Histone Abnormalities in Adult Acute Leukemias

By Lawrence Kass

Arginine-rich and lysine-rich histones were extracted from various cytologic types of leukemic blasts and from preparations rich in normal monocytes. On polyacrylamide disc electrophoresis, the patterns of normal monocyte histones closely resembled those found in acute histiomonocytic leukemia (Schilling type). The electrophoretic patterns of histones obtained from leukemic blasts in acute myelomonocytic leukemia (Naegeli type) were similar to those found in both acute myeloblastic leukemia and chronic granulocytic leukemia. The results support the concept that acute myelomonocytic leukemia may be closely related to, or a variant of, acute myeloblastic leukemia, and that acute histiomonocytic leukemia is most probably a monocytic rather than a myeloblastic disorder. In addition to accepted morphologic and enzymatic criteria, the present studies suggest that differences in histone patterns might be useful in further distinguishing between histiomonocytic, myeloblastic, and myelomonocytic leukemias.

This report describes differences in the electrophoretic patterns of lysine-rich and arginine-rich histones extracted from the nuclei of blasts in various cytologic types of adult acute leukemia. In addition to the accepted morphologic and cytochemical criteria for differentiating between the various types of adult acute leukemia, the present study suggests that differences in the composition of histones might be employed as additional criteria in separating the acute myeloblastic, myelomonocytic, and histiomonocytic leukemias.

MATERIALS AND METHODS

Twenty milliliters of heparinized peripheral venous blood samples were obtained from two patients with acute lymphoblastic leukemia, six patients with acute myelomonocytic leukemia (Naegeli type), three patients with acute histiomonocytic leukemia (Schilling type), two patients with acute myeloblastic leukemia, and one patient with chronic granulocytic leukemia. All of the patients had white blood cell counts of approximately 80,000/cu mm. In the case of the acute leukemias, the peripheral blood differential counts showed a homogeneous population of cells (e.g., myeloblasts, lymphoblasts, “histiomonocytes,” and “myelomonocytes,” respectively). In the case of chronic granulocytic leukemia, the leukocytes consisted primarily of progranulocytes and myelocytes.

The tubes containing the peripheral blood were centrifuged at 2000 rpm for 15 min to separate white cells. The buffy coat layers were washed twice with Hanks’ solution (BBL, Cockeysville, Md.). Lysine-rich and arginine-rich histones were extracted from the buffy-coat leukocytes by the method of Gershey et al., and equivalent amounts of histones from each patient (approximately 2.0 mg/gel) were subjected to electrophoresis in polyacrylamide gel according to the method of Panyim and Chalkley. More reproducible electrophoretic patterns occurred if the initial samples of histone were incorporated into a stacking gel having the same composition as...
the separating gel. Electrophoresis was performed in a Canalco analytic disc electrophoretic apparatus (Canalco, Rockville, Md.) for 4 hr at 1 mA/tube. The gels were fixed in 0.9\% acetic acid and stained for histones with alkaline fast green (National Aniline Division, Allied Chemical Corporation, New York, N.Y.) according to previously described methods.8

Preparations rich in normal monocytes were prepared by the method of Bennett and Cohen9 from the pooled buffy coats of 3000 ml of fresh ACD blood from six donors who were presumed to be normal. These preparations contained approximately 75\%–80\% monocytes, and the remainder of the cells were polymorphonuclear leukocytes and lymphocytes. Histones were extracted from the preparations rich in normal monocytes and subjected to polyacrylamide disc electrophoresis by the methods described above.

In all cases of acute leukemia, films of capillary peripheral blood and bone marrow were made between methanol-cleaned glass cover slips at the time of diagnosis and stained with Wrights' stain for conventional light-microscopic examination. In all instances, cover slip films were stained for glycogen with PAS (periodic acid-Schiff) reagent.5 Separate cover slip films were stained for nonspecific esterase activity using alpha-naphthyl acetate as substrate to demonstrate monocytic properties of the cells, and for specific esterase activity using ASD-chloroacetate as substrate to demonstrate granulocytic properties of the cells. Both esterase reactions were performed on the same cover slips according to the method of Yam et al.10 Esterase and PAS stains were also performed on cover slip films containing suspensions of monocytes from the six donors who were presumed to be normal.

