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Abstract

Leukemic cells were cytologically studied in the human bone marrow culture by the utilization of vital staining of Janus green B and neutral red. The minute cellular morphology of various types of leukemia was studied with special reference to their alterations in the course of the culture. The cytologic deviation of leukemic cells from the corresponding normal blood cells was clarified on monocytic leukemia, chronic myelogenous leukemia with the blastic crisis, chronic myelogenous leukemia, and acute and chronic lymphocytic leukemias.

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A STUDY ON THE CYTOMORPHOLOGIC STRUCTURE OF BLOOD CELLS BY VITAL STAINING

II. LEUKEMIC CELLS IN THE BONE MARROW

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Leukemia is one of the most important diseases among various hematologic discrasias, because we are confronted by its fatal termination and its augmented incidence in the recent years. Much effort has been made by many investigators to characterize this disorder, but the diagnostic difficulty of leukemia is still encountered in certain cases. Despite extensive studies of the morphologic characteristics of leukemic cells on smear preparations^{2,4,5,10}, discrimination of the pathologic myeloblast, monoblast and lymphoblast from each other is a controversial problem with consequent confusion in classifying the type of leukemia.

The supravital staining introduced by COWDRY¹¹ has been utilized by SABIN⁷, SIMPSON⁸, SUGIYAMA⁹, AMANO^{2,3}, etc., for the cytologic investigation of leukemic cells. However, cytologic difference between leukemic cells and their counterparts of the normal blood cells is not always clear in their studies. SIMPSON et al⁸. point out that many of the cells which have all the cytologic features of blast on smear preparations have neutral red particles in the cytoplasm by the supravital staining. Hence, the morphologic characteristics of neutral red particles seem to contribute to the differentiation of leukemic types as well as the diagnosis of leukemia. However, a method such as supravital staining is not sufficient for the study of detailed cellular structure, since the cells which are exposed to the dyes in a high concentration as in the general cases of the supravital staining do not survive for a long period of time and the cellular degeneration occurs quickly.

It is the purpose of the present paper to present the features of leukemic cells and their cytologic deviations from the corresponding normal

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blood cells by means of the culture of the leukemic bone marrow with Janus green B and neutral red in a low concentration in its medium.

MATERIALS AND METHODS

The bone marrow was obtained from 11 cases with various types of leukemia admitted to our clinic from September, 1958 to May, 1959. These were composed of three cases of monocytic leukemia, five cases of chronic myelogenous leukemia including one with the blastic crisis, two cases of acute lymphocytic leukemia and a case of chronic lymphocytic leukemia. They had been diagnosed by phase contrast microscopic study on the leukemic cells in tissue culture. The method employed for the present study was the same as used in the previous study⁶, i. e. the bone marrow culture in conjunction with vital staining with Janus green B and neutral red.

RESULTS

Monocytic Leukemia

In the culture, fine neutral red particles and mitochondria in a small number of leukemic cells of monocytic leukemia begin to stain faintly as early as five minutes after the start of the culture with neutral red and Janus green B, respectively and become fairly deep 30 minutes later. At the 3rd to 4th hour of the culture, the cells having migrated out of the explant form around it a dense growth zone whose outer border is sharply outlined from the culture medium. At this time, all the cells in the growth zone exhibit a deep coloration of mitochondria and neutral red particles. The predominant cell type composing the growth zone is the promonocyte along with a small number of mature monocytes. The normal blood cells which are seen among the leukemic cells in the growth zone vary in cases from small to moderate in number.

The leukemic promonocyte is a small or large round cell, occasionally protruding short needle-like processes from the cytoplasmic margin (Figs. 1, 2). It usually has no motility, but sometimes migrates actively in the form of mature monocytes, mature neutrophils or mature lymphocytes. The nucleus is oval or irregular in shape. There are one to three nucleoli in the nucleus, but they are not frequently seen. The nucleoli are round in shape and somewhat yellowish in color. All the neutral red particles seen in the cytoplasm are the minute granules, i. e. exceedingly fine neutral red particles. They are usually numerous and stain a deep red. There is a variety of the distribution of the minute granules, but the most

characteristic appearance seems to be a large aggregation of the minute granules in the wide portion of the cytoplasm. In a small number of the promonocytes, the minute granules are diffusely scattered in the cytoplasm. When only a few minute granules are present, they gather near the nucleus, or they are scattered in the cytoplasm. The distribution of the minute granules shows no definite relation to the Golgi zone. The mitochondria are usually numerous, staining a deeper greenish blue in color than those of the normal mature monocyte. They are small to intermediate in size and short plump rod or spherical in shape. The mitochondria usually form a large aggregation which is located in a crescent shape in the distal cytoplasm or closely to the aggregation of minute granules in the wide portion of the cytoplasm.

