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Human purine metabolism: some recent advances and relationships with immunodeficiency

GEORGE NUKI

From the Rheumatic Diseases Unit, Department of Medicine (WGH), University of Edinburgh

Studies of human purine metabolism began some 200 years ago with Scheele's identification of uric acid as a constituent of a renal calculus[1] and Wollaston's demonstration of urate in a tophus from his own ear.[2] Just as these were among the earliest experiments in the new science of biochemistry, so Garrod's (1854)[3] thread test must rank as the first assay in what we now call the discipline of clinical chemistry. Seegmiller's discovery, 15 years ago, that the severe purine overproduction and gout observed in boys with Lesch-Nyhan syndrome resulted from a specific defect of the purine salvage enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT)[4] led to what has been described as the 'purine revolution' and was soon followed by the identification of other inborn errors of metabolism associated with primary purine overproduction and gout. Oxypurine and deoxypurine bases, nucleosides, and nucleotides do, however, have much wider physiological importance as intermediates in energy metabolism, components of coenzymes, vasoactive and neurotransmitter substances, mediators of hormonal action, and as the building blocks for transmitters of genetic information (DNA and RNA). Most recently interest has been focused on the role of purines, and especially deoxypurines, in the regulation of immune responses.

Gout and hyperuricaemia

In most individuals with gout or asymptomatic hyperuricaemia the rise in serum urate concentration is the result of a decrease in renal urate clearance. In 15% or less there is evidence of excessive purine synthesis and hyperuricosuria. A 24-hour urinary urate excretion greater than 0·6 g (3·6 mmol) while on a 10·9 MJ (2600 kilocalorie), 70 g protein, purine-free diet is generally taken to be abnormal,[5] but recent data on healthy Australians on low purine diets showed mean urinary urates of 3·9 mmol (0·65 g)/24 hrs for men and 2·9 mmol (0·5 g)/24 hrs for women.[6]

A variety of mutations of three enzymes are associated with excessive purine synthesis in patients with gout, but these account for only a small proportion of those identified as primary overproducers of urate.

Purine nucleotide synthesis in mammalian cells is regulated by a balanced interaction of several biochemical pathways (Fig. 1). Purine biosynthesis de novo consists of a series of 10 enzymatic steps in which glutamine, glycine, carbon dioxide, aspartate, and 1-carbon-formyl derivatives of folate are added to the ribose moiety of phosphoribosyl pyrophosphate (PRPP) to form inosinic acid (IMP). Adenosine monophosphate (AMP) and guanosine monophosphate (GMP) are each formed from IMP by two enzymatic steps before phosphorylation to their respective diphosphates and triphosphates. Alternatively, the

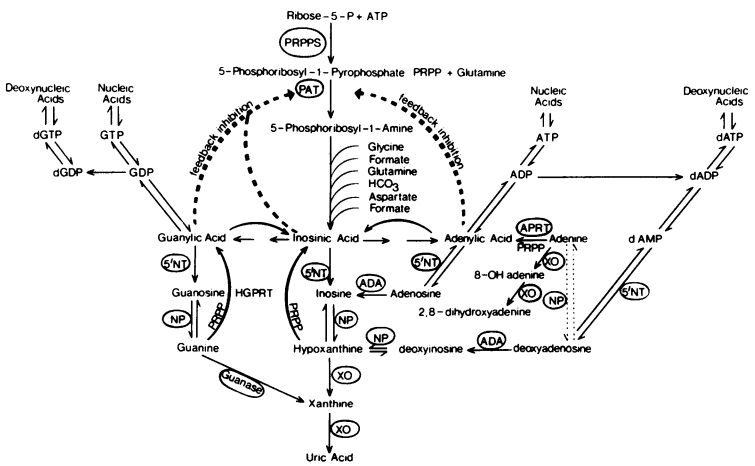

Fig. 1 *Pathways of purine metabolism in man.*

ADA = Adenosine deaminase.
APRT = Adenine phosphoribosyl transferase.
HGPRT = Hypoxanthine-guanine phosphoribosyl transferase.
NP = Nucleoside phosphorylase.
5'NT = 5'nucleotidase.
PAT = Phosphoribosyl pyrophosphate amidotransferase.
PPRPS = Phosphoribosyl pyrophosphate synthetase.
XO = Xanthine oxidase.

