
Study B	($N = 9$)	($N = 8$)
Peripheral mononuclear cells Premonocyte depletion	17.6 (12.6–24.8)	6.4 (2.2–8.8)
Postmonocyte depletion	24.8 (15.2–40.2)	7.6 (5.5–11.0)

[a] Values represent mean activity for each cell population with range of values in parentheses.

TABLE 4
5'-NUCLEOTIDASE IN CULTURED DIPLOID FIBROBLASTS

Subject	5'-Nucleotidase (μmol/hr/10^6 cells)	
	Normal	Congenital agammaglobulinemia
1	36.0	12.0
2	16.3	9.3
3	13.4	7.9
4	10.0	7.1
Mean value	18.9	9.1

In order to discern whether 5'-nucleotidase deficiency was unique to lympho-
cytes or whether the deficiency occurred in other tissues, cultured diploid fibro-
blasts were grown from skin biopsies from four normal subjects and four boys
with congenital agammaglobulinemia. The activity of 5'-nucleotidase was mea-
sured in the intact fibroblast monolayers through all stages of cell growth. High
levels of enzyme activity were present in the early logarithmic phase of cell
growth. Plasma membrane 5'-nucleotidase activity diminished at the time of con-
fluent cell growth and became stable during the late confluent stage of growth.
During late confluent cell growth (day 16 postpassage) in fibroblast cell lines,
5'-nucleotidase was 900-fold more active than in lymphocytes (Table 4). The over-
lapping enzyme activity in fibroblasts from the two patient populations did not
demonstrate a definitive difference.

Purine Nucleotide Degradation

Plasma urate levels and urinary uric acid excretion were normal in patients with
lymphocyte 5'-nucleotidase deficiency. Purine nucleotide degradation was mea-
sured directly by the excretion of radioactively labeled purine compounds in the
urine after labeling the adenine nucleotide pool with tracer doses of [8-^{14}C]adenine
(27). The cumulative radioactivity excretion in the urine during the first 5 days
ranged from 5.5 to 13% in six subjects with lymphocyte 5'-nucleotidase deficiency
and was 11.8 and 10.8% in two young males with common variable immunodefi-
ciency and normal 5'-nucleotidase (Fig. 3). Thus there was no evidence of a
systemic reduction in the degradation of nucleotides in patients with 5'-nucleo-
tidase deficiency using this measure.

The infusion of D-fructose, a sensitive provocative test, was used to examine
purine nucleotide degradation *in vivo* (4, 13). Six patients with 5'-nucleotidase
deficiency and two patients with common variable immunodeficiency and normal
enzyme activity were first injected with [8-^{14}C]adenine and then given intravenous
fructose 4 days later. Urinary radioactivity increased rapidly during the first hour
following fructose infusion in both groups of patients (Fig. 4). In six patients with
lymphocyte 5'-nucleotidase deficiency, total urinary purine (the sum of uric acid,
xanthine, hypoxanthine, and inosine) increased from baseline levels of 2.4 ± 0.8
millimol/g creatinine to 9.1 ± 4.0 millimol/g creatinine in the first hour following
fructose administration. The mean increase of 6.7 millimol/g creatinine was com-
parable to the elevated purine excretion of 7.5 ± 4.2 millimol/g creatinine ob-
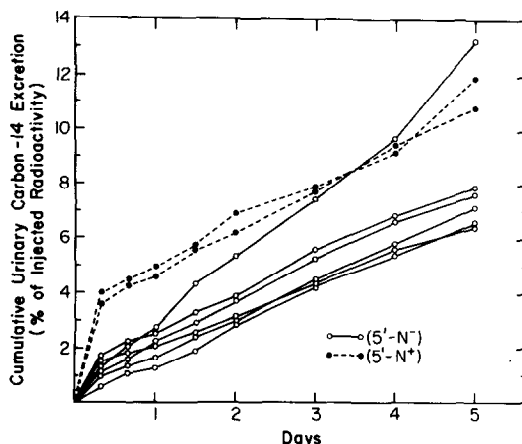served in eight adult control subjects. The two subjects with common variable

FIG. 3. Cumulative urinary radioactivity excretion following intravenous [8-¹⁴C]adenine. At day 0, 15 μCi of [8-¹⁴C]adenine (total infusion = 23.2 × 10⁶ cpm in our liquid scintillation spectrometer) is administered to subjects and urinary radioactivity excretion is measured. The slope of radioactivity excretion in six patients with lymphocyte 5'-nucleotidase deficiency (5'-N⁻) is similar to that seen in two age-matched patients with hypogammaglobulinemia and normal lymphocyte 5'-nucleotidase (5'-N⁺).