Morphologic diagnoses were made by two independent observers, and classification of acute leukemias into lymphoblastic, myeloblastic, myelomonocytic, and histiomonocytic types were made according to established criteria.3–5 The cytochemical reactions were characteristic of the particular cytologic type of acute leukemia,3–5 and, in the case of the monocytic leukemias, had the expected differential reactions of specific and nonspecific esterases: namely, positive specific and nonspecific esterase activities in myelomonocytic leukemia, and negative specific but positive nonspecific esterase in histiomonocytic leukemia. In this study, only typical cases were used in which both the morphology and cytochemistry were clear-cut.

RESULTS

Figure 1A is a photomicrograph of a typical normal monocyte-rich preparation stained with Wrights' stain. The nonspecific esterase reaction was
Fig. 2. (A) Lymphoblasts, acute lymphoblastic leukemia. Wrights’ stain × 1200. (B) Disc electrophoretic pattern of histones extracted from lymphoblasts. Left, lysine-rich histone; right, arginine-rich histone.

strongly positive, and specific esterase was negative. The electrophoretic pattern of histones extracted from preparations rich in normal monocytes is shown adjacent to the photomicrograph (Fig. 1B). The lysine-rich and arginine-rich fractions each contained three bands in the lower part of the gel. Several faintly staining slow-moving bands were sometimes observed in the upper part of the gel containing the arginine-rich histone.

In the two patients with acute lymphoblastic leukemia, typical lymphoblasts were seen, as illustrated in Fig. 2A. The reactions for both specific and nonspecific esterases were negative, but the reaction for glycogen using the PAS reagent was strongly positive in a granular pattern. The histones obtained from lymphoblasts showed two bands in the electrophoretic pattern in the lysine-rich fraction, and three bands in the arginine-rich fraction (Fig. 2B).

In the two patients with acute myeloblastic leukemia, large numbers of myeloblasts were found in the peripheral blood (Fig. 3A). The PAS and nonspecific esterase reactions were negative, but the reaction for specific esterase was strongly positive. The electrophoretic pattern of the histones obtained from myeloblasts is seen in Fig. 3B. The lysine-rich fraction demonstrated six bands, and the arginine-rich fraction showed eight bands. In most cases, seven or eight bands were seen in the arginine-rich fractions.

“Monocytoid blasts” and “myelomonocytes” from the peripheral blood of six patients with acute myelomonocytic leukemia (Naegeli type), are shown in Fig. 4A. The PAS reaction was negative, but positive reactions for both specific and nonspecific esterases were seen in most of the leukemic cells. The histones obtained from these leukemic cells yielded electrophoretic patterns as seen in Fig. 4B. The lysine-rich fraction showed six bands, and the arginine-rich fraction showed eight bands. In several cases, seven bands were seen in the arginine-rich fractions.
Fig. 3. (A) Myeloblasts, acute myeloblastic leukemia. Wright's stain × 1200. (B) Disc electrophoretic pattern of histones extracted from myeloblasts. Left, lysine-rich histone; right, arginine-rich histone.

Fig. 4. (A) "Myelomonoblasts," acute myelomonocytic leukemia. Wright's stain × 1200. (B) Disc electrophoretic pattern of histones extracted from blasts in acute myelomonocytic leukemia. Left, lysine-rich histone; right, arginine-rich histone.
In the cases of acute histiomonocytic leukemia (Schilling type), typical “histiomonoblasts” were found, as illustrated in Fig. 5A. The PAS and specific esterase reactions were negative, but the reaction for nonspecific esterase was strongly positive. The electrophoretic patterns of the histones extracted from these “histiomonocytes” and “histiomonoblasts” are shown in Fig. 5B. The lysine-rich histones and the arginine-rich histones each demonstrated three and, in some cases, four bands in the lower part of the gel, strongly resembling the electrophoretic pattern found in normal monocyte histones.

