PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 163, 76–80 (1980)

Eosinophil Colony Formation in Acute Nonlymphoblastic Leukemia (40726)

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In recent years, extensive studies have been made on human leukemic cells using various culture systems, such as the semisolid cultures for the colony growth (1–10), the liquid suspension culture (11–15), and the diffusion chamber cultures (16, 17) to elucidate whether impaired differentiation of blast cells of acute nonlymphoblastic leukemia (ANLL) could be due to either a cellular or a regulatory defect. However, results thus obtained and their interpretation are conflicting. This may reflect a wide variation in potential for differentiation and maturation of leukemic blasts among patients. At least in some ANLL cases, blastic cells appear to retain the ability to differentiate to mature granulocytes.

In a series of the agar culture studies on 52 ANLL cases, marrow cells from 14 patients formed variable numbers of eosinophil colonies. Of these, marrow cultures of one patient showed a significant delay in the formation of clusters and colonies, which consisted almost exclusively of mature eosinophils, and an extraordinarily high plating efficiency. Additional analyses including the liquid culture studies suggested that leukemic cells of this case had potential to differentiate mainly into mature eosinophils under appropriate culture conditions, although further studies are necessary to state this equivocally.

There have been few reports with special reference to eosinophilic differentiation of leukemic blasts in ANLL, thus our present case may contribute to our understanding in this area.

Materials and methods. Bone marrow cells from 52 adult patients with ANLL, at diagnosis or relapse, were collected by aspiration; 26 of these were myeloblastic, 6 promyelocytic, 3 monoblastic, 11 myelomonocytic, and 6 erythroleukemia. For normal controls, marrow samples were obtained as portions of diagnostic specimens with informed consent from 15 subjects with no abnormalities of granulopoiesis. Marrow cells from 11 patients with acute lymphoblastic leukemia (ALL) and 9 patients with reactive eosinophilia (3 eosinophilic granuloma of the soft tissue, 2 bronchial asthma, 2 collagen diseases, and 2 chronic renal failure on hemodialysis) were also used as controls.

The agar culture method for marrow cells was performed according to that of Pike and Robinson (18). Briefly, 5×10^4 or 2×10^5 cells were plated in 1 ml of a 0.3% agar layer over a 0.5% agar feeder layer containing 1×10^6 normal blood leukocytes in a 35-mm Falcon culture dish. Cultures were incubated in humidified 7.5% CO_2 in air at 37°C. Clusters (8 to 50 cells) and colonies (more than 50 cells) were counted at intervals up to 35 days with an Olympus inverted microscope. When the number of aggregates was too numerous, counts were performed on the dishes in which 5×10^4 cells were plated. Counts were done on duplicate or triplicate plates. For cell morphology, single clusters and colonies removed from the agar were examined after staining Wright's, peroxidase or nonspecific esterase stains. Most of the eosinophil (E-) clusters and colonies could be distinguished without much difficulty from those of other morphological types (blastic, neutrophil, mononuclear, and mixed) by their characteristic appearance as described by Shoham *et al.* (19). They were more compact, more sharply limited and darker than other types of aggregates.

0037-9727/80/010076-05$01.00/0

At 3 to 4 weeks of culture, the scoring of E-colonies is much easier in our hands than at earlier times, because the E-colonies appear much darker and most of the other types of colonies have died or dispersed by this time. When ambigious, however, they were confirmed by cytological studies as described above.

Liquid culture of marrow cells from 27 out of the 52 patients with ANLL and 8 out of the 15 normal controls were also done using the method described by Golde and Cline (11). Briefly, the culture apparatus consists of a glass bulb closed at one end by dialysis membrane and mounted in a 125-ml Erlenmeyer flask. The bulb contains the bone marrow cells (3×10^6), and the flask is filled with approximately 50 ml of the culture medium. The flasks were incubated at 37°C in humidified 7.5% CO_2 in air. Cultures were harvested at intervals up to 21 days for viable cell counts (trypan blue) and differential counts of Wright-stained cells.

Electron microscopic studies were performed on the cells before the culture and from the colonies after 28 days of culture. The cells in the colonies were fixed by overlaying agar plates with 1% glutaraldehyde in 0.1 *M* phosphate buffer at 4°C for 24 hr. They were then washed with the buffer, refixed with 1% OsO_4 at room temperature for 1 hr, dehydrated, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi HS-9 electron microscope.

