CONCISE REPORT

Effect of Interferon on Colony Formation in Culture by Blast Cell Progenitors in Acute Myeloblastic Leukemia

By Raymond Taetle, Ronald N. Buick, and Ernest A. McCulloch

The effect of purified human fibroblast interferon on primary and secondary colony formation by blast progenitors from the peripheral blood of patients with acute myelogenous leukemia was examined. Interferon inhibited blast progenitors and normal granulocyte/macrophage progenitors (CFU-C) in a dose-dependent manner. The magnitude of this effect on blast progenitors and CFU was similar. Interferon also inhibited secondary plating of blast progenitors (self-renewal). This effect was in marked contrast to the effect of adriamycin, which reduced primary plating efficiency of blast progenitors but did not affect self-renewal. Inhibition of blast progenitor proliferation by interferon was markedly reduced when interferon was added after 24 hr of culture and was absent when added after 72 hr. Inhibition of self-renewal was observed even when interferon was added at 72 hr. We conclude that interferon inhibits both primary proliferation and self-renewal of blast progenitors and that this effect is not due to reduction in the number of primary colonies. These experiments provide an example of how cell culture techniques may be used to test antitumor agents for effects on important cellular events other than general cytotoxicity.

RECENTLY, interferons, originally identified by their antiviral activity, have been proposed as antineoplastic agents.1 3 We have examined the antiproliferative activity of interferon using a cell culture assay that permits colony formation by blast progenitors obtained from the peripheral blood of patients with acute myeloblastic leukemia (AML).2 The assay is flexible; it permits not only evaluation of the effect of potential antineoplastic agents on the limited number of cell divisions leading to colony formation, but also upon the self-renewal process that is required for extensive growth.5 We found that interferon inhibits blast colony formation in a dose-related fashion similar to that observed for committed marrow progenitors.6 7 In addition, we observed that self-renewal was also inhibited. This finding with interferon is contrasted with observations using Adriamycin; colonies surviving exposure to this agent showed no reduction in their content of new blast progenitors.

MATERIALS AND METHODS

Patient Material

Blood was obtained by venipuncture from 8 patients with AML with greater than 40% circulating blast cells; heparin was used as an anticoagulant. Marrow was aspirated from the sternum or iliac crest from 4 patients without leukemia; these patients were 2 marrow transplant donors, a marrow transplant recipient 3 yr after successful engraftment, and 1 patient under investigation for anemia.

Blast Progenitor Assay

The assay for blast progenitors was that described by Buick et al.4 as modified by Minden et al.8 Mononuclear cells were collected from peripheral blood by centrifugation through Ficoll-Hypaque (density 1.070) at 1200 g for 20 min at 4°C. Cells found at the interface were collected, washed, and incubated with sheep red blood cells (SRBC). A second Ficoll-Hypaque centrifugation was used to obtain SRBC rosette negative cells; at this stage the cells were stored frozen in 10% fetal calf serum (FCS) and 10% dimethyl sulfoxide (DMSO). Survival of colony formation after cryopreservation was regularly between 30% and 60%. Cells were removed and plated at cell concentrations of 3 or 4 x 10^5 cells/ml in 35 mm plastic (Lux) Petri dishes in a medium consisting of 0.8% methylcellulose in alpha medium, 15% FCS, and 10% medium conditioned by phytohemagglutinin (PHA) stimulated peripheral blood mononuclear cells (PHA-LCM).9 The cultures were incubated at 37°C in 7.5% CO₂ for 5-7 days; colonies containing more than 20 cells were counted using an inverted microscope. Cells within colonies were characterized as blast-like on the basis of their appearance on Wright-Giemsa stain. The cells were negative or weakly positive when stained for chloroacetate esterase11 and did not have SRBC receptors.

Assay for Self-Renewal

The capacity of self-renewal of blast progenitors3 was assessed by harvesting colonies from 35 mm Petri dishes; the cells were resuspended, washed, and plated in microtiter plates at a cell concentration of 2 x 10^6 cells in 0.1 ml of methylcellulose and growth medium in the same proportions used for the primary cultures. Interferon or adriamycin was not added to these secondary cultures. The plates were incubated for 5-7 days and then colonies evaluated using the criteria described above.

Assay for CFU

The assay for granulocyte progenitors (CFU) was performed as described by Iscove et al.12 Mononuclear cells were obtained from marrow cell suspensions using the Ficoll-Hypaque separation proce-
dure described earlier. The cells were washed and plated at $2 \times 10^3$ cells/ml in medium of the same composition as that used for blast colony assay, except that standard leukocyte-conditioned medium, prepared by incubating mononuclear cells without lectin, was used rather than PHA-LCM. Colonies containing more than 20 cells were counted after 10 days of incubation at 37°C in 7.5% CO₂. Cells within such colonies were mature or maturing granulocytes when examined in Wright-Giemsa stained preparations.

