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Influence of Interferon Preparations on the Proliferative Capacity of Human and Mouse Bone Marrow Cells *in Vitro*

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ABSTRACT

The toxicity of interferon to bone marrow was studied by the use of *in vitro* colony forming assays for hemopoietic cells. In the same study the relative inhibitory effects of two clinically common interferon preparations, leukocyte and fibroblast interferons, were compared with regard to their effect on both myeloid [colony-forming unit, culture (CFU_c)] and erythroid [colony-forming unit, erythroid (CFU_e)] progenitor cells. CFU_e formation in human bone marrow cells *in vitro* appeared to be fairly resistant to both interferons. Only high doses of both interferons gave a marked inhibition of CFU_e.

However, the toxicity of leukocyte and fibroblast interferon was divergent for CFU_c in human bone marrow. Leukocyte interferon appeared to be considerably more inhibitory for CFU_c than was fibroblast interferon.

The effects of mouse interferon, induced in L929 cells, on the growth of CFU_c and CFU_e in murine bone marrow cells were comparable with those of fibroblast interferon on human cells.

The toxicity of human and murine interferon was species specific. Except for the toxicity of leukocyte interferon to CFU_c in human bone marrow, the toxicity of interferon was marked only with concentrations of interferon far exceeding the amount necessary to produce an antiviral state *in vitro*.

INTRODUCTION

Viral infections present a major problem in primates (rhesus monkey and human patients) with primary immune deficiencies and those subjected to immunosuppressive therapy. It has become evident that the severity and incidence of viral infections are particularly prominent in patients who have been treated with immunosuppressive regimens following allogeneic bone marrow transplants (2, 19). The further development of bone marrow transplantation in humans is largely dependent on the possibility for control or prevention of these complications (18). Recently, the successful application of interferon as an anti-viral drug in humans was reported (7, 9, 11).

Interferon preparations in high concentrations, however, inhibit cell division. Therefore one must be aware of possible bone marrow toxicity (6) because, particularly in patients treated with bone marrow transplantation and in patients treated with cytotoxic agents, the bone marrow reserve may be severely limited. Testing of the clinically applied interferon preparations for bone marrow toxicity prior to clinical use will be important. This can hardly be achieved *in vivo* in man. Therefore one must rely on *in vitro* assays that determine the proliferative capacity of hemopoietic cells. Colony-forming cultures, in which progenitor cells are induced to proliferate and differentiate towards clones of daughter cells, may meet the criteria of a convenient assay in this respect.

Human leukocyte interferon and human fibroblast interferon and their effects on hemopoietic myeloid and erythroid progenitors in culture were studied. The inhibition of the formation of granulocytic-macrophage colonies and erythroid colonies during exposure of marrow cells to interferon was investigated. The effect of mouse L-cell interferon on the formation of erythroid as well as granulocytic colonies in mouse bone marrow was also included in the study in order to compare the species specificity of all tested interferon preparations.

MATERIALS AND METHODS

Interferon Preparations

Human Interferon. Partially purified leukocyte interferon was kindly supplied by Dr. Cantell, Helsinki, Finland.

The interferon was induced and semipurified as described before (5). The leukocyte interferon batch used had an activity of 6×10^6 IRU[1]/ml and a protein concentration of 7 mg/ml.

Human fibroblast interferon was kindly supplied by Dr. V. G. Edy, Leuven, Belgium.

The interferon was induced and semipurified as described before (3) and had a specific activity of 10^6 IRU/20 mg of protein. A control preparation was prepared from human plasma protein Fraction V, following the same procedures as used for the manufacture of the interferon preparations. This preparation was also used as a control in *in vivo* studies in patients.

Mouse Interferon. Mouse interferon was induced with Newcastle disease virus in mouse L929 cells and prepared as described before (1).

The interferon had a specific activity of 10^6 IRU/mg of protein. Control preparations were prepared from normal L929 cell supernatant following the same procedures as those used to prepare the interferon preparations.

The interferon preparations were diluted to the desired concentration in Dulbecco's MEM and were added to the cell suspensions as a part of the culture medium wherein the cells were cultured.

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[1] The abbreviations used are: IRU, International reference unit; MEM, minimal essential medium; CFU_c, colony-forming unit, culture; CFU_e, colony-forming unit, erythroid.