In the case of chronic granulocytic leukemia, the peripheral blood leukocytes were primarily progranulocytes and myelocytes (Fig. 6A). They were PAS- and specific esterase-positive and nonspecific esterase-negative. The histones extracted from these leukemic cells produced the electrophoretic patterns illustrated in Fig. 6B. The lysine-rich histones showed six bands, and the arginine-rich histones showed nine bands, closely resembling the histone patterns found in acute myeloblastic and acute myelomonocytic leukemia.

DISCUSSION

The present studies have demonstrated differences in the electrophoretic patterns of histones extracted from various cytologic types of leukemic blasts in adult acute leukemia. The strong resemblance of the histones obtained from leukemic blasts in acute myelomonocytic leukemia to those obtained from myeloblasts in acute myeloblastic leukemia suggests that, in terms of their basic nucleoproteins, acute myeloblastic leukemia and acute myelomonocytic leukemia appear to be closely related, and that acute myelomonocytic leukemia may be a cytologic variant of acute myeloblastic leukemia. This concept is in
accord with recent morphologic and cytochemical studies of leukemic blasts in acute myelomonocytic leukemia.1–5

The present report also indicates that the electrophoretic pattern of histones obtained from leukemic blasts in acute histiomonocytic leukemia strongly resembled the pattern of histones obtained from presumed normal monocytes, and did not resemble the electrophoretic pattern of myeloblast histones. These findings lend themselves to the interpretation that, in terms of basic nucleo-proteins, acute histiomonocytic leukemia is probably a monocytic rather than a myeloblastic disorder, as suggested by Reschad and Schilling many years ago11 and substantiated by recent morphologic and cytologic studies.1,5

In the case of chronic granulocytic leukemia, in which the histones were extracted predominantly from myelocytes and progranulocytes, the electrophoretic patterns of these histones were similar to the electrophoretic patterns of myeloblast histone. This single case suggests that primitive granulocytic precursors (myeloblasts) share similar histone characteristics with better differentiated granulocytic cells such as myelocytes and progranulocytes. The electrophoretic pattern of lymphoblastic histone differed from normal monocytic histone in that several additional slow-moving bands were often found in the monocytic histone.

Most of the available evidence to date supports the concept that histones are involved in the regulation of gene function and control of cellular differentiation.12 Histones are believed to modulate the physical state of chromatin such that changes in histone might be expected to occur when cells undergo major metabolic changes.13 Recently it has been shown that phosphorylation of histone is one of the earliest biochemical events associated with the conversion of nonproliferating cells to proliferating cells, and that two phosphoryla-
tion events are involved with the condensation of interphase chromatin into mitotic chromosomes.¹⁴

In a study using human leukemic bone marrow cells, Frenster¹⁵ found that acridine orange bound to DNA exclusively within the active extended euchromatin of the nucleus. Histones inhibited the binding of acridine orange. It was stated that the avid binding of acridine orange to the euchromatin occurred as a result of the fact that histones in the euchromatin were less tightly bound to DNA than were the histones within the repressed heterochromatin. Additional recent studies on histones obtained from megaloblastoid erythroid precursors in the DiGuglielmo syndrome⁶,⁷ indicated that these histones differed from those obtained from erythroid precursors in a variety of other types of anemia in that they had a unique electrophoretic pattern⁶ and contained arginine in a methylated form.⁷ Methylated arginines have also been detected cytochemically in the histones of DiGuglielmo erythroid precursors.¹⁸

The present report suggests that there may be differences in the electrophoretic pattern of histones obtained from various cytologic types of leukemic blasts, and that perhaps these differences in histone patterns might be used, in addition to accepted morphologic and cytochemical criteria, to further distinguish between acute myelomonocytic, myeloblastic, and histiomyeloblastic leukemia. In future studies it will be necessary to examine a larger number of cases to find out whether the histone analysis merely parallels enzyme studies in typical cases or will be helpful in classifying all instances of adult acute leukemia. In this regard, it will also be important to examine the histones obtained from blasts in patients with leukemic reticulum cell sarcoma⁹ and erythroleukemia to see whether or not they possess unique features.

REFERENCES

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