The leukemic mature monocyte whose nucleus is lobulated migrates actively with broad thin pseudopods formed along the entire cytoplasmic margin. The minute granules are numerous, i. e. much more increased in number than in normal mature monocytes, but sometimes they are very few. Many of them are located around the nucleus. The mitochondria are very fine rod in shape and stain a bluish green. They may be distributed around the nucleus and the minute granules.

The monoblast where the neutral red particles are completely absent has hardly been encountered in the present cases of monocytic leukemia.

As time passes in the culture, the minute granules of the mature monocyte quickly develop into neutral red vacuoles which are arranged in a rosette pattern. This rosette is larger and more numerous in the number of neutral red vacuoles than that of the normal mature monocyte. All of the minute granules of the promonocyte, on the other hand, gradually increase in diameter and at the 10th to 20th hour of the culture, each of them develops a definite neutral red vacuole. At about the 30th hour, the entire cytoplasm is occupied by a great number of large neutral red vacuoles, many of them being about equal in size (Figs. 3, 5). Thereafter, the neutral red vacuoles undergo decoloration. The mitochondria of leukemic mature monocyte and the promonocyte are kept stained much more longer than that of the normal mature monocyte. At about the 15th hour from the onset of the staining, the mitochondria stain a dirty deep blue. Subsequently, in a case where the large type of mitochondria is present in leukemic cells, the mitochondria become thinner and elongated. And also they decrease in the intensity of the coloration, showing two or three constrictions on the body of the mitochondria. Unstainable vacuoles start to develop in the peripheral cytoplasm at about the 24th hour from the onset of the culture. At the 50th to 60th hour of the culture, all the cells

in the preparation show no stained particles.

In all of the present cases of monocytic leukemia, large atypical monocytes are observed to arise at about the 10th hour from the start of the culture (Fig. 4). These cells are large and thin, measuring approximately 15 to 20 μ or greater in diameter. The peripheral cytoplasm is very thin and wavy in appearance. The nucleus is usually bilobular and edematous without including any nucleoli. In the cytoplasm, numerous neutral red vacuoles which are small to large in size and stain uniformly salmon-red, are arranged in a large rosette pattern around the large Golgi zone. The mitochondria are usually not seen, although the fading mitochondria may be present at the periphery of the cytoplasm. A small number of unstainable vacuoles are usually present in the peripheral cytoplasm. This type of cells increases in number as time elapses. A small number of Auer body are present in some of the promonocytes and mature monocytes. They have needle-like appearance and stain a bright light red with neutral red. Decoloration of Auer body occurs in about 30 hours from the start of the culture, leaving a refractile colorless crystal in the cytoplasm.

Chronic Myelogenous Leukemia with the Blastic Crisis

The predominant cells seen in the culture are atypical promyelocytes (Figs. 6, 7). They start to stain as early as 30 minutes from the start of the culture, best at the 3rd to 10th hour. These atypical promyelocytes vary in size: many are small and some large. One or two nucleoli are present in the nucleus in about a half of them and some of the nucleoli are quite large in size. The nucleus is round in many of the cells, being irregularly indented in some. The cytoplasm is scanty and outlined with a round saw-toothed margin. The granules have a very peculiar appearance. They are angular in shape, stain a deep orange red and vary from small to large in size, many of them being much larger than those of the normal promyelocyte. They are sparsely scattered around the nucleus, forming one to three small aggregations of a few granules. These granules usually keep their appearance stationary until the cellular degeneration occurs. The mitochondria are numerous and fine rod in shape, staining a greenish blue with Janus green B. They usually form an irregular aggregation in the wide portion of the cytoplasm with a small number of mitochondria scattered around the nucleus.

Chronic Myelogenous Leukemia

In the present four cases of chronic myelogenous leukemia, many of the cytologic features of the constituting cells are similar to those of the normal blood cells. There are, however, some cytologic characteristics

pertaining to this disease as follows :

1) In some of the neutrophilic promyelocytes, the nucleus is constricted, forming a large bilobular nucleus. The minute granules gather near the constricted portion of the nucleus along with mitochondria.

