Metabolic Basis for Disorders of Purine Nucleotide Degradation

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Purine nucleotide degradation refers to a regulated series of reactions by which human purine ribonucleotides and deoxyribonucleotides are degraded to uric acid in humans. Two major types of disorders occur in this pathway. A block of degradation occurs with syndromes involving immune deficiency, myopathy or renal calculi. Increased degradation of nucleotides occurs with syndromes characterized by hyperuricemia and gout, renal calculi, anemia or acute hypoxia. Management of disorders of purine nucleotide degradation is dependent upon modifying the specific molecular pathology underlying each disease state.

An explosion of information about disorders of purine nucleotide degradation in humans has occurred during the past 8 yr. The discovery of seven new enzyme abnormalities associated with specific clinical syndromes and the intensive research to elucidate the underlying molecular pathology have provided the basis for the major advances. New concepts have related tissue ATP levels and their depletion by hypoxic or metabolic mechanisms to common clinical abnormalities.

Disorders of purine nucleotide degradation now encompass a range of previously unsuspected disease associations. Immunodeficiency, myopathy, renal calculi, hyperuricemia and gout, anemia, central nervous system dysfunction and tissue hypoxia occur with abnormalities of this biochemical pathway. In this review, diseases of purine nucleotide degradation will be described. Two major types of disorders occur; blocks of purine nucleotide degradation and increased activity of this pathway. The metabolic basis underlying altered regulation of purine nucleotide degradation to uric acid in these diseases will be described as it is understood at the present time focusing on the more recent advances.

REGULATION OF PURINE NUCLEOTIDE DEGRADATION

Purine nucleoside monophosphate derivatives are degraded to uric acid in humans by a final common pathway (Fig. 1). Complex regulation of this pathway is evident from experiments which increase the degradation of purine nucleotides. Activation of nucleotide degradation to inosine and hypoxanthine occurs in ascites tumor cells following incubation with 2-deoxyglucose or glucose. There is rapid utilization of ATP during the phosphorylation of these compounds. The sudden diminution of intracellular ATP concentrations and the resulting elevation of AMP and IMP levels appear to be triggering factors for the activation of nucleotide degradation.

Experimental evidence in humans indicates that altered regulation of the pathway may accelerate the degradation of purine nucleotides. A model for activation of purine nucleotide degradation in humans is provided by the rapid infusion of fructose. In less than 60 min after intravenous fructose there is an increase of the serum urate concentration and an elevation of urinary uric acid, oxypurine (hypoxanthine and xanthine) and inosine excretion (Fig. 2). In human liver there is a depletion of total adenine nucleotides, predominantly ATP, and inorganic phosphate within 30 min of a fructose infusion. These observations suggest that the rapid phosphorylation of infused fructose by ATP leads to hepatic ATP degradation to the purine compounds measured in blood and urine.

Vigorous muscular exercise in man causes a rise of the serum urate level and an elevation of plasma and urinary oxypurines. This may be related to an increase of uric acid synthesis from the degradation of muscle ATP during exercise, since there is a diminution of vastus lateralis muscle ATP concentrations. These observations are compatible with an activation of purine nucleotide degradation, initiated by a sudden decrease of intracellular ATP and inorganic phosphate concentrations during exercise.

Dephosphorylation

The dephosphorylation of nucleoside 5'-monophosphates (Fig. 1, reaction 1) is the first committed and irreversible reaction of purine nucleotide degradation. It has been proposed to be the major site of regulation of the pathway. Four distinctive subcellular types of 5'-nucleotidase have been recognized to hydrolyze

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Received for publication October 28, 1980.

This work supported in part by USPHS grants AM 19674 and SM01RR42, and grants from the Michigan Heart Association and the Warner Lambert Company.

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Metabolism, Vol. 30, No. 6 (June), 1981
Fig. 1. Pathway of purine nucleotide degradation. Purine nucleoside monophosphate derivatives are degraded to uric acid in humans by a final common pathway. Nucleotides are dephosphorylated to form the nucleoside derivatives. Specific 5'-phosphomonoesterase (E.C.3.1.3.5) and non-specific phosphatases (E.C.3.1.3.2) hydrolyze AMP, IMP, GMP or their deoxynucleoside monophosphates to the nucleoside or deoxynucleoside derivatives (reaction 1). AMP is deaminated to IMP by AMP deaminase (E.C.3.5.4.6, reaction 12). Inosine, deoxyinosine, deoxyguanosine, and guanosine are converted to purine bases and ribose-1-phosphate or deoxyribose-1-phosphate by purine nucleoside phosphorylase (E.C.2.4.2.1, reaction 3). Adenosine and deoxyadenosine are deaminated to inosine and deoxyinosine by adenosine deaminase (E.C.3.5.4.6, reaction 2). GMP is deaminated to inosine by guanine deaminase (E.C.3.5.4.3, reaction 5). Hypoxanthine and xanthine are oxidized to uric acid by xanthine oxidase (E.C.1.2.3.2, reaction 4) located primarily in the liver and jejunum of mammals. These pathways are interrupted by reactions which allow the resynthesis of nucleotides. Nucleoside kinases catalyze the conversion of adenosine to AMP, deoxyadenosine to dAMP, deoxyinosine to dIMP and deoxyguanosine to dGMP (reaction 6). Hypoxanthine-guanine phosphoribosyltransferase (E.C.2.4.2.8, reaction 7) converts hypoxanthine or guanine to IMP or GMP. Adenine phosphoribosyltransferase (E.C.2.4.2.7, reaction 11) catalyzes the formation of AMP from adenine. The precursor substrates of the final common pathway of purine nucleotide degradation are formed from the digestion of dietary nucleoprotein, the degradation of nucleic acids to nucleoside monophosphates and the formation of nucleotides by de novo purine synthesis and the purine salvage pathways. In addition, S-adenosylhomocysteine is degraded to adenosine and homocysteine by S-adenosylhomocysteine hydrolase (E.C.3.3.1.1, reaction 8) in an essential step of S-adenosylmethionine mediated methylation reactions (reaction 9). S-adenosylmethionine may also be decarboxylated to enter polyamine metabolism (reaction 10). Degradation of deoxyribonucleotides follows a similar pattern as indicated. However, dAMP may be converted to dIMP at only 6% of the V_{max} of AMP (2321 (reaction 12), while dIMP may be converted to dGMP or dAMP at virtually the same V_{max} as IMP (233-236). dGMP may be converted back to dIMP at about 1% of the V_{max} of GMP (237). The dephosphorylation of deoxynucleotides, the deamination of deoxyadenosine and the phosphorolysis of deoxyinosine or deoxyguanosine are all similar to the reactions of the ribosyl derivatives (Reviewed in Reference 14). GMP, guanosine 5'-monophosphate; dGMP, deoxyguanosine 5'-monophosphate; IMP, inosine 5'-monophosphate; dIMP, deoxyinosine 5'-monophosphate; AMP, adenosine 5'-monophosphate; dAMP, deoxyadenosine 5'-monophosphate.