Preparations of Cells

Human bone marrow was obtained aseptically from patients requiring a diagnostic puncture. Patients were suffering from a variety of diseases, predominantly malignant tumors and infections; some patients manifested a mild anemia. Human blood was collected by venipuncture. The erythrocytes were removed from the nucleated marrow and blood cells by sedimentation at unit gravity in 0.1% methyl cellulose (Methocel McDow Chemical Co., Midland, Mich.) in Dulbecco's MEM (Flow Laboratories, Irvine, Ayrshire, Scotland).

Mouse bone marrow was obtained by dissecting the femurs of 6- to 8-week-old female BALB/c mice. One of the ends was cut with scissors, and the marrow was flushed from the shafts. All cells were carefully washed with Hanks' balanced salt solution after collection.

Myeloid Colony Cultures for Human Cells

To determine the number of CFU_c, we adopted the culture method of Pike and Robinson (17).

The underlayers were prepared by mixing 10^6 leukocytes with 1 ml of culture medium (0.5% agar, 20% serum, and Dulbecco's MEM (Flow Laboratories). The serum component contained equal parts of horse serum, fetal calf serum, and 3% Trypticase Soy Broth (Difco Laboratories, Detroit, Mich.). Thereafter, an overlayer containing the marrow cells in 0.25% agar in Dulbecco's MEM, supplemented with 20% serum, was pipeted on top of the leukocyte underlayers. After 14 days of incubation in a humidified atmosphere of 5% CO_2-95% air, the colonies were counted with an inverted microscope.

Three Petri dishes were used for each experimental point.

Erythroid Colony Cultures from Human Cells.

Colony formation in plasma clots was determined according to the method of McLeod *et al.* (13). Nucleated bone marrow cells in a concentration of 2×10^3/ml were suspended in medium containing Dulbecco's MEM, 20% fetal calf serum, 1% bovine albumin (Fraction V; Sigma Chemical Co., St. Louis, Mo.), 2% asparagine, 0.25 unit erythroprotein (Step III; Connaught Medical Laboratories, Willowdale, Ontario, Canada), and 10% bovine citrated plasma (Grand Island Biological Co., Grand Island, N. Y.). The cells were cultured in microtiter plates (C. A. Greiner und Söhne, D-7440 Nürtingen, Germany), and 0.1 ml of the cell suspension was incubated per well.

The cultures were placed in an incubator under the same conditions as those used for the myeloid colony method. After 2.5 days of incubation, the clots were removed from the microtiter plates and stained with benzidine after fixation with 5% glutaraldehyde in phosphate-buffered saline (0.01 M isotonic Na_2HPO_4-KH_2PO_4 buffer, pH 7.0 to 7.2).

Cell aggregates containing more than 8 cells were counted with the light microscope. The aggregates consisted of differentiated erythroblasts. Each experimental point represents the average colony counts of at least 10 whole clots.

Granulocytic-Macrophage Colony Cultures of Mouse Cells

The system was originally described by Bradly and Metcalf (4). Nucleated bone marrow cells were suspended in an agar solution (0.3%) in Dulbecco's MEM supplemented with 20% serum (consisting of equal parts of horse serum, fetal calf serum, and 3% Trypticase Soy Broth solution).

One ml of this mixture containing 10^5 cells was plated per plastic Petri dish (diameter, 3.5 cm). As a stimulator 0.1 ml of mouse fibroblast-conditioned medium was added to each plate. The dishes were incubated for 6 days under the same conditions as those for the other cultures. Thereafter, colonies were counted.

RESULTS

Human leukocyte and human fibroblast interferons, when added to the culture, inhibit human CFU_c. The extent of inhibition is linearly related to the dosage of interferon (Chart 1). Human leukocyte and human fibroblast interferon preparations also suppress the formation of granulocyte-macrophage colonies (CFU_c) (Chart 2). The degree of inhibition of CFU_c and CFU_c by human fibroblast interferon is approximately equivalent. Colony growth from both cell populations appears to be reduced by approximately 50% in the presence of 10^4 IRU/ml.

