Erythroid Histones in Chronic Erythremic Myelosis[1] (37704)

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Originally described by DiGuglielmo (1, 2), acute erythremic myelosis is a fulminant disorder characterized by refractory macrocytic anemia and striking erythroid hyperplasia of the bone marrow. Chronic erythremic myelosis (3, 4) shares many of the hematologic findings of the acute form but has a more indolent clinical course. Chromosomal aberrations (5), decreased amounts of heme synthetase and delta alanine synthetase (6), periodic acid-Schiff positivity of proerythroblast cytoplasm (7), and ultrastructural abnormalities in iron storage (8) have been reported in both acute and chronic erythremic myelosis. The pattern of block-like chromatin aggregates and coarse-appearing chromatin strands in the erythroid precursors of erythremic myelosis has been called megaloblastoid (4). To date, the basic factors leading to the development of megaloblastoid erythropoiesis remain largely unknown. A substantial number of patients with erythremic myelosis subsequently develop myeloblastosis, myelomonocytosis, or erythroleukemia (9). Consequently megaloblastoid erythropoiesis may reflect an underlying neoplasia.

The present studies describe abnormalities in the histones of primarily proerythroblasts and megaloblastoid erythroid precursors obtained from the bone marrow of three patients with chronic erythremic myelosis. It is suggested that the histone abnormalities to be described may contribute to the development of megaloblastoid-type erythropoiesis in patients with this disorder.

Materials and Methods. As defined by the criteria of Dameshek and Gunz (9), the three patients with chronic erythremic myelosis had macrocytic anemia, leukopenia, thrombocytopenia, and splenomegaly, and their anemia was refractory to known hematinic agents. Bone marrow was also obtained from patients with other disorders of erythropoiesis. These included five patients with classical untreated pernicious anemia, two patients with severe folate deficiency, and two patients with autoimmune hemolytic anemia. These marrows served for comparison to the erythremic myelosis marrows. A "second pull" specimen through the original diagnostic needle puncture was aspirated into a lightly heparinized glass syringe. Marrow particles and sinusoidal blood were expressed into a disposable plastic test tube (Falcon Plastics, Los Angeles). In Wright's stained films of bone marrow, erythroid precursors constituted up to 80% of the marrow cells. Because of the sample limitations imposed by a routine diagnostic marrow aspiration and "second pull," fractionation of the marrow cells was not attempted.

Ten milliliters of a solution of 0.3% saponin in 0.162 *N* NaCl was added to the marrow suspension to hemolyze erythrocytes, and the marrow flecks were washed in Hanks' solution (BBL, Cockeysville, MD). Approximately 30–50 mg of marrow flecks remained. The tubes were agitated gently, centrifuged at 1200 rpm for 5 min and the supernatant discarded. The lysine-rich and arginine-rich histones were extracted from the nuclei of marrow cells (predominantly erythroblasts) by the method of Gershey *et al.* (10). They were subjected to electrophoresis in polyacrylamide gel according to the method of Panyim and Chalkley (11) using a Canalco

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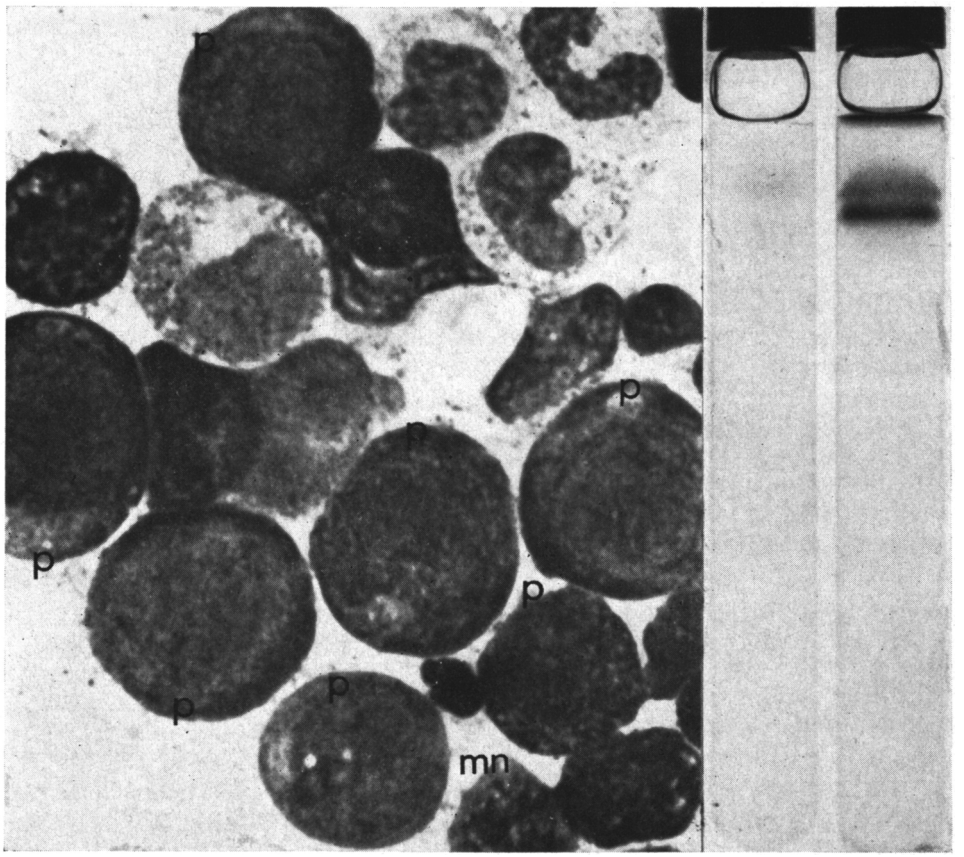