purine nucleoside 5'-monophosphates. These include enzymes in plasma membrane, microsomes, lysosomal membrane, and cytoplasm. The plasma membrane 5'-nucleotidase has been the focus of many studies although its function is not clear. The enzyme faces the outer surface of the plasma membrane. Substrate specificity is localized to 5'-monophosphate compounds. The enzyme is inhibited by nucleoside diphosphate and triphosphate derivatives and concanavalin A. Extracellular nucleoside 5'-monophosphates are degraded to their nucleoside derivatives in preparation for uptake, since the negatively charged nucleotides are not transported across the cell membrane under usual circumstances. A vectorial transport system for the adenosine component of AMP into rat myocardial cells has been suggested during perfusion with AMP. Plasma membrane 5'-nucleotidase may be an important site for the regulation of purine nucleotide degradation, since activation of this pathway occurs with a depletion of ATP, an inhibitor of this enzyme, and an accumulation of IMP and AMP, substrates for this enzyme. However, recent observations indicate that plasma membrane 5'-nucleotidase may not influence intracellular nucleotide degradation.
Fructose - l-P

\[ \text{Fructose} \rightarrow \text{ADP} + \text{ADP} \]

\[ \text{Fructose-1-P} \rightarrow \text{AMP} \]

\[ \text{Inorganic Phosphate (Pi)} \rightarrow \text{Adenosine} \]

\[ \text{Imp} \rightarrow \text{Hypoxanthine} \]

\[ \text{Ribose-1-P} \rightarrow \text{Uric Acid} \]

Fig. 2. Mechanism of fructose-induced purine nucleotide degradation. Fructose triggers the rapid breakdown of purine nucleotides to uric acid in the liver. The phosphorylation of fructose to fructose-1-phosphate causes ATP to be degraded to ADP. Fructose-1-P tends to accumulate and thus traps inorganic phosphate. ADP is converted back to ATP by the mitochondrial electron transport system or glycolysis, which use inorganic phosphate, or by adenylate kinase. The latter reaction also forms AMP. The net result is a diminution of intracellular ATP and inorganic phosphate and buildup of AMP. The elevated AMP concentrations also lead to increased IMP concentration. Dephosphorylation of 5'-nucleotidase is triggered. If AMP and IMP concentrations are high enough, then non-specific phosphatase can be activated. Once dephosphorylation is activated, there is a cascade of nucleotide degradation through the catabolic pathways leading to increased synthesis of uric acid and accounting for hyperuricemia and the elevated urinary excretion of inosine, hypoxanthine, xanthine and uric acid. Inhibition is indicated by dotted lines. Vertical arrows beside ATP, Pi and AMP show changes caused by the fructose infusion (From Fox, Reference 14).

AMP Deamination

The deamination of AMP (Fig. 1, reaction 12) has been proposed as the rate-limiting reaction in adenine nucleotide degradation instead of 5'-nucleotidase. Inorganic phosphate and GTP at physiological concentrations inhibit AMP deaminase by 95% and are decreased in concentration during fructose infusion. This is proposed to activate the enzyme and lead to adenine nucleotide degradation. However, in contrast to this proposal, under physiological conditions or in intact cells a rise in AMP, ADP and H⁺ concentrations is the major factor increasing the activity of AMP deaminase, while altered ATP, GTP and GDP levels do not change the activity. Therefore, it remains unclear whether AMP deamination is limiting for purine nucleotide degradation. The complicated regulation of this enzyme, its important location in the pathway, and the fact that adenine nucleotides constitute the majority of free cellular nucleotides make this a serious consideration.

AMP deaminase has an additional important role as a component of the purine nucleotide cycle in skeletal muscle. This reaction sequence causes the release of ammonia during muscle contraction and the resynthesis of AMP from IMP. The sequence may be important to energy metabolism and ATP synthesis during muscle contraction.

Reutilization

The major regulatory mechanism of purine nucleotide degradation after dephosphorylation may be reutilization pathways. Guanine and hypoxanthine are...
resynthesized to their respective nucleotides by hypoxanthine-guanine phosphoribosyltransferase (Fig. 1, reaction 7). This enzyme is regulated by the availability of intracellular phosphoribosylpyrophosphate and product inhibition. The importance of this reaction is illustrated by its deficiency, which leads to excessive synthesis of uric acid in part, from an inability to reutilize the substrate purine bases.38

Adenosine, deoxyadenosine, deoxyinosine and deoxyguanosine can be phosphorylated to their respective nucleoside 5'-monophosphates by kinase enzymes (Fig. 1, reactions 6). Adenosine phosphorylation may be important in maintaining normal intracellular ATP concentrations. In erythrocytes with increased adenosine deaminase activity there is a decrease in ATP concentrations and hemolytic anemia.39 The removal of adenosine by its deamination in this disease suggests that erythrocytes require adenosine phosphorylation to synthesize normal quantities of ATP. Adenosine kinase is regulated by adenosine, ATP and Mg concentrations, and is inhibited by ADP, AMP, deoxyadenosine and S-adenosylhomocysteine.40 Therefore, alterations in kinase activity may occur when adenosine or deoxyadenosine accumulates. Attention has been focused upon these reactions in adenosine deaminase deficiency, where both adenosine and deoxyadenosine accumulate in affected patients. Although there is evidence to suggest that adenosine and deoxyadenosine are phosphorylated by the same enzyme, some experiments demonstrate the existence of a distinct enzyme for deoxyadenosine phosphorylation41 possibly associated with deoxycytidine kinase activity.42 Observations with highly purified adenosine kinase suggest that adenosine kinase and deoxyadenosine kinase are distinct enzymes, with the former also having only a small amount of deoxyadenosine phosphorylating activity.43

MEASUREMENT OF PURINE NUCLEOTIDE DEGRADATION

The development of methods for the quantitation of purine nucleotide degradation in whole cells has lead to refined techniques to measure this pathway in humans.

