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ARGININE METHYLTRANSFERASE ACTIVITY IN CHRONIC ERYTHREMIC MYELOSIS (DIGUGLIELMO SYNDROME)

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Summary

Activity of *S*-adenosylmethionine-dependent arginine methyltransferase was substantially higher in sonicated bone marrow samples from 6 patients with chronic erythremic myelosis than in bone marrow from 3 patients with untreated pernicious anemia, 2 patients with autoimmune hemolytic anemia, and 4 normal persons. Increased activity of this enzyme may be one of the factors contributing to the pathogenesis of methylated arginines in histones of erythroblasts from patients with chronic erythremic myelosis.

Previous reports have indicated abnormalities of arginine-rich histone in erythroblasts from patients with chronic erythremic myelosis [1—3]. Seemingly unique to this disorder was the presence of methylated arginines in the histone [4, 5]. As yet, mechanisms responsible for pathogenesis of these substances in chronic erythremic myelosis are unknown. One possible mechanism may be increased production of methylated arginines as a result of increased enzyme catalysis by *S*-adenosylmethionine-dependent arginine methyltransferase (protein methylase I), an enzyme that preferentially methylates the guanidino group of arginine contained in histones [6—10]. To explore this possibility, measurements of arginine methyltransferase activity were made in sonicated samples of bone marrow obtained from patients with chronic erythremic myelosis, other disorders of erythropoeisis and normal marrows that served as controls.

Bone marrow was obtained at the time of diagnosis in heparinized syringes from sternum or iliac crest of 6 patients with chronic erythremic myelosis (DiGuglielmo syndrome). These patients had refractory macrocytic anemia with hemoglobins between 7 and 9 g per 100 ml, and marrow composed of 50% proerythroblasts, 40% megaloblastoid intermediate macronormoblasts, and 10% granulocytic precursors and megakaryocytes. Large numbers

of ringed sideroblasts and periodic acid-Schiff reagent positive erythroblasts were found in all patients.

As controls, bone marrow from patients with other erythropoietic disorders characterized by marked erythroblastic hyperplasia of the marrow were used. These included 3 patients with severe untreated pernicious anemia who had hemoglobins between 3 and 5 g per 100 ml, serum B_{12} ranging from 5 to 20 pg/ml, and marrow composed of 40% proerythroblasts, 50% intermediate megaloblasts, and 10% granulocytic precursors and megakaryocytes; and 2 patients with autoimmune hemolytic anemia who had hemoglobins between 4 and 6 g per 100 ml, and strongly positive direct antiglobulin tests due to warm-reacting IgG antibody, and bone marrow composed of 30% proerythroblasts, 60% intermediate normoblasts, and 10% granulocytic precursors and megakaryocytes. As additional controls, bone marrow was obtained from presumed normal persons undergoing evaluations for disorders that did not ultimately disclose hematologic abnormalities. These bone marrows contained approx. 1% proerythroblasts, 30% intermediate normoblasts, 67% granulocytic precursors, and 2% megakaryocytes and plasma cells. In all instances, 200—400 mg bone marrow particles were obtained.

To minimize cellular disruption and loss of cells by fractionation, marrow particles rather than cellular suspensions were used. As noted above, bone marrow flecks from patients with disorders of erythropoeisis were composed largely 90% or greater) of erythroblasts. Bone marrow particles were washed in Hank's solution, suspended in approx. 1 ml 80 mM phosphate buffer (pH 7.1) and subjected to two 5-s microprobe sonications using a Branson sonicator. The sonicated samples were centrifuged at 10 800 × *g* for 30 min in a Sorvall refrigerated centrifuge and dialyzed overnight in the phosphate buffer at 2—5°C. Protein concentrations (11) in the dialysates ranged from 2.1 to 18.4 mg/ml. Highest protein concentrations were found in patients with disorders of erythropoiesis.

S-Adenosyl[^{14}C]methionine (specific activity 53—59 mCi/mmol) obtained from Amersham Searle was used as the source of radiolabeled methyl donor. Calf thymus histone type II-A (Sigma, St. Louis) served as the substrate and sonicated bone marrow was the source of the enzyme. Activity of the *S*-adenosylmethionine-dependent arginine methyltransferase (protein methylase I) was assayed under conditions of pH and substrate that permitted distinction of protein methylase I from other protein methylases [6, 7]. Incubations were carried out at final protein concentrations ranging from 0.4 to 11 mg/ml and at time intervals ranging from 20 to 120 min. Two types of control incubations were performed, one in the absence of sonicated bone marrow as source of the enzyme and the second in the absence of exogenous histone as substrate.