FIG. 1. Bone marrow, chronic erythremic myelosis, Wright's stain. Large proerythroblasts (p) can be seen along with several megaloblastoid early intermediate macronormoblasts (mn) and several granulocyte precursors. ×1200.

The polyacrylamide gel electrophoretic patterns of histones extracted from these cells are shown to the right of the photomicrograph. The gels were stained with alkaline Fast green to identify histone bands. The gel immediately adjacent to the photomicrograph illustrates the electrophoretic pattern of the histone extracted at pH 1.8 (lysine rich). There are two lightly stained bands near the upper part of the gel. The remaining gel contains the electrophoretic pattern of histones extracted at pH 1.0 (arginine rich). There are two darkly stained bands, especially the lower of the two bands.

disc electrophoresis apparatus (Canalco, Rockville, MD). Approximately 1–2 mg of protein as determined by the method of Lowry *et al.* (12) were applied to each gel.

The gels were fixed in saturated picric acid for 1 hr, then in 0.05 *M* Veronal buffer, pH 9.0, for 1 hr according to the method of Tidwell *et al.* (13). The gels were stained in a solution of 1% Fast green FC in 0.05 *M* Veronal buffer, pH 9.0, for 1 hr to specifically identify histone bands (13). The excess stain was removed by immersion in several changes of the Veronal buffer. Duplicate gels were fixed in 7% acetic acid and stained with Coomassie blue to compare with the Fast green-stained gels. This procedure determined the presence of nonhistone protein in the fractions tested. Samples were also subjected to amino acid analysis by methods described previously (14).

Results. A photomicrograph of a bone marrow sample stained with Wright's stain from a representative patient with chronic erythremic myelosis is seen in Fig. 1. Proerythro-

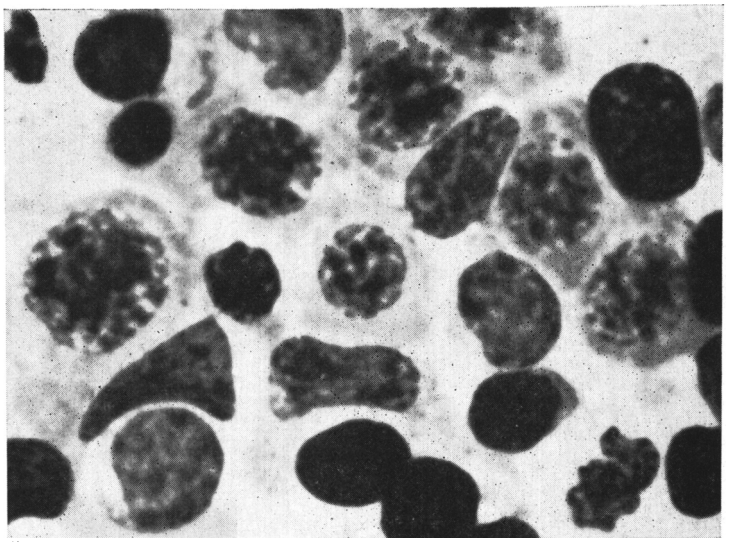

FIG. 2. A group of early intermediate macronormoblasts with megaloblastoid chromatin pattern from the bone marrow of a patient with chronic erythremic myelosis. Wide chromatin strands and block-like aggregates of chromatin impart a coarsely fenestrated appearance to the nucleus. ×1200

blasts constituted approximately 50–60% of the marrow differential cell count. The chromatin pattern in the early intermediate macronormoblasts was megaloblastoid (Fig. 2).