In Vitro Measurements

The basis for the measurement of purine nucleotide degradation in cells is to alter the normal regulation of the pathway by causing a breakdown of ATP.1-4 This initiates a cascade of purine nucleotide degradation with a flow of metabolites through the pathway to adenosine, inosine, and hypoxanthine. Deoxyglucose or inhibitors of the mitochondrial electron transport system and oxidative phosphorylation have been used to initiate degradation. The measurement of intracellular nucleotides and the quantitation of hypoxanthine, inosine, and adenosine allow an assessment of the activity of this pathway. A refinement of this technique is to prelabel the adenine nucleotide pool by incubation with radioactively labeled adenine.12 The stimulation of the breakdown of ATP under these conditions allows a clear documentation of the pathways of ATP degradation through the purine catabolic pathways.

In Vivo Measurements

Plasma and urinary purines. Measurements of plasma and urinary purines in man by standard spectrophotometric methods and more recently by high pressure liquid chromatography allow an assessment of disorders of the nucleotide degradation pathway. An increase in the serum urate concentration and in the urine uric acid excretion indicates increased activity of the purine nucleotide degradation pathway, since it demonstrates the increased synthesis of uric acid. On the other hand, a diminution of the serum urate concentration with very low urine uric acid excretion may indicate a block in uric acid synthesis. An accumulation of purine catabolic intermediates may localize such a block. For example, the excessive excretion of hypoxanthine and xanthine and hypouricemia may indicate a block at xanthine oxidase. Excretion of inosine, deoxyinosine, guanosine, and deoxyguanosine and hypouricemia may indicate a block at purine nucleoside phosphorylase (Fig. 1). Thus, simple measurements of plasma and urinary purines may provide a clue to an underlying disorder of purine nucleotide degradation. Other research methods are summarized below.

Fructose induced purine catabolism. A probe of the intactness and activity of the purine catabolic pathway may be carried out by the use of a fructose infusion.10 Fructose administered rapidly intravenously activates purine nucleotide degradation (Fig. 2). A block in the pathway will be reflected by a change of the normal pattern of urinary purine excretion following the fructose infusion. Alterations in the urinary excretion following fructose infusion have been observed in deficiencies of purine nucleoside phosphorylase, xanthine oxidase and hypoxanthine-guanine phosphoribosyltransferase.44-46

Radioactive labeling of the adenine nucleotide pool. A radioactive isotope of adenine is administered intravenously to patients. This small quantity of adenine does not alter pool sizes and is incorporated into the adenine nucleotide pool. As the adenine nucleotide pool is turned over every day, there is a flow of radioactively labeled compounds through the purine pathway.
nucleotide degradation pathway. The intermediates of purine nucleotide degradation are labeled separately from purines formed by the pathway of purine synthesis de novo. Some of these radioactively labeled purines will be excreted in the urine and can be easily measured. Block of the pathway or alteration of reutilization steps may change the quantity and pattern of urine radioactive purine excretion. This method has been used to demonstrate decreased hypoxanthine reutilization in Lesch-Nyhan Syndrome.48

**Adenine labeling combined with fructose infusion.** The two techniques described above may be combined for a sensitive probe to assess for alterations of the purine nucleotide degradation pathway in humans. The adenine nucleotide pool may be pre-labeled three to four days prior to fructose infusion. Under these conditions the normal response to a fructose infusion is a 10-fold elevation of the urinary radioactivity over the baseline excretion (Edwards NL and Fox IH, unpublished results); the greatest increase of radioactivity is found in inosine and hypoxanthine in subjects with normal enzyme activity.48 Alteration in the amount or pattern of radioactivity excretion following the fructose infusion may indicate disorders of the purine nucleotide degradation pathway. Such an alteration has been discerned in hypoxanthine-guanine phosphoribosyltransferase deficiency,48 but not in patients with lymphocyte plasma membrane 5'-nucleotidase deficiency.49

## DISORDERS OF PURINE NUCLEOTIDE DEGRADATION

Two major types of abnormalities of human purine nucleotide degradation occur; a block in the pathway and an increased activity of the pathway. Table 1 outlines a classification of disorders associated with alteration of purine nucleotide degradation. Blocks of purine nucleotide degradation may lead to an accumulation of the intermediates or their metabolites in human body fluids or cells. The accumulation of such intermediates may be toxic to certain cells and lead to clinical disorders such as immunodeficiency. An elevated rate of purine nucleotide degradation may lead to an increase in the end-products of the pathway. On the basis of the observations with fructose-induced purine nucleotide degradation which is triggered by massive ATP utilization (Fig. 2), an elevated rate of purine nucleotide degradation may be expressed by the following metabolic variables: an increase in the serum urate concentration and an elevation of urinary uric acid, inosine, and oxypurine excretion.49

### Blocks of Purine Nucleotide Degradation

**5'-Nucleotidase deficiency of lymphocytes.** The surface 5'-phosphomonoesterase activity of peripheral lymphocytes is decreased in certain patients with congenital agammaglobulinemia, common variable hypogammaglobulinemia, and selective IgA deficiency.49,51-53 The enzyme deficiency is related to a decrease in the number of 5'-nucleotidase positive lymphocytes.54,55 The enzyme deficiency is measured in peripheral sheep erythrocyte rosette-forming lymphocytes49,56-58 and in peripheral mononuclear cells depleted of monocytes56-59 and represents a block in the ability of these cells to degrade extracellular nucleoside 5'-monophosphates. The diminished activity of lymphocyte 5'-nucleotidase in these diseases appears to be related to decreased 1-lymphocyte enzyme activity and decreased numbers of B-lymphocytes.