2) There is an increased affinity to Janus green B of the mitochondria of the mature neutrophils in comparison with those of the normal mature neutrophils. The mitochondria of mature neutrophils of this disorder stain a light blue as early as 30 minutes from the start of the culture and keep this coloration for 2 to 4 hours. The mitochondria appear to be increased in number comparing with those of normal neutrophils.

3) There is also an increased affinity to neutral red of the granules of the mature neutrophils, so that the appearance of A type and B type granules is very much similar to those of the normal myelocytes.

4) Several neutral red vacuoles develop in some of the mature eosinophils.

5) There may be small mature basophils in which the granules vary in size and are less in number than in the normal mature basophils. These granules undergo the same swelling and pole formation as seen in normal basophils.

6) In a few of the cells, possibly neutrophils, in one of the present cases, there are crystals which are small to large lozenge in shape and stain a bright orange red with neutral red. The crystals resemble closely to Auer body seen in monocytic leukemia in shape and stainability to neutral red.

Acute Lymphocytic Leukemia

The predominant cells observed in the tissue culture are atypical lymphocytes (Fig. 9). There are a small number of lymphoblasts (Fig. 8). The remainder of the composing cells are normal blood cells. In the culture, the atypical lymphocytes start to stain in about 1 hour from the start of the culture. They are small to intermediate, round cells and often migrate in a hand-mirror shape like the normal lymphocytes. The nucleus is usually irregularly indented and outlined with a clear nuclear membrane. The nucleolus is usually not present. The cytoplasm is scanty and usually protrudes needle-like processes or blebs. The minute granules are usually less than 20 in number, staining a dark red and varying in size. They are distributed around the nucleus, or gather in the nuclear indentation. The mitochondria are 20 to 30 in number and variable in size from small to intermediate ones, staining equally a dark greenish blue. They are diffusely scattered in the cytoplasm, but sometimes gather in the nuclear indentation.

The lymphoblast includes a fairly distinct nucleolus, but other cytologic features are similar to the atypical lymphocyte except for the absence of the neutral red particles.

As time elapses, the minute granules develop into neutral red vacuoles which vary from small to large in size (Fig. 10). The largest vacuoles may exceed 1.5μ in diameter. The mitochondria of the lymphoblasts and the atypical lymphocytes undergo swelling and gradually lose the affinity to Janus green B. All the coloration of the atypical lymphocytes and the lymphoblasts in the preparation fades at about the 30th to 50th hour of the culture.

Chronic Lymphocytic Leukemia

The majority of the cells seen in the preparations consists of lymphocytes (Fig. 11). These mature lymphocytes are small round cells which have a round, oval or occasionally sharply indented nucleus without a nucleolus. The cytoplasm is scanty and outlined with a saw-toothed margin. The minute granules are usually less than 20 in number, staining a deep red and distributed around the nucleus or gathering in a small clump. In a small number of the lymphocytes, these minute granules are absent in the cytoplasm. The mitochondria are round in shape and uniform in size, but far smaller than those of the normal lymphocytes, staining a deep greenish blue with Janus green B. They are rather numerous: i. e. they are much more increased in number than in normal mature lymphocytes and usually are collected in the wide portion of the cytoplasm. As time elapses, all of the minute granules simultaneously develop into neutral red vacuoles. All of the cells fade in about 50 hours from the start of the culture.

DISCUSSION

On the cytologic features of monocytic leukemia, BESSIS⁴, using phase contrast microscope, states that pathologic monocytes frequently develop a horseshoe or twisted nucleus. AMANO^{2,3} emphasizes the occurrence of the nuclear indentation specific to the monocytic series in the monoblast and the presence of the rosette in the promonocyte. This characteristic of the nuclear shape is mostly accepted and has been confirmed in the present study. The rosette, however, is not always observed in the promonocyte. The features of the promonocyte which have been observed in the course of the bone marrow culture can be summarized as follows:

- 1) The minute granules are numerous and aggregate in a portion of

the cytoplasm without surrounding the Golgi zone. All of the minute granules gradually develop into deep salmon-red stained vacuoles which finally occupy the entire cytoplasm.