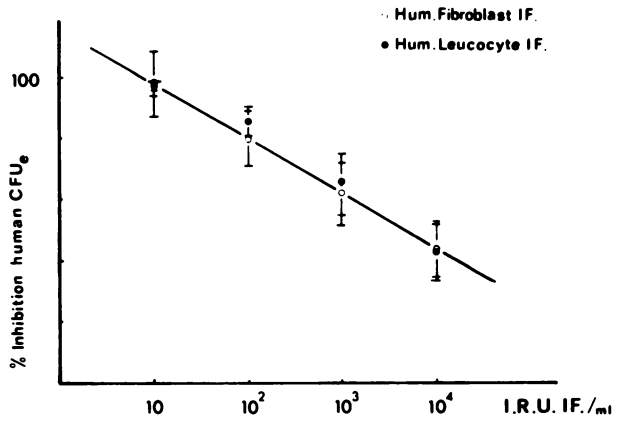

Chart 1. Cytotoxicity of human (*Hum.*) leukocyte and human fibroblast interferon (*IF*) for human CFU_c. Nine experiments: *bars*, S.D.

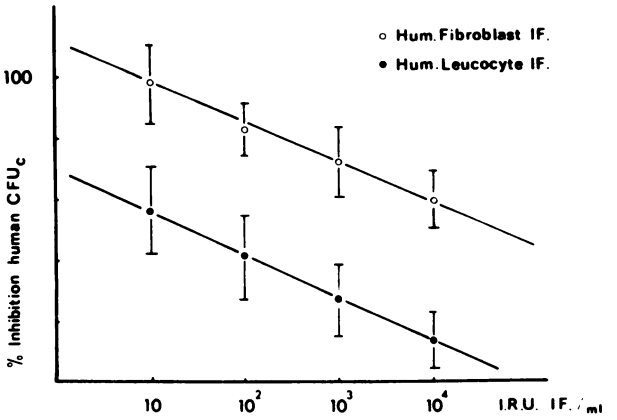

Chart 2. Cytotoxicity of human (*Hum.*) leukocyte and human fibroblast interferon (*IF*) for human of CFU_c. Nine experiments; *bars*, S.D.

Table 1
Specificity of the toxicity of interferon preparations

	CFU_c and CFU_e of mouse bone marrow cells[a]			
	Human fibroblast interferon		Human leukocyte interferon	
	$CFU_e/10^5$ cells	$CFU_c/10^5$ cells	$CFU_e/10^5$ cells	$CFU_c/10^5$ cells
Control	202.3 ± 24.7[b]	100.0 ± 10.0	202.3 ± 24.7	100.0 ± 10.0
1×10^4 IRU	194.7 ± 23.7	94.4 ± 7.4	131.3 ± 20.1	58.8 ± 9.6
1×10^3 IRU	203.4 ± 26.6	98.6 ± 11.4	177.1 ± 25.9	91.5 ± 9.1
1×10^2 IRU	209.9 ± 24.4	101.5 ± 7.4	191.2 ± 21.9	102.9 ± 10.4
1×10 IRU	198.7 ± 26.6	99.6 ± 11.3	208.3 ± 35.5	102.7 ± 10.3
Mock interferon	197.1 ± 20.1	102.4 ± 8.2		

[a] Human bone marrow cells were not inhibited by mouse L-cell interferon preparations.
[b] Mean ± S.D.

However, human leukocyte interferon diverges clearly from fibroblast interferon: (*a*) it is significantly more toxic to human CFU_c than to CFU_e; (*b*) this interferon shows no or only a minor inhibitive effect on murine CFU_c and CFU_e (Table 1).

A third interferon preparation, produced in mouse L929 cells, was also investigated for its effect on human and mouse progenitor cells. Whereas mouse interferon shows inhibition of the colony growth of mouse CFU_e and CFU_c in a linear dose-effect relationship (Charts 3 and 4), it is not toxic for human bone marrow (results not shown).

This indicates that the toxicity of human interferon may be species dependent.

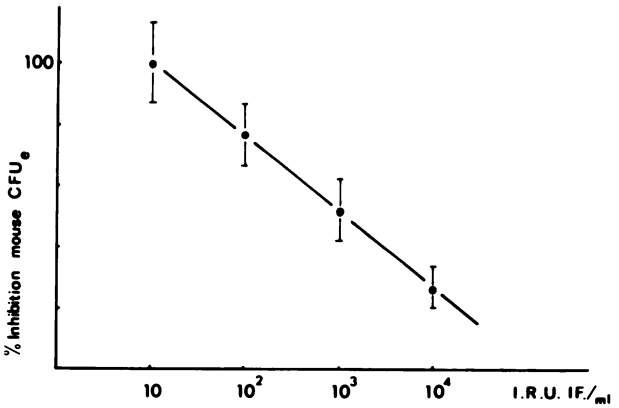

Chart 3. Cytotoxicity of mouse L-cell interferon (*IF*) for mouse CFU_e. Five experiments; *bars*, S.D.