immune deficiency and normal 5'-nucleotidase levels had increased purine excretion of 8.4 and 10.9 millimol/g creatinine in the first hour following fructose infusion. Serum urate concentration increased similarly following fructose infusion in all three groups of patients. Thus, the *in vivo* isotope and fructose studies showed no consistent evidence for a systemic deficiency of 5'-nucleotidase in subjects with decreased lymphocyte 5'-nucleotidase.
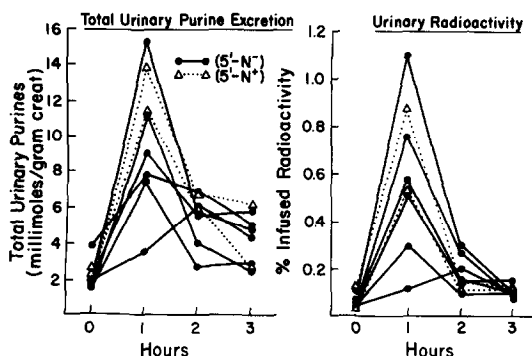
FIG. 4. Effect of intravenous D-fructose on purine excretion in hypogammaglobulinemia. Fructose (0.5 g/kg) is administered intravenously starting at time 0 for 10 to 15 min. Urine is collected for 1 hr prior to and 3 hr following fructose infusion. Total urinary purine excretion (left) includes the sum of uric acid, hypoxanthine, xanthine, and inosine. Baseline purine excretion for 5'-N⁻ patients is 2.39 ± 0.81 mmol/g creatinine and for 5'-N⁺ patients is 3.00 and 2.79 mmol/g creatinine. The increase in urinary radioactivity (right) following fructose infusion reflects the degradation of adenine nucleotides previously labeled with [8-¹⁴C]adenine. Baseline radioactivity excretion for 5'-N⁻ patients is 0.065 ± 0.026% of the administered dose and for 5'-N⁺ patients is 0.051 and 0.062%. Patients with nucleotidase deficiency (5'-N⁻) or with normal enzyme activity (5'-N⁺) respond similarly to fructose.

TABLE 5
In vitro SYNTHESIS OF IgG BY NORMAL PERIPHERAL BLOOD LYMPHOCYTES

Concentration of AOPCP (μM)	IgG secretion[a] (ng/10^6 lymphocytes)	
	Subject A	Subject B
0	162 ± 57	497 ± 121
10	170 ± 20	495 ± 97
15	180 ± 40	727 ± 187
25	171 ± 33	687 ± 237
100	151 ± 39	790 ± 262

[a] Values express the mean ± standard deviation of two experiments performed on each subject in duplicate.

Immunoglobulin Synthesis and 5'-Nucleotidase Inhibition

To assess whether 5'-nucleotidase activity regulates immunoglobulin synthesis, IgG secretion by activated lymphocytes was assayed with and without a potent inhibitor of 5'-nucleotidase (Table 5). Although the average secretion of IgG appears to be stimulated by the higher concentrations of AOPCP, there were no statistical differences among the values for each subject by Student's t test. Thus, there was no simple relationship between IgG secretion *in vitro* and 5'-nucleotidase activity.

DISCUSSION

A reduction of lymphocyte 5'-nucleotidase occurs in patients with congenital agammaglobulinemia (Fig. 2) (8), other forms of hypogammaglobulinemia (Fig. 2) (6–8), acute and chronic lymphatic leukemia (30–32), and acute infectious mononucleosis (31). In general, the patients in our study with congenital agammaglobulinemia or common variable immunodeficiency and lymphocyte 5'-nucleotidase deficiency have an earlier clinical onset of immune dysfunction, lower numbers of circulating B-lymphocytes, and a greater propensity for familial occurrence (Tables 1 and 2) than do patients with similar diagnoses and normal lymphocyte 5'-nucleotidase activity.

In contrast to deficiencies of adenosine deaminase and purine nucleoside phosphorylase, there is no detectable alteration of systemic purine metabolism in patients with 5'-nucleotidase deficiency and no tissue other than peripheral circulating mononuclear cells exhibits the enzyme deficiency. Our patients with lymphocyte 5'-nucleotidase deficiency have no alteration of purine nucleotide degradation as shown by the normal values for plasma urate concentration, urinary uric acid excretion, and urinary radioactivity excretion and the response to D-fructose infusion.

An etiologic relationship between lymphocyte 5'-nucleotidase deficiency and abnormally low immunoglobulin synthesis has not been established. In the immune deficiency states associated with adenosine deaminase deficiency or purine nucleoside phosphorylase deficiency, an accumulation of purine nucleosides and deoxynucleosides leads to increased concentrations of tissue purine deoxynucleoside triphosphates (9, 33–37). These latter compounds are toxic to lympho-

cytes. An accumulation of deoxynucleoside triphosphates does not occur in lymphocytes and erythrocytes from patients with 5'-nucleotidase deficiency (38). Therefore, it appears unlikely that there is a shared mechanism of immune toxicity occurring in 5'-nucleotidase deficiency and the other forms of purinogenic immunodeficiency. However, our recent observations suggest that 5'-nucleotidase activity may be a plasma membrane marker for the mature T-lymphocyte, since human thymocytes have very low levels of this enzyme (39). Thus, 5'-nucleotidase deficiency may be the consequence of altered lymphocyte subpopulations rather than a cause of the disease itself.

The role of lymphocyte 5'-nucleotidase in cellular metabolism is not clear. This enzyme degrades nucleoside 5'-monophosphates to their nucleoside derivatives in the following reaction: nucleoside monophosphate $+ H_2O \rightarrow$ nucleoside $+ P_i$. It is known that highly charged nucleoside monophosphate derivatives are not transported across cell membranes in usual circumstances. Nucleosides released by the 5'-nucleotidase reaction, however, are freely transported into the cell, and recent studies suggest that 5'-nucleotidase may actually function as an adenosine translocase (40).

Whether 5'-nucleotidase deficiency leads to hypogammaglobulinemia, is a marker for patients that share a common maturational block of lymphocyte development, or connotes an alteration of lymphocyte subpopulations is not currently known. Further clinical observations are necessary to distinguish among these possibilities. However, the measurement of lymphocyte 5'-nucleotidase may provide both a rational basis for classifying the primary hypogammaglobulinemias and a useful clinical diagnostic technique.

ACKNOWLEDGMENTS

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