Assessment of Interferon and Adriamycin Effects

Two lots of fibroblast-produced interferon were used in these studies, both provided through the courtesy of Drs. W. A. Carter and S. Leong, Roswell Park Memorial Institute, Buffalo, N.Y. The first, obtained from HEM Research Inc., Rockville, Md., contained $1.5 \times 10^4$ U/mg protein; the second, obtained from the Roswell Park Memorial Institute, contained $1.5 \times 10^5$ U/mg protein. Both lots gave very similar results, and accordingly, the data are presented together.

Lyophilized interferon was dissolved in distilled water, diluted with alpha medium containing 10% fetal bovine serum (FBS, Flow Laboratories), and frozen at -80°C until use. The interferon was thawed at 37°C and added to culture plates just prior to plating or after 24 or 72 hr. Adriamycin was obtained from Adria Laboratories, Mississauga, Canada. Leukemic cells were exposed to the drug for 10 min, washed twice, and then plated for colony formation as described previously.

RESULTS

Interferon Inhibition of Primary Colony Formation

When interferon was added at the initiation of cultures designed to promote colony formation by blast progenitors or CFU, a dose-dependent inhibition was observed. Figure 1 contains data from 11 experiments using leukemic blood and 4 experiments using normal marrow. The normalized means and standard deviations are shown as a function of interferon dose. The resulting log-dose effect curve is shallow, no difference is seen between the interferon inhibition of colony formation by blast progenitors and CFU. Control values without interferon showed patient-to-patient variation and ranged from 1721 to 53 (518 ± 567; mean ± 1 SD) for blast colony formation and from 76 to 164 (116 ± 37) for CFU.

The Effect of Interferon on Blast Cell Self-Renewal

In 7 experiments, colonies of blast cells grown in the presence of increasing concentrations of interferon were pooled, and cell suspensions prepared and plated in microwells in quadruplicate to estimate self-renewal. The data are presented in Table 1. It is apparent that colonies grown in the presence of interferon contained many fewer new progenitors than controls, indicating that self-renewal occurred less frequently in these plates. Although patient-to-patient variation was observed, self-renewal decreased with increasing interferon dose.

The Effect of Adriamycin on Blast Cell Self-Renewal

The reduction in self-renewal observed in interferon-containing cultures might be nonspecific, that is, self-renewal might be reduced under any conditions that were inhibitory to colony formation. To test this possibility, colonies surviving exposure to a chemotherapeutic drug (adriamycin) were tested for their self-renewal capacity. The data from four experiments are presented in Table 2. It is apparent that, even though primary colony formation was markedly reduced, no reductions in secondary plating efficiency were seen.

Time Course of Interferon Effects

To determine the time course of the interferon effect, the agent was added to cultures of leukemic blasts at time 0 or after 24 or 72 hr of incubation. The

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cultures were assessed for primary and secondary colony formation. Data form four experiments are given in Table 3. It is apparent that inhibition of primary colony formation was not seen when interferon was added after 72 hr. Inhibition of self-renewal was still present, though reduced, at 72 hr.

DISCUSSION

We have suggested that cell divisions occurring during blast cell colony formation may have two different consequences. On the one hand, self-renewal may occur with the production of blast progenitors with properties very similar to those present in leukemic blood. On the other hand, the progeny of blast progenitors may enter into a series of terminal divisions leading to cells of blast-like morphology but without proliferative potential. The major finding of the present study is that exposure of blast progenitors to interferon during colony formation may separate these two functions; the observation is that self-renewal is inhibited even when sufficient divisions are possible to produce a colony containing at least 20 cells.

The inhibition of primary granulocyte colony formation observed in these experiments was similar to that reported by others. Our findings differed from those of Verma et al., however, in that marked patient-to-patient variation in the effect of interferon on blast progenitor and CFU proliferation was not seen, nor was decreased granulopoietic maturation found in interferon-treated colonies from marrow CFU.

Certain technical explanations of the observations have been considered. First, it might be more difficult to obtain representative cell suspensions from plates containing reduced numbers of colonies. In previous experiments, we have provided evidence that secondary plating efficiency is not dependent on the number of colonies used to prepare the suspensions from which the value is determined. This observation is confirmed in the present study, where reduced numbers of colonies in plates from cells exposed to adriamycin yielded suspensions of cells with colony-forming capacity equivalent to that of controls. This observation also provides evidence that interferon has some specificity; that is, every agent that is capable of reducing primary colony formation does not selectively affect blast cell renewal.

The time course experiments provide evidence that interferon acts upon cellular events that occur early in colony formation. It is during these early stages that self-renewal is particularly important; indeed, the apparent specificity of interferon for self-renewal may reflect this early action rather than direct interaction of the agent with molecular events required for renewal. Regardless, self-renewal must occur if clones of malignant cells are to maintain themselves. Thus, preferential effect of interferon on self-renewal may explain its therapeutic efficacy, even though it is not highly toxic and does not possess specificity for leukemic cells.

Finally, the experiments described in this article provide an example of the extent to which the antitumor agents may be tested using cell culture methodology. Not only can general cytotoxicity be tested, but also the effect of an agent on distinct, but important, cellular events may be demonstrated.

REFERENCES


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R Taetle, RN Buick and EA McCulloch