The question arises whether 5'-nucleotidase deficiency causes immune dysfunction or results from abnormal lymphocytes. The data suggest that abnormal lymphocytes are the basis for the enzyme deficien-
cy. No structural alteration of the deficient enzyme has yet been demonstrated.\textsuperscript{52} The enzyme deficient patients have no evidence for a systemic disorder of purine nucleotide degradation and appear to have the deficiency only on lymphocytes.\textsuperscript{49} Erythrocyte and lymphocyte deoxynucleoside triphosphates are normal in 5'-nucleotidase deficiency.\textsuperscript{60} Finally, earlier stages of lymphocyte maturation may be characterized by lower values of S-nucleotidase, supporting the possibility that abnormal lymphocyte populations cause 5'-nucleotidase deficiency.\textsuperscript{58,61}

A deficiency of lymphocyte 5'-nucleotidase has also been observed in chronic lymphocytic leukemia, acute lymphoblastic leukemia and transiently in acute infectious mononucleosis.\textsuperscript{62-65}

\textbf{Adenosine deaminase deficiency.} In 1972, the deficiency of adenosine deaminase was found in two unrelated patients with severe combined immunodeficiency disease.\textsuperscript{66} The enzyme deficiency accounts for about half of the patients with this syndrome and autosomal recessive inheritance. In 85\%-90\% of adenosine deaminase deficient patients there is severe lymphopenia, failure to thrive, and infections, diarrhea, malabsorption, and candidiasis with atrophic tonsils, adenoids, and thymus in the first few months of life. In 10\%-15\% of patients the disease may become evident later than 3-6 mo and immunoglobulin retention may occur.\textsuperscript{57,68} X-rays demonstrate evidence of osteoporosis, small or absent thymus gland, and chondro-osseous dysplasia at costochondral junctions, apophysis of iliac bones and vertebral bodies. There is a disorder of decreased platelet aggregation.\textsuperscript{69-72} Neurological abnormalities including nystagmus, head lag, spasticity, athetosis, and developmental delay occur in 10\%-15\% of patients.\textsuperscript{71}

Biochemical abnormalities appear to account for the clinical syndrome. Elevated adenosine and deoxyadenosine concentrations in the urine, plasma and erythrocytes result from the adenosine deaminase deficiency. Increased concentrations of dATP and dADP in erythrocytes, lymphocytes and bone marrow cells and, in some instances, decreased concentration of ATP in erythrocytes from these patients result from the deoxyadenosine accumulation and may have toxic properties toward the immune system.\textsuperscript{50,72-76} Increased lymphocyte and platelet cyclic-AMP concentrations may also contribute to the dysfunction of these cells.\textsuperscript{77} A secondary severe deficiency of erythrocyte S-adenosyl-homocysteine hydrolase has been observed.\textsuperscript{78,79} This may be related to the deoxyadenosine accumulation, since deoxyadenosine has been shown to inactivate S-adenosylhomocysteine hydrolase.\textsuperscript{78} A possible interruption of methylation reactions by this inactivation may potentially contribute to the immunodeficiency and central nervous system disease. How these biochemical abnormalities lead to immune dysfunction is discussed below.

Adenosine deaminase is structurally altered in tissues from patients with severe combined immunodeficiency disease.\textsuperscript{68,69} The properties of the mutant enzymes provide support for genetic heterogeneity of the mutations involving the structural gene coding for adenosine deaminase. Structural alterations of adenosine deaminase also occur in patients with the enzyme deficiency and normal immune function.\textsuperscript{86,87} In these patients white cell adenosine deaminase is less severely deficient; this amount is adequate for normal function of the immune system.

Experimental therapies attempt to restore immune function in patients with severe combined immunodeficiency and adenosine deaminase deficiency. The results of these trials support an etiological relationship between the enzyme deficiency and the immune disorder. About 50\% of the patients with severe combined immunodeficiency disease and adenosine deaminase deficiency respond to enzyme replacement therapy with packed irradiated erythrocytes.\textsuperscript{88-91} The response includes an increase in the absolute lymphocyte count and improved responsiveness to the mixed lymphocyte culture and to phytohemagglutinin stimulation. As well, there is a diminution in the tissue deoxyadenosine triphosphate concentrations, a decrease in the excretion of adenosine and deoxyadenosine and an elevation of erythrocyte S-adenosylhomocysteine hydrolase.\textsuperscript{78,89-91} Bone marrow transplantation corrects most of the immunological and biochemical abnormalities\textsuperscript{81-82} and appears to be the treatment of choice. A low purine diet, which reduces the urinary excretion and the plasma concentrations of adenosine and deoxyadenosine,\textsuperscript{93} and thymosin administration may be useful adjuncts to the management of these patients.\textsuperscript{94}

The molecular basis for the immune dysfunction has been assessed in cell culture models simulating adenosine deaminase deficiency. Cytotoxicity or immunosuppression follow the addition of adenosine or deoxyadenosine to cultured diploid fibroblasts, human lymphoblasts, lymphosarcoma T-cells, Chinese hamster ovary cells, and mouse fibroblasts (Reviewed in Reference 95). These compounds also inhibit mitogen-mediated lymphoblastogenesis and monocyte to macrophage transformation. Toxicity from adenosine or deoxyadenosine is potentiated with inhibition of adenosine deaminase by specific inhibitors, coformycin, deoxycoformycin or erythro-9(2-hydroxyl-3-nonyl) adenine. A block of adenosine deaminase alone, or addition of deoxyguanosine may also have similar cytotoxicity or immunosuppressive effects.\textsuperscript{95,96-100}
The biochemical features of cells susceptible to deoxynucleoside toxicity have been defined using cultured human lymphoblast cell lines. Deoxyadenosine is selectively toxic to cultured T-lymphoblasts during adenosine deaminase inhibition. Deoxyadenosine mediated cytotoxicity in T-lymphoblasts is accompanied by increased concentrations of dATP. T-lymphoblasts have a 20–45-fold greater capacity to accumulate deoxyadenosine nucleotides than B-lymphoblasts at deoxyadenosine concentrations of 50 μM. The accumulation of deoxyadenosine nucleotides in T-lymphoblasts may result from the small quantity of 5'-nucleotidase activity either on the plasma membrane or in the cytoplasm. Similarly, the lack of deoxyadenosine nucleotide accumulation in B-lymphoblasts may result from the high activity of 5'-nucleotidase in the cytoplasm or plasma membrane. The high 5'-nucleotidase activity may degrade dAMP back to deoxyadenosine, preventing its conversion to dATP in B-lymphoblasts. A close correlation exists between plasma membrane 5'-nucleotidase and the ability to accumulate dATP, supporting a relationship between these two variables in murine lymphocytes. Since human thymocytes have 5'-nucleotidase activity as low as cultured T-lymphoblasts, human thymocytes may share common properties with T-lymphoblasts and may be a cell at risk for toxicity when deoxynucleosides accumulate.

