Mitotic Ability of Leukemic Leukocytes in Chronic Myelocytic Leukemia*

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SUMMARY

In vitro culture of leukemic blood leukocytes from patients with chronic myelocytic leukemia (CML) showed them to be capable of division in the absence of phytohemagglutinin. In the presence of the latter, lymphocytes in the blood of these subjects were preferentially stimulated into mitosis. In the absence of the mitogenic stimulant only the immature leukemic granulocytic cells underwent division. The capacity of the leukemic leukocytes in CML to divide in vitro without an exogenous mitogenic agent was a characteristic of both Ph1-positive and Ph1-negative CML cells. The results indicate that immature granulocytic cells in the blood of patients with CML, and possibly in the blood of nonleukemic subjects, are capable of division without exogenous mitogenic substances.

It has been amply demonstrated that a factor in phytohemagglutinin (PHA) or other mitogenic substances are necessary for the growth of certain blood cells (i.e., lymphocytes) in short-term culture (48—72 hr.), rendering them suitable for karyotypic examination (1—3, 5—10, 12, 13, 18). In previous studies we have shown that acute leukemic blood cells did not undergo mitosis, either with or without PHA, to a sufficient degree to gain a representative picture of the chromosome patterns of these cells from blood cultures (17). In the presence of PHA, the majority of metaphases originated from nonleukemic cells and had a normal karyotype. On the other hand, the leukemic cells, which were characterized by aneuploid or pseudodiploid modes in the bone marrow, constituted, at best, a very small percentage of the metaphases, or failed to divide altogether. Our observations have recently been substantiated by Fitzgerald et al. (4) in a group of adult patients with acute leukemia. These authors found a dichotomy between the very aneuploid cells observed in leukemic bone marrows and the preponderantly diploid metaphases seen following culture of blood from the same patients. In the absence of PHA, both normal and acute leukemic blood cells failed, for all practical purposes, to undergo mitosis, at least in our hands and under the conditions employed (24- to 72-hr. cultures). The present study was undertaken to ascertain whether blood cells from patients with chronic myelocytic leukemia (CML) require exogenous mitogenic factors. Since not all patients with CML have the Philadelphia (Ph1) chromosome, the possible influence of this abnormal autosome on cell division was investigated.

MATERIALS AND METHODS

Blood cells were cultured for 48—72 hr. according to technics previously described (11, 15—17). Blood was obtained from seven patients with Ph1-positive CML and from three subjects with Ph1-negative CML. In each case the blood contained substantial numbers of immature leukemic cells (myeloblasts, promyelocytes, myelocytes) and lymphocytes. The presence of the latter was necessary, since the division of these cells had to serve as an indicator of the number of metaphases with normal karyotypes observed under various conditions. All the patients with CML had been treated with various chemotherapeutic agents, and their white blood cell counts, when elevated, were not excessively so (8—30,000/cu mm). Whether the patients were Ph1-positive or Ph1-negative had been established in the past on the basis of examination of metaphases from bone marrow and in cultured blood cells (16).

RESULTS

A study of the morphology of the cells following culture revealed immature lymphocytic and lymphoblastic-like cells in the smears derived from normal blood (3, 9) and immature granulocytic cells (myeloblasts, promyelocytes, and myelocytes) in the blood of patients with CML grown without phytohemagglutinin. The CML blood cultured with phytohemagglutinin revealed a mixture of immature lymphocytic and immature granulocytic cells. In all smears dividing cells at various stages of mitosis were observed.

Crucial to the interpretation of the initial results was the...
identification of metaphases with the Ph¹ chromosome. Cytogenetic evidence indicates that the abnormal Ph¹-autosome is confined to those hemopoietic elements which are of bone marrow origin (granulocytic, megakaryocytic, and possibly the erythroblastic series) and is not present in lymphocytes and in cultured cells of skin and other tissues (16, 19). Metaphases were labeled as Ph'-positive only if the abnormal autosome could be identified with certainty and when it met the morphologic criteria of the Ph¹ chromosome. Metaphases and karyotypes with and without the Ph¹ chromosome are shown in Figures 1, 2. As can be seen, the abnormal Ph¹ can be easily differentiated from the other G-group acrocentrics and from the Y.

Preliminary studies showed that the number of metaphases in CML blood grown in vitro without phytohemagglutinin was higher (twenty per 1000 cells) after 48 hr. of culture than after 72 hr. (ten per 1000 cells); and that in the presence of PHA there was no significant difference. Hence, the results to be presented are based on cells cultured for 48 hr.

The incidence of the Ph¹ chromosome in blood grown for 48 hr. with and without phytohemagglutinin and the percentage of cells in the blood of patients with CML capable of division are shown in Table 1. It is apparent that, in the absence of PHA, a much higher (92 per cent) percentage of metaphases was Ph¹-positive as compared with the incidence of metaphases with the Ph¹-autosome (39 per cent) in PHA-treated cultures.

To test the possibility that the presence of the Ph¹ chromosome increased the mitotic capacity of the leukemic leukocytes to undergo mitosis in the absence of added mitogenic factors, blood cells from three subjects with CML and devoid of the Ph¹ autosomes were cultured with and without PHA. Since the incubated cells did not contain an abnormal “marker” chromosome and were not aneuploid, it cannot be assumed with certainty that the metaphases observed in the absence of PHA are of leukemic origin.