To verify the presence of ^{14}C-labelled methylated arginines formed by enzymatic catalysis of arginine methyltransferase, acid hydrolysates of the incubation mixtures were subjected to amino acid analysis (using a Beckman automated amino acid analyser) and fractions counted for radioactivity [7]. Reference standards included monomethyl-L-lysine, dimethyl-L-lysine, 1-methylhistidine, 3-methylhistidine, monomethyl-L-arginine, N^G-dimethyl-L-arginine, and N^G,N'^G-dimethyl-L-arginine (Sigma, St. Louis). Of labeled amino acids, greater than 90% were ^{14}C-labelled methylated arginines. Enzyma-

tic activity was expressed as pmol *S*-adenosyl[^{14}C]methionine used/min per mg protein [6, 7].

Table I illustrates activities of the enzyme in various disorders. In chronic erythremic myelosis, activity ranged between 2.59 and 5.35 pmol *S*-adenosyl[^{14}C]methionine used/min per mg protein. In autoimmune hemolytic anemia, activities were 2.39 and 2.25, respectively. In pernicious anemia, activities ranged from 1.86 to 2.4 and in normal bone marrow, activities ranged from 1.41 to 2.03. Mean and standard error of mean for each group of disorders are given in Table I. In all instances, enzymatic activity was proportional to protein concentration up to 11 mg (final concentration) and constant up to 60 min incubation. In control incubations performed either in the absence of exogenous histone or sonicated bone marrow, little, if any, enzymatic activity was detectable.

In terms of protein concentration of bone marrow extract and time of incubation, arginine methyltransferase found predominantly in bone marrow erythroblasts in disorders of erythropoiesis and in preparation of normal bone marrow showed properties similar to those reported in other tissues [6—10]. The present studies suggest that activity of this *S*-adenosylmethionine-dependent enzyme is substantially greater in sonicated bone marrow from patients with chronic erythremic myelosis than in other disorders characterized by marked erythroid hyperplasia of the marrow. Further partial purifications of crude sonicated bone marrow might lead to refinements of technique and perhaps additional information regarding properties of the enzyme in various disease states.

Recent studies have described cytochemical [1], ultrastructural [2], and electrophoretic [3] abnormalities of arginine-rich histone in erythroblasts from patients with chronic erythremic myelosis. Methylated arginines have also been demonstrated in histones of these erythroblasts by cytochem-

TABLE I

ACTIVITY OF S-ADENOSYLMETHIONINE-DEPENDENT ARGININE METHYLTRANSFERASE EXPRESSED AS PMOL *S*-ADENOSYL [^{14}C] METHIONINE USED/MINUTE PER MG PROTEIN

Disorder	Activity	Mean
Chronic erythremic myelosis	5.35	
	5.06	
	4.13	3.90 ± 0.47
	3.48	
	2.81	
	2.59	
Autoimmune hemolytic anemia	2.39	2.32 ± 0.07
	2.25	
Pernicious anemia	2.40	
	1.90	2.05 ± 0.17
	1.86	
Normal	2.03	
	2.01	1.81 ± 0.15
	1.78	
	1.41	

ical [4] and electrophoretic [5] methods. As yet, factors contributing to production of increased amounts of arginine-rich and methylated arginine-rich histone in chronic erythremic myelosis are not understood. Histone is the preferred substrate for *S*-adenosylmethionine-dependent arginine methyltransferase [8—10]. In the present studies, increased activity of this enzyme has been shown in the bone marrow from patients with chronic erythremic myelosis. As yet, it has not been demonstrated that increased methyltransferase activity is responsible for increased methylated arginines found in histones of erythroblasts in this disorder. Elevated enzymatic activity may be a secondary phenomenon and bear no direct causal relationship to methylarginine-rich histones in chronic erythremic myelosis. Additional studies will be necessary to determine what effects, if any, methylated arginines have upon processes of gene readout in chronic erythremic myelosis and its evolution, in some instances, into acute myeloblastic or myelomonocytic leukemia.

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