2) The mitochondria form an aggregation in the cytoplasm which is located in the wide portion of the cytoplasm or in the portion of the cytoplasm most distant from the wide portion. In a typical promonocyte, the aggregation of minute granules and of mitochondria is situated side by side in the wide portion of the cytoplasm.

3) The peripheral cytoplasm has a great tendency to form thin membranous pseudopods along the entire cytoplasmic margin and frequently the cells move actively in the form of a normal mature monocyte. It is very interesting that the migration form of some of the promonocytes closely resembles that of a mature neutrophil or a mature lymphocyte.

4) Large thin atypical monocytes arise in the course of the culture. These cells are presumed to be degenerating promonocytes.

5) Cytologic variability from one cell to others is very great. When a vital staining preparation is observed, one would unquestionably agree that the predominating cells belong to only one cell series. With a careful observation, it is noticed that one cell has a small number of minute granules, whereas another has numerous minute granules without a great cytologic difference between them, namely these cells being in the same developmental stage. In one cell, minute granules and mitochondria form respective aggregation, but one may find other cell in which they are diffusely mingling with each other in the cytoplasm. Hence, the author strongly feels that the great cytologic fluctuation of the cells of the same developmental stage is characteristic of acute leukemia.

The mature leukemic monocyte is usually not present in a large number in monocytic leukemia. This seems to be due to the presence of the maturation arrest. The mature monocyte seen in monocytic leukemia fails to show a significant cytologic difference from the normal mature monocyte. In a leukemic mature monocyte, however, the rosette is larger and the neutral red vacuoles are more variable in size than in a normal mature monocyte.

The features of minute cellular morphology of chronic myelogenous leukemia do not appear to have sufficiently been described hitherto. The author has clarified that there is a cytologic difference between the normal blood cells and the blood cells of chronic myelogenous leukemia in minute cellular morphology. It has been pointed out that there are some cytologic features of chronic myelogenous leukemia, such as the presence of atypical basophils and an increased affinity of granules and mitochondria of

mature neutrophils respectively to neutral red and Janus green B, and that some characteristics of this condition with the blastic crisis, namely, the presence of large angular granules and fine mitochondria in the promyelocyte. As stated in the previous paper⁶, the granules and mitochondria of normal mature neutrophils have a poor affinity to neutral red and Janus green B, respectively, whereas those of normal myelocytes have a strong affinity. Hence, it may be concluded on the basis of the increased affinities to the dyes of the mature leukemic neutrophils that there is maturation arrest of the neutrophils in chronic myelogenous leukemia.

BESSIS⁴ claims the presence of malformation of the nucleus in lymphocytic leukemia. ACKERMAN and BELLIOS¹ state that the number of mitochondria and cytoplasmic granules is greater than in normal mature lymphocytes. In this study, the author has confirmed these findings and demonstrated that the neutral red vacuoles vary in size in the lymphocytes of acute lymphocytic leukemia and the mitochondria are smaller in size in the lymphocytes of acute and chronic lymphocytic leukemia than in normal lymphocytes.

SUMMARY

Leukemic cells were cytologically studied in the human bone marrow culture by the utilization of vital staining of Janus green B and neutral red. The minute cellular morphology of various types of leukemia was studied with special reference to their alterations in the course of the culture. The cytologic deviation of leukemic cells from the corresponding normal blood cells was clarified on monocytic leukemia, chronic myelogenous leukemia with the blastic crisis, chronic myelogenous leukemia, and acute and chronic lymphocytic leukemias.