Chromosome studies were performed on the marrow cells before culture and after 10 days of liquid culture, according to the method previously described (20).

Results. Fourteen of fifty-two patients with ANLL showed the formation of various numbers of E-colonies in agar cultures of bone marrow cells (Fig. 1). Of these, one patient (Y.K.) is of special interest in respect that the marrow cells formed almost exclusively E-colonies with an extremely high plating efficiency (520 per 10^5 marrow cells, about 24 times the normal control value (22 ± 8)). Although the incidence of E-colonies in four other patients were also greater than that in the normal controls, the incidence of blastic colonies with

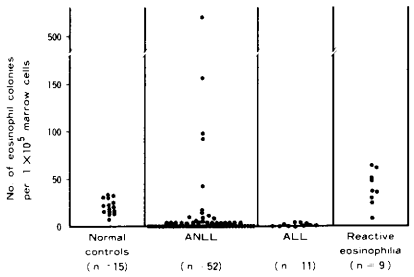

FIG. 1. The incidence of eosinophil colonies formed by 10^5 marrow cells from the normal controls and patients with ANLL, ALL, and reactive eosinophilia.

impaired maturation was much more than that of E-colonies. No correlation was noted between the percentage of eosinophils in the marrow and the incidence of E-colonies. On the other hand, marrow cells from all the 11 patients with ALL showed a great decrease in the formation of E-colonies (0 to 5 per 10^5 marrow cells). An increase in the incidence of E-colonies was observed in some patients with reactive eosinophilia, but the degree of the increase was much lower than that seen in the patient Y.K.. We describe here the results of further investigations on this patient.

When the studies were performed, the patient, 20-year-old man, was at the first relapse, 10 months after complete remission by chemotherapy. The white blood cell count was 19.8×10^9/liter with myeloblastosis (75%). The bone marrow aspirate was hypercellular, with a preponderance of myeloblasts and neutrophilic promyelocytes (95.2%). Less than 1% eosinophils, either immature or mature, were noted in the Wright-stained smears of the peripheral blood or bone marrow. Myeloblasts with Auer rods were seen occasionallly.

Figure 2 shows the comparison of the growth patterns of E-aggregates (clusters and colonies) formed by the marrow cells from the patient and 15 normal controls. In the normal controls the formation of E-colonies reached a peak at 3 to 4 weeks of culture, 1 to 2 weeks later than that of other types of colonies. This significant delay in the development of E-colonies in comparison with other types of colonies in agar culture of normal human marrow cells have been reported by Dresch *et al.* (21). The

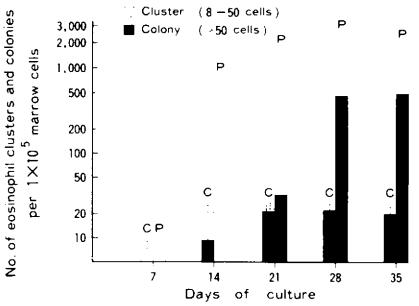

FIG. 2. Comparison of development of eosinophil aggregates in agar cultures of marrow cells from the patient Y. K. at the first relapse (mean of duplicate cultures) and the 15 normal controls (mean ± SA).

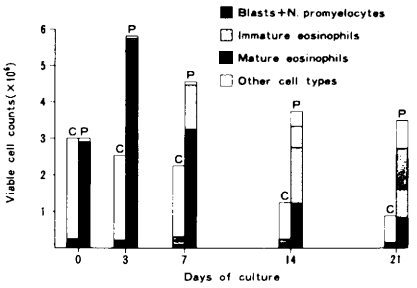

FIG. 3. Comparison of cellular proliferation and differentiation in liquid cultures of marrow cells from the patient Y. K. at the first relapse (P) and the eight normal controls (C, mean data).

maximum formation of E-aggregates by the patient marrow was observed 1 to 2 weeks later than that by the normal control marrows. The maximum incidence of E-aggregates formed by 10^5 marrow cells from the patient was 2420, about 1000 times more than the number grown from the normal controls (26 ± 9). Cytological studies revealed that the aggregates at 4 to 5 weeks consisted of all mature eosinophils.

Ultrastructural study of 25 separated marrow cells from the patient before culture revealed 18 myeloblasts, 5 neutrophilic promyelocytes, and 2 immature cells with eosinophilic granules. All the cells from colonies examined after 28 days of culture had larger granules resembling eosinophilic granules. The characteristic crystalloid structure of mature eosinophilic granules were not noted. The absence of the crystalloid structure in most eosinophils grown in agar or methylcellulose culture of normal marrow cells has been reported (19, 22). This observation suggests that the phenomenon may be a result of environmental factors rather than an abnormality of the cell itself.

