Growth Inhibition of Human Lymphoblastoid Cells by Interferon Preparations, Obtained from Human Leukocytes

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The growth inhibition of human lymphoblastoid cells by human interferon preparations (HIF) and a mock-interferon preparation (Mock-IF) was studied. HIF was induced in suspensions of primary human leukocytes by Newcastle Disease Virus. Mock-IF was prepared from uninduced cell suspension of a comparable batch. The highest degree of inhibition of cell propagation was obtained on the EBV producer line P3HR1, the inhibition of the cell lines Raji and SKL 1 was significantly lower, no effect was observed on the cell line RPMI 1788 originating from a healthy donor. Mock-IF also caused a growth inhibition but to a lower degree as HIF. HIF and Mock-IF are similar in the following properties: 1. Stability at pH 2 at 4 °C for four days, 2. sensitivity to tryptic digestion, and 3. molecular weights in the range between 5,000 and 40,000 daltons.

I. Gresser *et al.* have described experiments showing that mouse interferon preparations, in addition to the antiviral effect, had an inhibitory activity on the multiplication of mouse cells *in vitro* [1, 2]. A similar effect was observed by K. Cantell [3] who found an inhibition of propagation of human lymphoblastoid cells in cultures which had been incubated with human interferon for at least several weeks. E. V. Gaffney *et al.* have reported growth inhibition of SV 40 transformed human amniotic cells by human interferon, while there was no effect on established human cell lines as HeLa or FL [4].

We studied the *in vitro* anticellular activity of human leukocyte interferon preparations on different lymphoblastoid cell lines from normal or tumor patients. In addition, we wanted to elucidate whether the antiviral and anticellular activity of our different interferon preparations were properties of the same substance. We used four different lymphoblastoid cell lines. One of these was established by G. E. Moore from a healthy donor Burkitt 1788) [5, 6]; two others mere isolated from Burkitt lymphoma patients (Raji [8], P3HR1); and the fourth was obtained from a patient with monocytic leukemia (SKL 1) [7]. Both Burkitt lymphoma cell lines contain Epstein Barr Virus (EBV) genome [10], but only the P3HR1 is an EBV producer cell [9].

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Materials and Methods

Interferon

The induction of interferon was performed according to the method of H. Strander and K. Cantell [11] with Newcastle Disease Virus (NDV) strain Beaudette, which was propagated in embryonated chicken eggs and purified by twofold sedimentation in the ultracentrifuge. Washed human peripheral leukocytes ($7-9\times10^6$ cells/ml) were incubated with a multiplicity of 10 virus particles per cell at 37 °C for 13 hours in Eagle's Medium MEM supplemented with inactivated human serum (2%). After incubation, the cells were removed by low speed centrifugation, the supernatant adjusted to pH 2 and incubated for virus inactivation at 4 °C for four days. Thereafter the solution was chromatographed on Sephadex G75 (Pharmacia, Frankfurt, Germany) and eluted with 0.2 M Glycin/HCl-buffer pH 3.5. The fractions following the albumin were pooled, concentrated by ultrafiltration, sterilized by filtration and stored at −20 °C. The buffer of the pooled fractions was changed to 0.15 M NaCl by chromatography on Sephadex G25. One preparation (HIF2) was rechromatographed before the buffer was changed. Before the last concentration step by ultrafiltration, 1% Haemaccel, modified gelatine (Behringwerke AG, Marburg, Germany), was added to stabilize the interferon activity. This component was disregarded in the calculation of the specific antiviral activity (= antiviral activity per mg protein, see Table I).

Mock interferon

This preparation was obtained by the same procedure as interferon (HIF 3, Table I) using un-

induced leukocyte suspensions as starting material. We have defined the supernatant of 4×10^3 leukocytes after chromatography on Sephadex G75 and G25 and concentration by ultrafiltration a formal unit of our mock interferon preparation. This amount is equal to the supernatant from the same number of induced leukocytes, which had the antiviral activity of one unit of the British Research Standard A 69/19 [12].

Interferon assay

The determination of the antiviral activity of interferon was done as described by H. Strander and K. Cantell [11] in a plaque reduction assay. Our challenge virus was Semliki Forest Virus (SFV). Interferon was tested in serial twofold dilutions, each of which was given to three cell cultures. The titers were calculated according to the units of the British Research Standard Human Interferon A 69/19.