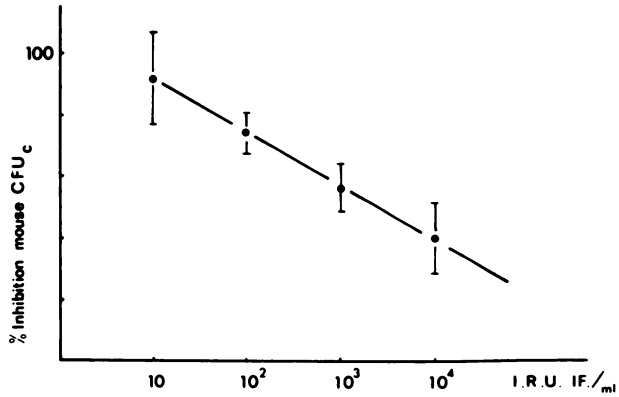

Chart 4. Cytotoxicity of mouse L-cell interferon (*IF*) for mouse CFU_c. Five experiments; *bars*, S.D.

DISCUSSION

Interferon preparations inhibit CFU_e and CFU_c *in vitro*. Human fibroblast interferon inhibits the formation of CFU_c and CFU_e *in vitro* at concentrations much larger than those needed for the establishment of the antiviral state of cells *in vitro*. The toxic concentration of fibroblast interferon also exceeds the serum titer of interferon in patients treated with high doses of fibroblast interferon [20×10^6 IRU/ml fibroblast interferon daily, a serum titer rise of 30 IRU/ml (V. G. Edy, personal communication)]. The effect of leukocyte interferon on CFU_e is similar to that of human fibroblast interferon, but human leukocyte interferon is comparatively more toxic to CFU_c. Only 10 units leukocyte interferon suffice to inhibit the growth of CFU_c by approximately 40%, whereas as much as 10^4 units of fibroblast interferon are needed to produce a similar reduction. Using a similar assay system, other investigators have reported that human leukocyte interferon preparations may be extremely toxic for granulocyte-macrophage colonies in human bone marrow cultures (10, 16). To our knowledge the effect on erythroid progenitors has never been investigated.

Others have demonstrated in a different experimental system that the cytotoxic and antiviral activities of interferon preparations share many physicochemical and biological characteristics (12, 15). This may suggest that interferon itself is responsible for the cytotoxic effects. However, in view of the nonavailability of completely purified interferon, one cannot be certain whether the cytotoxicity is due to impurities in the preparation. Comparison of the cytotoxic effect of human leukocyte interferon on mouse and human CFU_c, respectively, shows that this interferon is less toxic for mouse bone marrow, indicating a (cell- and species-) specific effect and not toxicity due to impurities. Therefore the cytotoxic effect could be closely related to the antiviral effect of interferon (compare Chart 2 and Table 1).

The toxicity of leukocyte interferon could pose a problem in clinical application because it is already apparent at lower than pharmacological concentrations.

The finding that leukocyte interferon is more toxic for CFU_c than for CFU_e is of interest in view of previous work of Einhorn *et al.* (8). They suggest that the inhibiting effects of interferon are specific for those target cells that are prevalent in the tissue in which the interferon is induced.

The effect of mouse interferon on murine CFU_e and CFU_c is essentially similar to that of human fibroblast interferon

on human bone marrow. The adverse effect of L-cell interferon on the colony formation has been reported to be less prominent than the effect of interferons from other murine sources. McNeill (14) explained this effect by a contamination of L-cell interferon with colony-stimulating activity, but in our material no significant colony-stimulating activity was present.

No doubt, it will be necessary in the future to test the interferon toxicity on bone marrow for *in vivo* relevance. This should be done in direct comparison with clinical and hematological parameters, and this should finally establish the value of the method. Until the toxicity ratio of interferon has been more thoroughly established in *in vivo* studies, one must rely largely on extrapolation from *in vitro* tests.

The above data imply that, in terms of marrow toxicity, human fibroblast interferon might represent a preferable choice over leukocyte interferon, especially in patients with a marrow insufficiency.

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