How does deoxynucleoside triphosphate accumula-

![Diagram](image-url)

*Fig. 3. Ribonucleotide reductase hypothesis. Ribonucleotide reductase (RR) is the enzyme that converts ADP, GDP, UDP, and CDP to dADP, dGDP, dUDP, and dCDP, respectively. (A) In adenosine deaminase (ADA) deficiency, deoxyadenosine cannot be deaminated to deoxyinosine, and is thus phosphorylated to dAMP, dADP, and dATP. The accumulation of dATP potently inhibits all the reactions of ribonucleotide reductase. This potent inhibitory effect may decrease the synthesis of dGDP, dUDP, and dCDP, reduce the substrates available for DNA synthesis and inhibit DNA synthesis. (B) In purine nucleoside phosphorylase (PNP) deficiency, deoxyguanosine, which ordinarily undergoes phosphorylation to guanine is phosphorylated to dGMP, dGDP, and dGTP. Deoxyguanosine triphosphate accumulates in purine nucleoside phosphorylase deficiency and is a potent inhibitor of three of the four components of the ribonucleotide reductase reaction. Inhibition of these reactions can lead to a reduction of DNA synthesis.*
tion cause cell toxicity? A block at ribonucleotide reductase is currently the most popular hypothesis (Fig. 3). This reaction leads to the synthesis of the deoxyribonucleoside triphosphate substrates necessary for DNA formation. It is potently inhibited by dATP. A block at ribonucleotide reductase from the accumulated dATP could explain immune dysfunction in the deficiencies of adenosine deaminase (Fig. 3). Resistance to deoxynucleoside toxicity with mutations of ribonucleotide reductase support the role of this enzyme in S-49 mouse T-lymphoma cells. The ribonucleotide reductase hypothesis is further strengthened by the reduction of DNA synthesis, but not RNA synthesis, during incubation with 50 μM deoxyadenosine and an adenosine deaminase inhibitor in cultured human cells. However, the pattern of inhibition of DNA synthesis in these experiments was not similar to that expected for ribonucleotide reductase inhibition. Therefore, an additional or alternative mechanism of block of DNA synthesis needs to be considered.

Inhibition of intracellular methylation reactions provides an additional hypothesis to explain immune dysfunction. This possibility originates from the secondary decrease of erythrocyte S-adenosylhomocysteine hydrolase in adenosine deaminase deficiency. An accumulation of S-adenosylhomocysteine, which may result from the hydrolase deficiency, inhibits methylation reactions and has cytotoxic and immunosuppressive activities. Although the exact mechanism for toxicity remains unclear, recent studies indicate that enzymatic methylation of phospholipids may be critical for the transduction of receptor mediated signals through cell membranes. A block of this reaction may also be toxic to cells of the human immune system. Deoxyadenosine, which accumulates in adenosine deaminase deficiency, irreversibly inactivates S-adenosylhomocysteine hydrolase and probably accounts for the secondary hydrolase deficiency (Fig. 4). However, this proposal remains unproven since elevated concentrations of S-adenosylhomocysteine have not been observed in cells from patients with adenosine deaminase deficiency. The selectivity of this mechanism may be directed toward B-lymphocytes.

The hypothesis that increased levels of cAMP concentrations account for the immune dysfunction has been proposed based upon elevated cAMP concentrations in leukocytes and platelets from patients with adenosine deaminase deficiency. Adenosine increases cAMP concentrations in cells derived from the immune system by activating an adenosine receptor and stimulating plasma membrane adenylate cyclase. Increased concentrations of cAMP inhibit immune responsiveness and immune cytolysis. Elevated concentrations of cAMP may be responsible for decreased platelet aggregation in adenosine deaminase deficiency providing evidence for the operation of this mechanism. Increased levels of cAMP in cells remain a possible mechanism of immunodeficiency in adenosine deaminase deficiency.

Biochemical mechanisms other than the ones discussed above may account for the association between purine enzyme defects and immune disorders or more than one mechanism may be involved. Direct proof for a particular mechanism by its demonstration in the tissues of the enzyme deficient patient is not yet available.

Purine nucleoside phosphorylase deficiency. Purine nucleoside phosphorylase deficiency was first described in 1975 in association with a disturbance of cellular immunity. His autosomal recessive disturbance of T-cell function is characterized by a marked reduction in T-lymphocyte numbers and by a lack of a proliferative response of lymphocytes to mitogens and allogenic cells. The normal induction of T-cell maturation in bone marrow precursors by human thymic epithelium conditioned medium or thymosin in two
brothers with purine nucleoside phosphorylase deficiency suggest normal T-cell generation and intact thymic epithelial function.123

Humoral immune function appears intact or enhanced as shown by normal or elevated immunoglobulin concentrations, normal numbers of peripheral B-lymphocytes, and normal antibody response to antigens. The occurrence of monoclonal gammopathy, rheumatoid factor, antinuclear antibody, and Coombs-positive hemolytic anemia indicate excessive antibody production.123-125 Features resembling systemic lupus erythematosus are evident in some of these patients, suggesting that the enzyme deficiency may provide a model for this autoimmune disorder.