Nevertheless, the nearly total absence of metaphases in cultured normal blood incubated without PHA would militate against the metaphases observed in CML bloods as being nonleukemic. In addition, in smears only immature granulocytic cells were observed. The number (range: fifteen to twenty metaphases per 1000 cells) of metaphases observed in the three blood samples did not differ materially whether or not PHA was added to the culture medium.

The experiments cited above indicate that cells present in the blood of patients with CML are capable of division in the absence of PHA regardless of whether the Ph¹ is present, in contrast to cells present in normal blood, which fail to undergo mitosis unless a mitogenic substance is added to the medium.

A series of experiments was performed with the aim of determining whether a mitogenic substance was present in the plasma of subjects with CML or was confined to the cells. Normal and CML leukocytes were incubated with normal or CML serum, in the presence or absence of PHA, and the number of metaphases was determined. CML leukocytes underwent division in both normal and CML serum, with the number of metaphases being higher, in this series of experiments, in the absence of PHA. Interestingly, in several instances a significant number (five to ten per 1000 cells) of metaphases was observed when normal leukocytes were incubated with CML serum and no PHA. Under the same conditions, and with PHA, the number of metaphases was somewhat smaller than in the absence of PHA. Not all (four out of seven) of the CML sera had this mitogenic effect. Whether this is related to previous therapy, stage or complications of the disease, quantity or quality of cells in the blood or marrow, or other factors is at present unknown.

An attempt was made next to determine whether the capacity of CML leukocytes to divide in vitro in the absence of PHA was peculiar to the leukemic state. To this end blood was obtained from several subjects who had

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<th>CASE NO.</th>
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<td>♀</td>
<td>9</td>
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Total: 103 187 210 19

TABLE 1

THE INCIDENCE OF METAPHASES WITH THE PH¹ CHROMOSOME IN CML BLOOD CULTURES WITH AND WITHOUT PHA

Also shown in the table are the percentages of cells capable of division present in the bloods as determined from differential counts.
myelocytes and other immature granulocytic cells in the blood (two subjects with myeloid metaplasia, one subject with a high leukocyte count due to infection complicating cancer of the lung). Even though the number of metaphases was not as high as that observed with CML cells, there was a significant number of metaphases (four to seven per 1000 cells) observed in the three blood samples incubated without PHA.

**DISCUSSION**

Recently published results and the data of the present experiments elucidate several parameters of leukocytic division in vitro. It would appear that the leukocytes of normal blood which are capable of mitosis—i.e., the lymphocytes—are triggered into that process by substances acting through immune mechanisms (1–3, 5–10, 12, 13, 18). In the absence of mitogenic substances, for all practical purposes no mitotic cells can be seen in normal blood cells cultured for short periods of time (48–72 hr.). In the present study, in six out of seven cases the nonleukemic leukocyte metaphases outnumber the leukemic ones (Ph'-positive cells) in the presence of PHA. It is unclear whether this is due to stimulating effects of PHA on lymphocytes and/or inhibition of division of the leukemic leukocytes, thus resulting in a higher rate of mitosis in the Ph'-negative cells. On the other hand, in the absence of PHA, only Ph'-positive cells were seen in mitosis. Thus, it appears that PHA is not necessary for leukocytes of CML to undergo division. The presence of the Ph1 chromosome did not seem to endow the leukemic leukocytes with any advantage of in vitro growth as compared with leukemic leukocytes devoid of the Ph1 autosome. In addition, it would appear that blood myelocytes and other immature granulocytic cell precursors of nonleukemic origin are capable of division in the absence of PHA. These findings indicate: (a) Blood lymphocytes require the presence of a mitogenic stimulator in order to undergo division in short-term cultures. It is thought that the mitotic stimulus is triggered through an immunological response. (b) Normal and leukemic granulocytic cells (myeloblasts, promyelocytes, myelocytes) are capable of division in the absence of exogenous mitogenic substances.

It is of more than passing importance to note that acute leukemic leukocytes did not undergo mitosis when incubated under the same conditions as were the leukocytes of normal or CML blood, regardless of whether PHA was added to the culture medium (4, 17). Thus, it would appear that considerable further information has to be gathered about the exact parameters controlling division of various leukocytes in vitro. The fact that mitosis of lymphocytes is initiated by immunological mechanisms may be of considerable importance in relation to the development of disorders involving these cells or their precursors. The fact that serum from CML was capable of initiating mitosis of normal lymphocytes may indicate that such serum contains mitogenic substances, which may have originated from the granulocytic cells, capable of inducing division in normal cells.

Even though immunological mechanisms may be responsible for initiating and sustaining mitoses in blood lymphocytes, it appears that each antigen may provoke the cells into division by diverse processes (2). For example, it has been shown that phytohemagglutinin and tuberculin induce mitosis in normal blood lymphocytes at different times and rates, suggesting different loci of action of these two substances. These observations indicate that the factors controlling and initiating mitosis of leukocytic cells may be highly diversified.

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