The electrophoretic pattern of the histones extracted from one of these representative bone marrow samples is seen in Fig. 1 adjacent to the photomicrograph of the bone marrow cells. In the sample extracted at pH 1.8 (lysine rich), two faintly stained bands are observed in the upper part of the gel. In the sample extracted at pH 1.0 (arginine rich), two prominent bands are observed. The lower of the two bands is especially darkly stained with alkaline Fast green.

In untreated pernicious anemia and severe folate deficiency, proerythroblasts constituted 60% of the marrow cells. Most of the remaining cells were large early intermediate megaloblasts and occasional (5%) giant metamyelocytes with aberrant-appearing nuclei. Electrophoresis of histones obtained from these developing erythroblasts (14) showed two separate histone bands in the fraction extracted at pH 1.8 (lysine rich). The material extracted at pH 1.0 showed two bands, the lower of which was faintly stained. The electrophoretic pattern of histones obtained from pernicious anemia marrows appeared indistinguishable from the pattern of histones obtained from severe folate-deficient marrows.

In autoimmune hemolytic anemia, approximately 90% of the bone marrow cells were late intermediate normoblasts with normoblastic maturation. The electrophoretic patterns of histones obtained predominantly from these late normoblasts showed a faintly visible band in the pH-1.8 fraction (lysine rich). In contrast, two darkly stained bands were seen in the pH-1.0 (arginine rich) fraction at a location below the faint band in the corresponding pH-1.8 gel.

In all 24 samples, Coomassie blue-stained gels did not reveal any additionally stained protein bands when compared to duplicate gels stained specifically for histone with alkaline Fast green.

Amino acid analyses. Amino acid analyses of the histone fractions appear in Table I. In comparing the pH-1.8 fractions, the highest amount of lysine was found in the pernicious anemia histones. Lysine was substantially lower in the pH-1.8 erythremic myelosis histones compared to the pH-1.8 pernicious anemia histones. Arginine and lysine were lowest in the autoimmune hemolytic anemia histones. Aspartic acid and serine were highest in pernicious anemia histones, and proline was unusually high in autoimmune hemo-

TABLE I.

Amino acid	Moles of amino acid/100 moles total amino acids							
	Pernicious anemia		Erythremic myelosis[a]				Autoimmune hemolytic anemia	
	pH 1.8	pH 1.0	pH 1.8		pH 1.0		pH 1.8	pH 1.0
			a	b	a	b		
Lys	10.8	7.9	6.8	7.4	7.6	8.3	5.8	6.0
His	3.4	3.7	2.6	3.0	2.7	3.1	2.8	2.3
Arg	5.0	4.8	4.5	6.2	4.3	5.0	3.1	35.7
Asp	10.5	9.3	8.6	9.4	12.4	9.8	6.6	9.1
Thr	4.3	5.8	4.6	5.7	5.4	5.8	3.0	4.0
Ser	10.3	8.3	6.8	6.0	7.5	7.3	4.2	5.5
Glu	11.2	11.6	12.6	11.3	11.5	11.3	7.5	8.1
Pro	5.0	5.2	8.0	4.6	4.9	6.3	42.2	4.5
Gly	11.2	8.5	10.9	7.8	9.8	8.6	5.7	7.0
Ala	9.8	9.7	11.9	9.8	11.6	9.6	6.9	2.2
Val	4.1	5.5	4.9	5.4	4.7	5.3	7.8	4.2
Met	0.0	0.0	3.0	1.8	1.0	1.6	0.1	0.1
Iso	1.8	1.6	1.6	2.9	2.7	2.3	0.9	1.2
Leu	8.0	10.7	8.2	10.3	9.1	8.3	5.3	7.0
Tyr	4.4	5.1	3.7	3.7	2.9	4.4	2.4	2.7
Phe	0.0	0.7	1.0	3.8	0.5	3.3	2.3	0.3

[a] In the case of erythremic myelosis, a and b represent different patients.

lytic anemia histones.

In the case of pH-1.0 histones, the amount of lysine in the erythremic myelosis histones was lower than in the pernicious anemia histones. Lysine, alanine, and tyrosine were lowest in the autoimmune hemolytic anemia histones, and arginine was unusually high in the latter histones. Glutamine and glycine were higher in pernicious anemia histones than in the others, and isoleucine was highest in erythremic myelosis histones.

The Lys/Arg ratios of these various histone fractions appear in Table II. The Lys/Arg ratio was highest in the pH-1.8 histones obtained from pernicious anemia marrows. The ratio was lower in the pH-1.8 fraction obtained from erythremic myelosis and autoimmune hemolytic anemia marrows. The lowest Lys/Arg ratio (0.2) occurred in the pH-1.0 histones obtained from autoimmune hemolytic anemia marrows.

Discussion. These studies complement earlier cytochemical studies relating to erythroid histone abnormalities in pernicious anemia and erythremic myelosis (15). In these studies, the ammoniacal silver stain revealed discrete punctate deposits of arginine-rich histones in the nuclei of proerythroblasts of erythremic myelosis. Such deposits were not present in the nuclei of pernicious anemia proerythroblasts.