Patients with this disorder have evidence of a block of purine nucleotide degradation. There is decreased synthesis of uric acid with hypouricemia, hypouricosuria and an excessive urinary excretion of inosine, guanosine, deoxyinosine, and deoxyguanosine.46,122,124-127 Fructose infusion indicates a block of purine nucleotide degradation by no change in serum urate concentration or urinary uric acid and oxypurine excretion and a massive increase in urinary inosine excretion.46 These patients are overproducers of purines despite the hypouricemia. The overproduction of purines is accompanied by an elevation in the concentration of phosphoribosylpyrophosphate in erythrocytes to a level similar to that observed in patients with a deficiency of hypoxanthine-guanine phosphoribosyltransferase.128 This is related to a loss of active reutilization of purine bases in purine nucleoside phosphorylase deficiency from the lack of formation of hypoxanthine or guanine, substrates for hypoxanthine-guanine phosphoribosyltransferase. Deoxyguanosine triphosphate concentrations are elevated in erythrocytes.60,129,130 This is believed to result from the accumulation of deoxyguanosine and its subsequent phosphorylation. Increased concentrations of dGTP may be the toxic factor to the immune system. The degree of abnormality in uric acid levels, nucleoside excretion, and dGTP concentrations reflect the severity of the enzyme deficiency.130 These patients also have an 80% decrease in erythrocyte S-adenosylhomocysteine hydrolase activity,79 but the significance of this observation remains unclear.

Structural alterations of the decreased purine nucleoside phosphorylase provides evidence for structural gene mutations and genetic heterogeneity in this disorder.130-134

Enzyme replacement therapy with blood transfusions have only partial success in patients with purine nucleoside phosphorylase deficiency.135-137 Two of four patients have modest improvement. There is an increase in the percentage of peripheral E-rosette forming cells without an increase in the lymphocyte count. The lymphocytes stimulate with phytohemagglutinin. A slight reaction to skin tests became evident in one patient. Transfusion therapy in these patients increases the serum urate concentration and the urine uric acid excretion and decreases urine nucleoside excretion.133,135,136 Partial and transient responses to thymosin administration or thymus epithelial transplants are reported.137 Administration of uridine and hypoxanthine with or without allopurinol has no apparent beneficial effect on the immune function. Since in vitro studies suggest that deoxyctydine may reverse deoxyadenosine or deoxyguanosine induced toxicity,43,96-100 it is possible that this therapy in patients may be helpful.

The molecular pathology by which the enzyme deficiency causes the immune dysfunction may be related to deoxyguanosine accumulation. Deoxyguanosine is toxic to T-lymphoblasts and not to B-lymphoblasts.96-98,138 T-lymphoblasts accumulate dGTP and B-lymphoblasts do not. Inhibition of ribonucleotide reductase by dGTP and subsequent inhibition of DNA synthesis is the major mechanism by which toxicity to the immune system may occur (Fig. 3B). The role of a decrease in S-adenosylhomocysteine hydrolase in this disease has not been defined.79

Myoadenylate deaminase deficiency. A new enzyme deficiency associated with myopathy has been recognized in a series of 5 cases of 250 biopsies.139 All patients were young males with muscle weakness or cramping after exercise, in many instances since childhood. Decreased muscle mass, hypotonia and weakness are evident on physical exam. There is a mildly elevated creatine phosphokinase and nonspecifically abnormal electromyograms. Although the patients release lactate into the venous blood during exercise, there is a failure to release ammonia. While the muscle biopsy is normal, the stain for adenyIate deaminase is negative. Homogenized muscle is deficient in adenylate deaminase, while the enzyme is normal in erythrocytes and neutrophils.

The original observations about this disorder have been expanded140,141 and now are confirmed by other workers.142 Males and females may have this disorder. Three of 6 female patients have a poorly understood associated collagen disease such as systemic lupus erythematosus, polymyositis or mixed connective tissue disease.143 Rabbit antiserum to human purified myoadenylate deaminase reacts with human enzyme from muscle, but not from erythrocytes, neutrophils or platelets.140 The data suggest a separate genetic origin for the muscle enzyme and explains the basis for a deficiency of the muscle enzyme alone.

The frequency (5–6 per 250 muscle biopsies)139,142 of
the enzyme deficiency and the diversity of patients involved have suggested that this enzyme deficiency may represent a normal variant or a subclinical state rather than an actual disease. However, recent experiments indicate an important possible role of adenylate deaminase in the maintenance of a normal adenine nucleotide pool. ATP depletion is accelerated in the contracting muscle from a patient with this enzyme deficiency. This may be related to the interruption of the purine nucleotide cycle of muscle.

Xanthine oxidase deficiency. Xanthinuria is a rare disorder characterized by low serum urate concentrations and urinary uric acid excretion and elevated urinary excretion of hypoxanthine and xanthine (Reviewed in Reference 145). This autosomal recessively inherited disease is associated with a gross deficiency of xanthine oxidase in the jejunal mucosa or liver. Xanthine stones, hypoxanthine and xanthine in muscle, and osteoarthritis have been observed in this disorder. Fructose infusion in a patient with xanthine oxidase deficiency shows an increase of urinary hypoxanthine and no change in the serum urate level or urinary uric acid excretion. These changes illustrate the block in purine nucleotide degradation at xanthine oxidase.

Guanine deaminase deficiency. The complete absence of guanine deaminase activity and of the inhibitory protein has been reported in a single newborn, full-term boy, who died two days after birth. No specific clinical features were described. It remains unclear what is the significance of this association of guanine deaminase with newborn death.

Increased Degradation

Increased nucleotide degradation may occur by a number of different mechanisms which alter the regulation of the pathway. If the subsequent purine catabolic pathway is not blocked, this may result in an increased synthesis of uric acid and may be clinically expressed as hyperuricemia, hyperuricosuria and gout. Not all disorders of nucleotide degradation ultimately alter uric acid synthesis.

Increased enzyme activity. A 45–70-fold increase of erythrocyte adenosine deaminase activity has been observed in the kindred with hereditary hemolytic anemia. Patients with this dominantly inherited entity have a mild anemia and a decrease of erythrocyte adenine nucleotide levels to less than 50% of that comparable with reticulocyte-rich blood. The decreased erythrocyte adenine nucleotide concentration appears to be responsible for the hemolytic anemia. This may result from diminished reutilization of adenosine to AMP as a result of excessive destruction of adenosine by elevated adenosine deaminase activity.