The growth pattern of peripheral blood cells from the patient was almost the same as that of bone marrow cells except for a slightly lower plating efficiency. The patient marrow at the second remission showed a growth pattern similar to that of the control marrows.

Figure 3 shows the patterns of cellular proliferation and differentiation in liquid cultures of marrow cells from the patient Y.K., and 8 of the 15 normal controls. In the patient the viable cell count increased steeply after 3 days of culture, reaching about twice as high as controls. This higher level was maintained throughout the culture. After 1 week of culture, immature eosinophils appeared, followed by gradual differentiation and maturation. At 3 weeks of culture, 51% eosinophils, 21% immature and 30% mature forms, were noted. Myeloblasts and neutrophilic promyelocytes accounted for 24%, and mature neutrophils for less than 1%. The remaining cells were mainly macrophages. In the eight controls values (mean ± SD) for the proportion of eosinophils were 8.3 ± 2.5% (range, 5.4 to 11.2%) at 14 days, and 4.0 ± 1.7% (2.2 to 6.0%) at 21 days. In 26 patients with ANLL other than the patient Y. K., the mean value for the maximal proportion of eosinophils between 14 and 21 days of culture was 4.4 ± 3.3% (range, 0 to 11.8%).

Chromosome studies on direct marrow preparation before the culture at the first relapse revealed a hypodiploid cell line missing chromosome 21 with a translocation between chromosome 4 and 9. The same hypodiploid constitution was noted in all of 15 metaphases examined after 10 days of the liquid culture. Morphology of the cells in mitosis was observed of the Wright-stained smears of the marrow cells at this time. Ten of the forty mitotic cells had immature eosinophilic granules, and the others Azur or no granules. The abnormal karyotype was not found at the time of complete remission.

Discussion. Previous authors have noted

the development of E-colonies in cultures of marrow cells of patients with ANLL. Dresch *et al.* (21) reported three cases of ANLL in relapse whose marrow cells formed small number of E-clusters but no colonies. Beckmann *et al.* (9) analyzed the cytology of 74 colonies formed by marrow cells from 12 patients with untreated ANLL and found an increase in pure E-colonies (16.5% of the total number of colonies) compared to normal controls (5.5%). They postulated that these E-colonies were derived from a residual population of normal stem cells.

The present studies have corroborated and expanded these previous findings. E-colonies were scored at 3 to 4 weeks of culture, 1 to 2 weeks later than other types of colonies, because of their maximum formation at this time. In the present studies, marrow cells from 14 of 52 patients with ANLL formed variable numbers of E-colonies, and the plating efficiency was higher than normal in 5 patients. The derivation of these colonies can not be fully determined from either the previous or present studies. For the moment, this must simply reside as an interesting finding. The patterns of proliferation, differentiation and chromosomal karyotype shown by the liquid culture of the marrow cells from patient Y. K. suggest that, in this case, the E-colonies may have been derived from the leukemic cell line. An extraordinarily high plating efficiency and a significant delay in the development of the E-colonies in the patient may also support this. However, further confirmation in more cases, such as the demonstration of specific karyotypic abnormalities in the E-colonies, is essential before this can be stated equivocally.

Summary. Agar culture of bone marrow cells from 52 patients with acute nonlymphoblastic leukemia showed the development of higher than normal incidence of eosinophil colonies in 5 patients. Of these, the growth pattern of one patient was of particular interest since the aggregates formed consisted almost exclusively of eosinophilis which matured by 4 weeks of culture. In this patient, the plating efficiency was extraordinarily high and the growth of eosinophil colonies was significantly delayed. In this single patient karyotypic analysis suggested that the eosinophil colonies may have been derived from a leukemic cell line. Further studies are, however, necessary to determine the nature and origin of eosinophil colonies with acute nonlymphoblastic leukemia.

We would like to thank Dr. Yasukazu Tanaka, Department of Experimental Pathology, Metropolitan Institute of Gerontrogy, Tokyo, for his assistance in the preparation of the electron micrographs and for his kind comments. We would also like to thank Professor W. A. Robinson, Department of Medicine, University of Colorado, Denver, for his useful comments and suggestions.

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Received March 16, 1979, P.S.E.B.M. 1980, Vol. 163.