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nucleotide monophosphates may be synthesised by phosphorylation of purine bases by phosphoribosyl transferase enzymes reacting with PRPP, and AMP may be formed by phosphorylation of adenosine by adenosine kinase. In vitro studies of amido phosphoribosyl transferase (PAT), the first enzyme in the de novo pathway, show it to have allosteric properties that one often associates with a rate-limiting enzyme, and have provided a molecular model for the mechanism whereby the rate of purine nucleotide synthesis may be regulated. The protein exists in two forms; an inactive dimer and a catalytically active monomer. PRPP converts the larger form of the enzyme to the active monomer, while purine nucleotide monophosphates have the reverse effect.[7]

HGPRT deficiency

In Lesch-Nyhan syndrome and the severe familial form of X-linked gout, which is also associated with deficiency of HGPRT, it seems logical to conclude that the considerably accelerated rate of de novo purine synthesis results from both accumulation of PRPP and diminished feedback inhibition of PAT by purine nucleotides. However, while intracellular concentrations of PRPP are increased in HGPRT-deficient cells, steady state purine nucleotide concentrations do not appear to be reduced,[8 9] and concentrations of pyrimidine nucleotides are actually increased.[9] A good deal of evidence suggests that PRPP, which is present in cells at limiting concentrations, is the major regulator of rates of de novo purine synthesis whenever purine overproduction occurs in vivo. On the other hand, the importance of purine base 'salvage' is emphasised by experiments which show that rates of purine synthesis in normal human lymphoblasts may be increased to those observed in HGPRT-deficient mutants if hypoxanthine is carefully excluded from the culture medium.[10] Moreover, Hershfield has demonstrated co-ordinated inhibition of IMP conversion to adenine nucleotides with enhancement of total guanine nucleotide synthesis after the addition of adenosine to human lymphoblasts in culture, emphasising the potential regulatory importance of the distal inosinic acid branch point.[11]

There is increasing evidence of genetic heterogeneity at the HGPRT locus.[12 13] Although the severity of clinical manifestations is usually proportional to the severity of the enzyme defect, this is not always apparently the case. In rare instances mutations resulting in abnormal enzyme kinetics may be associated on the one hand with the classic clinical Lesch-Nyhan phenotype and apparently normal red cell HGPRT activity assayed at saturating substrate concentrations, or on the other hand with X-linked gout without neurological features and apparently absent enzyme activity in standard assays. Immunochemical studies have shown that most mutations at the HGPRT locus are structural gene mutations resulting in catalytically defective enzyme protein that does not cross react with antibody raised against highly purified normal human HGPRT (CRM negative mutants).[14]

Recent studies have provided evidence for a single amino acid substitution in one such structural variant ($HGPRT_{munich}$),[15] while other groups have now succeeded in cloning the HGPRT gene.[16 17]

APRT deficiency

Adenine phosphoribosyl transferase deficiency is not associated with gout or purine overproduction. Although heterozygous, partial APRT deficiency was originally described in gouty patients it is clear from family studies that the enzyme defect and hyperuricaemia are inherited as independent traits.[18] The apparent association arose spuriously because APRT enzyme assays were undertaken only in patients with gout, although heterozygous APRT deficiency may occur in as many as 1% of the population.[19] More recently, a number of patients have been identified in whom severe, homozygous APRT deficiency was associated with symptomatic urinary lithiasis and calculi composed of 2, 8-dihydroxyadenine.[20 21] *X*-ray diffraction, infrared and ultraviolet absorption spectrometry, and ion exchange chromatography of HPLC extracts of these stones have been required to differentiate the adenine metabolites from uric acid.[21] These patients may be treated successfully with a low purine diet and allopurinol,[22] which inhibits the formation of 2,8-dihydroxyadenine.

Superactive PRPP synthetase

Structural mutations resulting in superactive phosphoribosyl pyrophosphate synthetase are now well established as a cause of X-linked gout and purine overproduction. Initially increased enzyme activity was associated with decreased sensitivity to feedback inhibition by purine nucleotides in one family[23] and a primary increase in catalytic activity per enzyme molecule of the mutant protein in another.[24] More recently the genetic heterogeneity and diversity of abnormalities in kinetic mechanisms leading to superactivity of this enzyme has been extended to include mutants with increased V max, various abnormalities of purine nucleotide feedback inhibitor responsiveness, increased affinity for the substrate ribose-5-phosphate, and a combination of catalytic and regulatory defects.[25]

Most affected males have presented with gouty arthritis and/or uric acid urolithiasis in early adult life, but two families have been described where homozygous boys developed symptoms in childhood and shared metabolic abnormalities and nerve deafness with their heterozygous mothers.[26 27]