Inhibition of cell growth by interferon and mock interferon

The lymphoblastoid cell line RPMI 1788 (purchased from Associated Biomedic Systems, Buffalo, N. Y., USA) and the cell lines Raji, P3HR1 and SKL 1 (obtained from Prof. zur Hausen, Institut für Klinische Virologie, Erlangen, Germany) were propagated in medium RPMI 1640 supplemented with 10% fetal calf serum, 200 μg/ml streptomycin, 200 μg/ml neomycin and 200 units/ml penicillin in a cell roller apparatus (Bellco Glass Inc., Vineland, N. J., USA). The final cell suspension of a concentration of $1.5 - 2.5 \times 10^6$ cell/ml was diluted by the addition of interferon or mock interferon in complete medium RPMI 1640 to a cell concentration of 2×10^5 cells/ml. These suspensions were incubated in Erlenmeyer flasks without agitation (a 200 ml flask was filled with 40 ml cell suspension) at 37 °C. Control cell suspensions contained the buffer solution used for the interferon preparations in the corresponding dilution. The cell growth was followed by cell counting and by [2-^{14}C]thymidine incorporation during a 24 hour pulse. Three 1 ml samples per Erlenmeyer flask were incubated in polysterene tubes (NunC, Rosluede, Denmark) with the addition of 70 nCi [2-^{14}C]thymidine (Amersham, England) in 50 μl. Two different lots with the specific activities of 6 mCi/mM (Fig. 1), and 60 mCi/mM (Fig. 3) were used. The samples

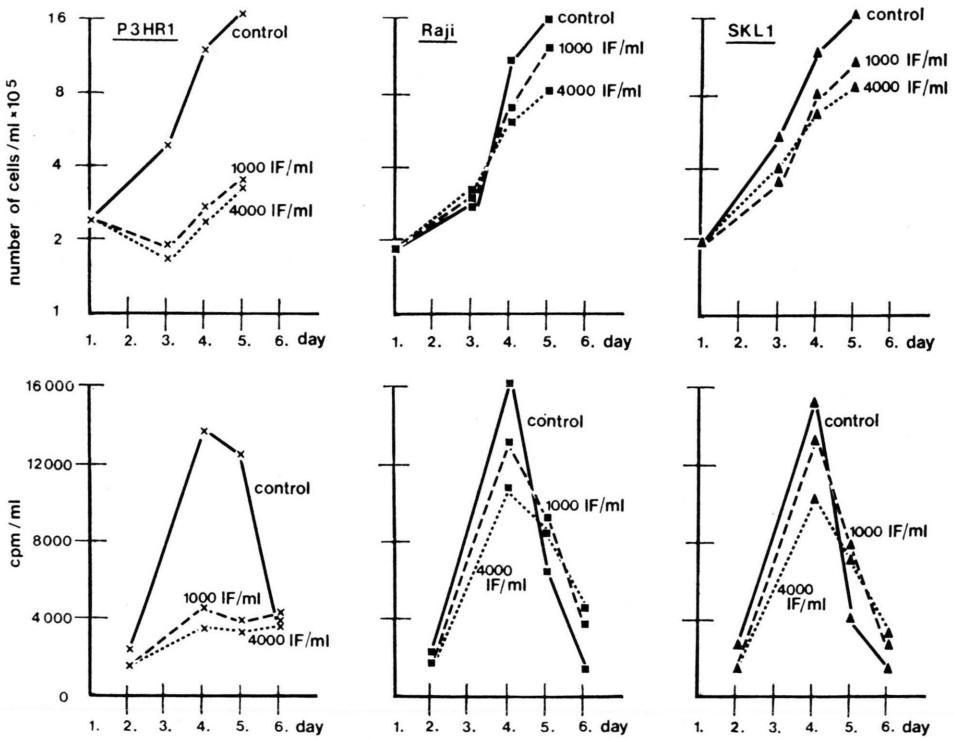

Fig. 1. Growth curves of control (———) and interferon (HIF 3)-treated (1000 units/ml (– – –), 4000 units/ml (· · ·)) cultures of the three lymphoblastoid cell lines P3HR1 (×), Raji (■), and SKL 1 (▲). Cell propagation was measured by cell counting and [2-^{14}C]thymidine incorporation during a 24 hour period before cell harvest.