Hepatic xanthine oxidase is increased in gouty patients who exhibit an overproduction of uric acid and in one patient with a partial deficiency of hypoxanthine-guanine phosphoribosyltransferase. This may be a secondary change related to the induction of xanthine oxidase. Administration of RNA, hypoxanthine, ethylaminothiadiazole or fructose to human subjects causes liver xanthine oxidase to increase from 2 to 4 times higher than the control group.

Decreased reutilization and increased substrate. Seventy-five percent of adenine nucleotide degraded to hypoxanthine in normal individuals is reutilized to IMP. This may represent an important homeostatic mechanism for the maintenance of the nucleotide pool, since a loss of this pathway leads to increased purine excretion and elevated IMP formation by de novo purine synthesis. The formation of increased quantities of nucleotides by this mechanism or by increased nucleic acid degradation will provide increased substrate for the purine catabolic pathway and will result in an accelerated rate of degradation.

The ability to reutilize hypoxanthine is lost in hypoxanthine-guanine phosphoribosyltransferase deficiency. Patients with this X-linked enzyme deficiency overproduce uric acid and develop hyperuricemia and hyperuricosuria. The inability to reutilize hypoxanthine is an important contributor to purine overproduction in these patients, since oxidation to uric acid then remains the only pathway for hypoxanthine metabolism. Inability to reutilize hypoxanthine and increased activity of purine biosynthesis de novo from increased intracellular phosphoribosylpyrophosphate together account for the overproduction of uric acid (Fig. 5). In the complete enzyme deficiency, patients have Lesch-Nyhan syndrome, a disorder characterized by self-mutilation, choreoathetosis, mental retardation, spasticity and uric acid calculi (Reviewed in Reference 149). In the partial enzyme deficiency, patients have Kelley-Seegmiller syndrome, a disorder characterized by gout, recurrent uric acid calculi and occasional mild central nervous system disorders (Reviewed in Reference 149). In purine nucleoside phosphorylase deficiency (described above), there is a secondary loss of hypoxanthine reutilization and increased de novo purine synthesis. This results from the inability to form hypoxanthine, a product of purine nucleoside phosphorylase, and increased intracellular phosphoribosylpyrophosphate levels, respectively.

The complete deficiency of adenine phosphoribosyltransferase is characterized by a loss of the ability to
reutilize adenine. There results an excessive excretion of adenine and its oxidation product, 2,8-dihydroxyadenine. The increased degradation of these compounds is small in relation to total purine excretion. However, 2,8-dihydroxyadenine is insoluble and stones composed of this compound are formed. The major feature of almost all patients with this autosomal recessively inherited disorder is recurrent renal calculi formed from adenine and its oxidation products. Since these stones are similar to uric acid, they may be erroneously identified as such. These patients have a normal serum urate concentration and no evidence for gout. In contrast to the complete deficiency, the partial deficiency of adenine phosphoribosyltransferase has no definite associated clinical features.

Increased substrate alone may cause elevated purine nucleotide degradation. An error of purine metabolism, increased activity of phosphoribosylpyrophosphate synthetase, leads to increased intracellular concentrations of phosphoribosylpyrophosphate and increased de novo purine synthesis (Reviewed in Reference 153). In this rare X-linked disorder there is a massive overproduction of uric acid with gout and uric acid calculi, but no evidence of central nervous system dysfunction. A large tumor or hyperplastic tissue may have increased turnover of nucleic acid (Reviewed in Reference 133) and generate increased nucleotide substrate for degradation. This may result in increased synthesis of uric acid. Hematological malignancies or other hematological disorders leading to bone marrow hyperplasia classically cause hyperuricemia on this basis. In psoriasis, the increased turnover of epithelial tissue may lead to hyperuricemia by means of increased synthesis of uric acid.

**Decreased ATP.** A sudden diminution of ATP concentration and the resultant elevation of AMP and IMP levels appear to activate a cascade of nucleotide breakdown to purine catabolic intermediates and uric acid. A substantial decrease in the intracellular concentration of ATP may result in profound morphological and functional changes with persistent ATP levels of 20%–25% of control values. In myocardial ischemia there is a correlation between a diminution of ATP content of muscle and impairment of left ventricular function. Hypophosphatemia leads to decreased ATP concentrations and heart failure or even rhabdomyolysis. In myocardial ischemia the degree of ATP loss is correlated with a lethal injury.

Hyperuricemia, gouty arthritis and uric acid calculi may complicate the clinical course of Glycogen Storage Disease Type 1. The glucose-6-phosphatase deficiency may activate a mechanism similar to fructose-induced hyperuricemia. In this disorder the triggering mechanism for increased production of uric acid may be the insulin counter-regulatory hormonal response to hypoglycemia and the inability to synthesize glucose (Fig. 6). Recent studies indicate that parenteral glucagon causes a rise in the serum urate level and urinary uric acid excretion in enzyme deficient patients. This activity of glucagon is accompanied by a reduction in hepatic ATP concentrations and a marked elevation of glucose-6-phosphate, fructose-6-phosphate and fructose-1,6-diphosphate. The depletion of ATP and the trapping of inorganic phosphate in the form of phosphorylated sugar compounds set the stage for activation of purine nucleotide degradation and may explain the increased synthesis of uric acid (Fig. 6). The elevated rate of purine biosynthesis de novo observed in this disease may result secondarily from the depletion of the adenine nucleotide pool. Increased lactate formation inhibits the renal excretion of urate and accentuates the hyperuricemia.
Fig. 6. Mechanisms for hyperuricemia in glucose-6-phosphatase (GL-6-P'ASE) deficiency. Hypoglycemia may be the trigger for the abnormalities in this enzyme deficiency. Hypoglycemia causes glucagon release and this activates glycogen phosphorylase to degrade glycogen to glucose-6-phosphate. There results an increased intracellular concentration of glucose-6-phosphate, fructose-6-phosphate and fructose-1,6-diphosphate. These events lead to a depletion of ATP concentrations, an activation of purine nucleotide degradation and increased synthesis of uric acid. The increased accumulation of phosphorylated sugars leads to hyperlacticacidemia and this decreases the renal excretion of urate.