Type I glycogen storage disease

Glucose-6-phosphatase deficiency is associated with both overproduction and underexcretion of uric acid, so that gout and hyperuricaemia become appreciable clinical problems in affected individuals fortunate enough to survive to adult life. Persistent lactic acidaemia is the major cause of impairment of uric acid excretion and the simultaneous increase in de novo purine synthesis observed in these patients is thought to result from excessive production of PRPP after 'shunting' of metabolites through the pentose phosphate pathway.[28] It has been difficult to test this hypothesis direct, as glucose-6-phosphatase activity is limited to the liver, kidney, and intestinal mucosa. Recently it has been suggested that increased purine

production in this disorder may be secondary to accelerated purine degradation after recurrent glycogenolysis and depletion of ATP[29]; rather like the mechanism accounting for the purine overproduction and hyperuricaemia which follow ingestion or infusion of fructose.[30] Van den Berghe and his colleagues believe that the hepatic catabolism of adenine nucleotides and the formation of uric acid are regulated by the activity of AMP deaminase.[31]

Type I glycogen storage disease is usually characterised by complete absence of hepatic glucose-6-phosphatase. We have recently reported the case of a child with a partial enzyme deficiency where there was a striking absence of hypoglycaemia.[32] This suggests that type I glycogen storage disease with partial deficiency of hepatic glucose-6-phosphatase should perhaps be considered in patients with gout or hyperuricaemia associated with hypertriglyceridaemia and lactic acidaemia, even in the absence of hypoglycaemia.

Other enzyme defects and gout

We are still unable to define the metabolic abnormality responsible for purine overproduction in most of the few patients with gout who are primary hyperexcretors of uric acid. Though some of these may well be attributable to subtle kinetic variations of known enzyme defects not detectable in standard in vitro assays, it seems probable that other primary purine enzyme defects remain to be discovered. For example, in vivo[33] and in vitro[34] studies with drugs that inhibit IMP dehydrogenase suggest that deficiency of this enzyme might well be associated with primary purine overproduction.

PURINE ENZYME ABNORMALITIES AND IMMUNODEFICIENCY

A great deal of interest has been recently aroused by the association of two inborn errors of purine metabolism with immune deficiency syndromes.

Severe deficiency of *adenosine deaminase* (ADA) was first reported in red cell haemolysates from two children with severe combined immunodeficiency in 1972.[35] Three years later Eloise Giblett also reported a gross deficiency of red cell *purine nucleoside phosphorylase* (PNP) in children with recurrent infections and a predominant T-lymphocyte deficiency.[36]

The causal relationship between these inherited enzyme abnormalities and the immunodeficiency syndromes is now established and has highlighted the need for an intact purine catabolic pathway to maintain normal cellular and humoral immunity. In patients with deficiencies of ADA and PNP plasma concentrations of deoxyadenosine[37] and deoxyguanosine[38] are raised. These deoxynucleosides are selectively phosphorylated and trapped by T-lymphocytes with high activities of deoxycytidine kinase and low activities of intracellular deoxynucleotidase. As a result deoxyadenosine[39] and dexoyguanosine[40] accumulate in the cells, leading to inhibition of ribonucleotide reductase and DNA synthesis. An alternative mechanism for the toxicity of deoxyadenosine in ADA deficiency has been proposed by Hershfield,[41] who showed that it causes irreversible 'suicide' inactivation of the enzyme S-adenosyl homocysteine hydrolase. Consistent with this hypothesis S-adenosyl homocysteine hydrolase activity is found to be considerably reduced in the red cells of children with ADA deficiency,[42] but an alternative explanation needs to be found to explain the T-cell deficiency in PNP deficiency, as deoxyguanosine does not inhibit the S-adenosyl homocysteine hydrolase enzyme.

To explain the difference in the immunodeficiency syndromes associated with ADA and PNP deficiency, evidence has recently been produced to show that intracellular ATP activities are depleted with accumulation of deoxyadenosine but not with deoxyguanosine, so killing the non-dividing helper T-lymphocytes.[43] Humoral immunity persists in patients with PNP deficiency because T-helper function is relatively proliferation independent.

Decreased activity of the *ectopurine 5' nucleotidase* (5'NT) enzyme has been associated with X-linked[44] and acquired adult onset (common variable) hypergammaglobulinaemia.[45] Deficiency of this enzyme does not, however, result from a primary gene mutation and there is no evidence to suggest that it is causally related to B-cell deficiency. 5'NT deficiency in X-linked agammaglobulinaemia largely reflects the deficit in circulating B-cells which have high 5'NT activity. B-cell numbers are, however, almost normal in common variable hypogammaglobulinaemia and in this situation it seems likely that reduction in 5'NT is associated with a population of relatively immature B-lymphocytes. 5'NT has been previously shown to be a marker of differentiation in both T[46] and B[47] lymphocytes.

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