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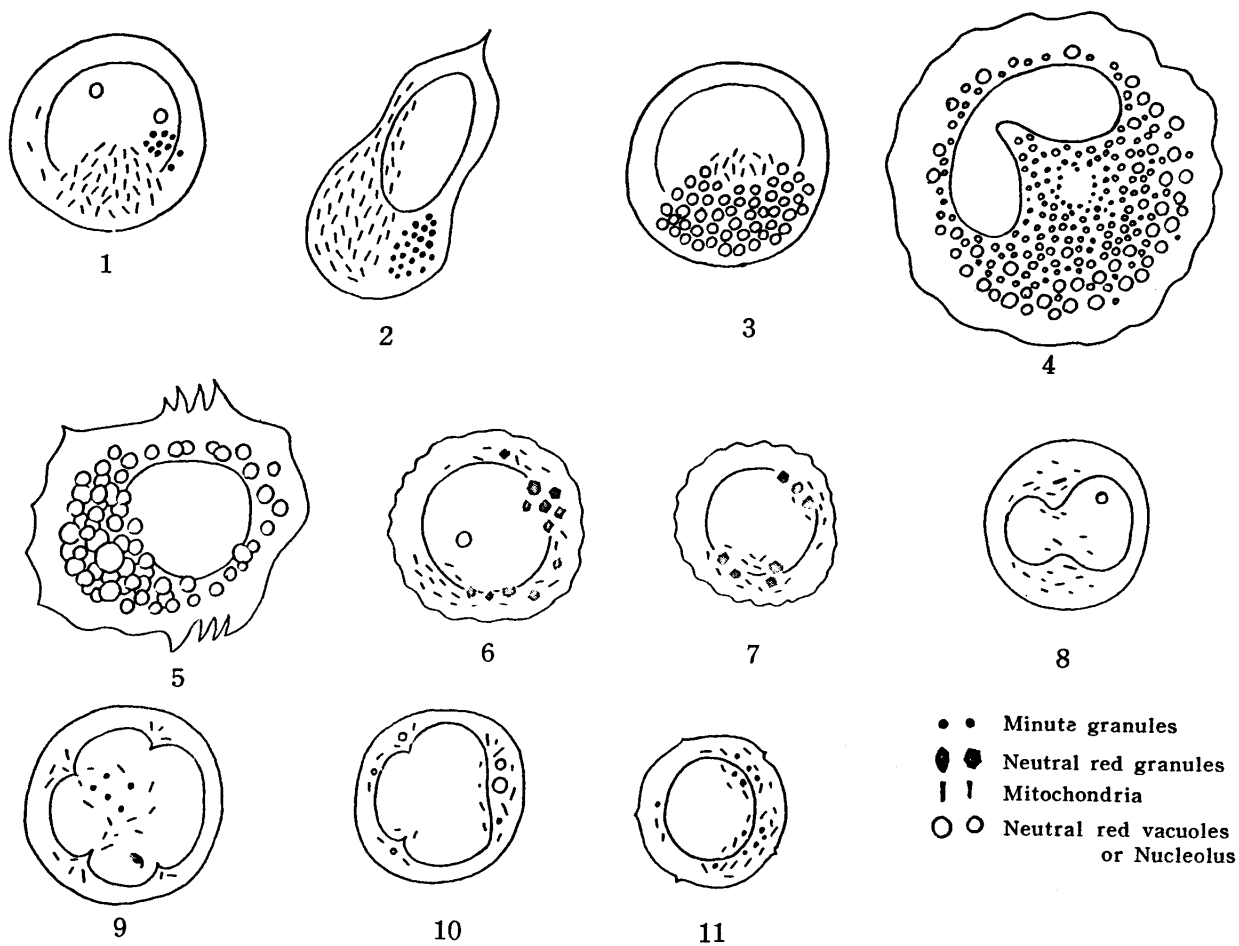
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EXPLANATION OF ILLUSTRATIONS

1. Promonocyte with an aggregation of minute granules and of mitochondria. Monocytic leukemia. 3rd hour of culture.
2. Promonocyte with an aggregation of minute granules and of mitochondria. Monocytic leukemia. 3rd hour.
3. Promonocyte with vacuolized minute granules. Monocytic leukemia. 36th hour.
4. Atypical monocyte with a large rosette formation. Monocytic leukemia. 33rd hour.
5. Degenerating promonocyte with a large number of neutral red vacuoles. Monocytic leukemia. 33rd hour.
6. Atypical promyelocyte with angular granules. Chronic myelogenous leukemia with the blastic crisis. 3rd hour.
7. Atypical promyelocyte with angular granules. Chronic myelogenous leukemia with the blastic crisis. 3rd hour.
8. Lymphoblast with mitochondria varying in size. Acute lymphocytic leukemia. 3rd hour.
9. Lymphocyte with a few minute granules. Mitochondria are collected in the sharp nuclear indentations. Acute lymphocytic leukemia. 3rd hour.
10. Lymphocyte with several neutral red varying in size. Acute lymphocytic leukemia. 10th hour.
11. Mature lymphocyte with numerous fine mitochondria. Chronic lymphocytic leukemia. 10th hour.

Schematic Illustrations of Leukemic Cells in the Bone Marrow.



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