A lack of any of these components will impair ATP synthesis. The resultant decrease of ATP concentration may trigger a cascade of purine nucleotide degradation as described above. Tissue hypoxia and hypophosphatemia are recognized causes of decreased ATP synthesis. The effects of hypophosphatemia were described above. The hyperuricemia accompanying disorders of fructose metabolism may result from similar mechanisms. Hypoglycemia complicates fructose-1,6-diphosphatase deficiency. This could contribute to the observed hyperuricemia by increased production of uric acid and decreased urate excretion from the hyperlacticacidemia in a manner similar to glucose-6-phosphatase deficiency. In hereditary fructose intolerance there is a block in the further metabolism of fructose-1-phosphate and this compound may accumulate. Fructose-induced hyperuricemia is more pronounced in these patients, whose disease state is worsened by administration of this sugar in any amount. Thus fructose intake may trigger increased uric acid synthesis from the phosphorylation of fructose, an accumulation of fructose-1-phosphate, subsequent depletion of hepatic ATP concentrations, and activation of purine nucleotide degradation. Hereditary fructose intolerance is treated by limiting fructose ingestion. This avoids the activation of purine nucleotide degradation.

(B) Decreased synthesis of ATP. ATP is formed by mitochondrial respiratory-chain phosphorylation, using ADP, O2, and inorganic phosphate as substrates according to the following overall equation:

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3\text{ADP} + 3\text{Pi} + 1/2\text{O}_2 \rightarrow 3\text{ATP} + 4\text{H}_2\text{O} + \text{NAD}^+ \]

Evidence for depletion of tissue adenine nucleotides and release of purine nucleotide degradation interme-
Elevated plasma,* cerebrospinal fluid,* urinary or gas mixture have been found to minimize cardiac congestive heart failure.* Inosine, a purine nucleotide, enhances the preservation of ischemic kidneys,* increases ATP levels in the infarcting myocardium,* and improves myocardial perfusion, augmenting ATP production by anerobic glucolysis with glucose-insulin-potassium and hypertonic glucose, and reducing myocardial ATP consumption by beta blocking agents and balloon counter-pulsation.* Glucose, insulin and potassium or inhalation of an oxygen rich gas mixture have been found to minimize cardiac necrosis and to maintain ATP levels in the infarcting myocardium* or to have an inotropic response in congestive heart failure.* Inosine, a purine nucleoside, improves the performance of ischemic myocardium* and enhances the preservation of ischemic kidney.* Inosine increases ATP levels by its degradation to hypoxanthine and ribose-l-phosphate, compounds which promote ATP synthesis. ATP-Mg increases ATP concentrations during hepatic ischemia.*

Allopurinol may be useful in the management of disorders of purine nucleotide degradation by blocking the pathway at xanthine oxidase and preventing the conversion of hypoxanthine to useless metabolic end products. The accumulated hypoxanthine could be reutilized to form nucleotides and ultimately ATP.* In tissues known not to contain xanthine oxidase, allopurinol may be active at another site either directly or as one of its metabolites.* In humans, allopurinol is rapidly oxidized to oxipurinol by xanthine oxidase. Allopurinol or oxipurinol may be phosphorylated to ribonucleotide derivatives.* A modification of purine nucleotide degradation by allopurinol is supported by its stimulation of hypoxanthine or adenine uptake into myocardial cell nucleotides.*

Pretreatment with allopurinol usually improves function and nucleotide levels, but treatment after the acute event may not be consistently effective. Sodium allopurinol in dosages ranging from 50–100 mg/kg has been shown to reverse the effects of experimental myocardial hypoxia,* and irreversible hemorrhagic shock in dogs treated with hypoxanthine.* In a similar experiment, allopurinol, adenine, hypoxanthine and oxaloacetate produced the best survival (43%).* Allopurinol has also been found to preserve kidneys,* small intestine* and to increase hepatic adenine nucleotide resynthesis after oligemia,* further demonstrating a potential role in maintaining normal cell integrity during ischemia. However, this drug did not modify infarct size when administered 15 minutes after infarction,* perhaps because an irreversible depletion of ATP had already occurred.

The application of these promising experimental observations to human disease processes that have proven refractory to current management appears to be indicated.

SUMMARY AND CONCLUSIONS

The pathway of purine nucleotide degradation is a regulated series of reactions by which purine ribonucleotides are degraded to uric acid in man. Two major categories of disorders occur. A block of degradation is associated with syndromes involving immune dysfunction, myopathy or renal calculi. Increased degradation of nucleotides occurs with syndromes characterized by hyperuricemia, gout, renal calculi, anemia or acute hypoxia. Some disorders associated with increased purine nucleotide degradation are characterized by marked decreases in intracellular ATP concentration and increases in the serum urate concentration and elevated uric acid, oxypurinol, and inosine excretion.

Management of disorders of purine nucleotide degradation is dependent upon an understanding of the metabolic mechanisms of the disease state. In blocks of the pathway, symptomatic therapy may
reverse the consequence of the block. In other instances, specific biochemical therapy to correct an enzymatic defect has been attempted, including enzyme replacement therapy. Patients with increased purine nucleotide degradation with overproduction of uric acid need protection from the adverse effects of excess uric acid production. This is achieved by inhibiting uric acid synthesis with allopurinol. Patients with disorders leading to decreased intracellular concentrations of ATP require therapy to reverse the underlying disorder, to inhibit further purine nucleotide degradation and to stimulate ATP synthesis.

Application of the promising experimental observations about the molecular pathology underlying disorders of purine nucleotide degradation will provide innovative approaches to diagnosis and management of the associated diseases. Already, concepts of enzyme blocks and alteration of immune function has lead to clinical trials of deoxycoformycin, a potent inhibitor of adenosine deaminase.22,23

REFERENCES

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114. Hershfield MS: Apparent suicide inactivation of human...


173. Guddharnason S, Raves KG, Mathes P: Metabolic changes in infarcted and non infarcted myocardium during the postinfarction period, in Bajusz E, Rona G (eds): Recent Advances in Studies on Cardiac Structure and Metabolism, vol 1, Baltimore, Park Press, 1972