The present amino acid analyses show that histones obtained primarily from proerythroblasts and megaloblastoid erythroid cells in erythremic myelosis are more arginine rich than those obtained from proerythroblasts and megaloblasts in untreated pernicious anemia. A preponderance of arginine-rich histones is characteristic of erythroid precursors considerably more mature in their development than the proerythroblast (10). In this

TABLE II.

Sample	Lys/Arg ratio[a]
Erythremic myelosis	
pH 1.8	1.5 a, 1.2 b
pH 1.0	1.8 a, 1.6 b
Pernicious anemia	
pH 1.8	2.2
pH 1.0	1.6
Autoimmune hemolytic anemia	
pH 1.8	1.6
pH 1.0	0.2

[a] a and b represent different patients.

sense, the findings described suggest that the proerythroblasts and early intermediate megaloblastoid macronormoblasts in erythremic myelosis may be "older" than might be anticipated from their morphological appearance. It also suggests that in both untreated pernicious anemia and erythremic myelosis, a characteristic histone abnormality is present in cells as early in development as the proerythroblast, even though in both instances these cells may be morphologically indistinguishable.

In addition, the electrophoretic patterns of the pH-1.8 and pH-1.0 histone fractions in erythremic myelosis strongly resemble the patterns of histones obtained from erythroid precursors of patients with *treated* pernicious anemia (14). In erythremic myelosis, the histones were extracted primarily from proerythroblasts and megaloblastoid intermediate macronormoblasts. In treated pernicious anemia, the histones were extracted primarily from late intermediate macronormoblasts.

Several explanations for the histone abnormalities in erythremic myelosis can be offered. It is possible that the proerythroblasts and early intermediate macronormoblasts in erythremic myelosis synthesize abnormally increased amounts of arginine-rich histones. Alternatively, the cells may produce normal amounts of arginine-rich histones but may not be able to degrade it. This could lead to an "accumulation" of arginine-rich histones in the nucleus. Increased amounts of arginine-rich histones could then bind chromatin strands into the block-like coarsely fenestrated chromatin pattern characteristic of megaloblastoid maturation. Abnormalities in a lysosomal histonase have been suggested as factors contributing to these aberrations.

Arginine was especially high in the pH-1.0 fractions obtained from the autoimmune hemolytic anemia marrows. The predominant cells in these marrows were late intermediate normoblasts. Therefore, the increased amounts of arginine in the histones extracted from these cells may reflect their increased stage of maturity and may relate to the compact-appearing densely aggregated chromatin of their nuclei.

Summary. Lysine-rich and arginine-rich histones were extracted from erythroid precursors of bone marrow obtained from three patients with chronic erythremic myelosis. On polyacrylamide gel electrophoresis, the histone patterns resembled the patterns of histones extracted from erythroid precursors in patients with treated pernicious anemia. Amino acid analyses of histone fractions demonstrated that the erythremic myelosis histones were arginine rich. Relationships between these findings and the pathogenesis of megaloblastoid erythropoiesis are discussed.

1. DiGuglielmo, G., Boll. Soc. Med. Chir. Ser. (Pavia) **1**, 665 (1926).

2. DiGuglielmo, G., Hematologica **9**, 301 (1928).

3. DiGuglielmo, G., and Quattrin, N., Hematologica **24**, 241 (1966).

4. Heilmeyer, L., and Schoener, W., Deut. Arch. Klin. Med. **187**, 223 (1941).

5. Kiossoglou, K. A., Mitus, W. J., and Dameshek, W., Blood **28**, 241 (1966).

6. Steiner, M., Baldini, M., and Dameshek, W., Blood **22**, 310 (1963).

7. Quaglino, D., and Hayhoe, F. G. J., Brit. J. Haematol. **6**, 26 (1960).

8. Schnitzer, B., and Kass, L., Amer. J. Clin. Pathol. **60**, 343 (1973).

9. Dameshek, W., and Gunz, F., "Leukemia." Grune and Stratton, New York (1964).

10. Gershey, E. L., Haslett, G. W., Vidali, G., and Allfrey, V. G., J. Biol. Chem. **244**, 4871 (1969).

11. Panyim, S., and Chalkley, R., Arch. Biochem. Biophys. **130**, 337 (1969).

12. Lowry, O. H., Rosebrough, M. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

13. Tidwell, T., Allfrey, V. G., and Mirsky, A. E., J. Biol. Chem. **243**, 707 (1968).

14. Kass, L., Blood **41**, 549 (1973).

15. Kass, L., J. Histochem. Cytochem. **20**, 817 (1972).

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