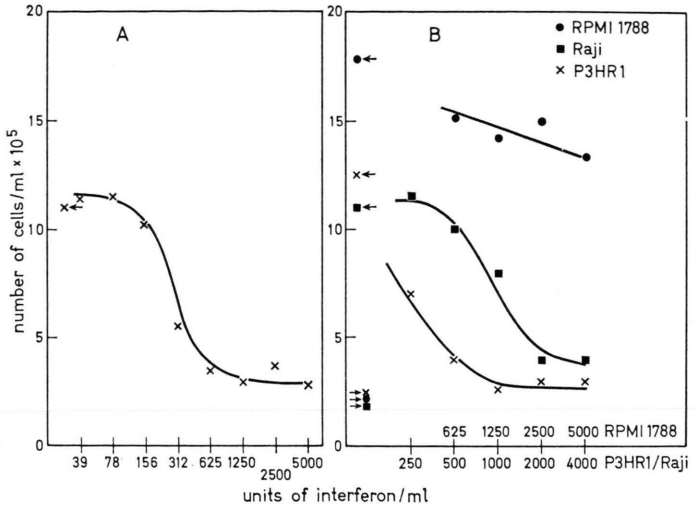

Fig. 2. Growth inhibition of the three cell lines P3HR1 (×), Raji (■), and RPMI 1788 (●) depending on interferon concentration determined by cell counting at the fifth day of cell propagation. Starting (→) and final (←) cell concentrations of the control cultures are marked by arrows. Two different interferon preparations HIF 1 (A) HIF 2 (B) have been used.

were incubated at 37 °C in a humidified atmosphere with 8% CO_2. The cells were then precipitated on glass fiber filters (Sartorius GmbH, Göttingen, Germany), and washed three times with excess distilled water. After drying the filters, they were immersed in a toluene scintillator, and the incorporated radioactivity was measured in a liquid scintillation spectrometer. Each point represents the average values of six samples. Viability tests were performed in a haemocytometer with the trypan blue exclusion test.

Results

The growth of the three cell lines P3HR1, Raji, and SKL 1 was reduced after the addition of 1000 and 4000 IF-units/ml (Fig. 1). The growth inhibition of the EBV producing cell line P3HR1 was considerably higher than that of the other two cell lines. Cell growth in relation to interferon concentration was studied by cell counting after a five day incubation period. The results for the cell lines P3HR1, Raji, and RPMI 1788 are given in Fig. 2. The data presented in Fig 1 and 2 were obtained with three different human interferon preparations (Table I). The cell line P3HR1 shows a similar sensitivity to the three preparations. As can be seen in Fig. 2, P3HR1 was most sensitive, while Raji was less sensitive to the anticellular activity. No growth inhibition was observed in the case of RPMI 1788.

We used mock interferon to investigate whether only preparations with a high antiviral activity inhibit cell proliferation. For comparison of the properties of our interferon and mock interferon preparations see Table I. Our assumption that anticellular activity is only linked to interferon, is contradicted by the data given in Fig. 3. The three interferon sensitive cell lines showed growth inhibition also by mock interferon. This effect is weak in the case of Raji and SKL 1, but of almost the same magnitude as the interferon effect on P3HR1.

Discussion

Three interferon preparations caused an inhibition of cell proliferation in three lymphoblastoid cell lines originally isolated from tumor patients. The greatest effect was obtained on P3HR1, whereas the sentivities of Raji and SKL 1 were

Table I. Properties of the interferon and mock interferon preparations.

Lot-No.	Purified on Sephadex G 75	Purified on Sephadex G 25	Antiviral activity * per ml	Antiviral activity * per mg protein	Anticellular activity sensitive to tryptic digestion
HIF 1	1 ×	n.d. **	1×10^5	1×10^4	n.d.
HIF 2	2 ×	1 ×	$1{,}5 \times 10^5$	1×10^5	n.d.
HIF 3	1 ×	1 ×	1×10^5	3×10^4	yes
mock-IF	1 ×	1 ×	20	9	yes

* Units as defined under interferon assay (Methods).
** n.d. = not done.

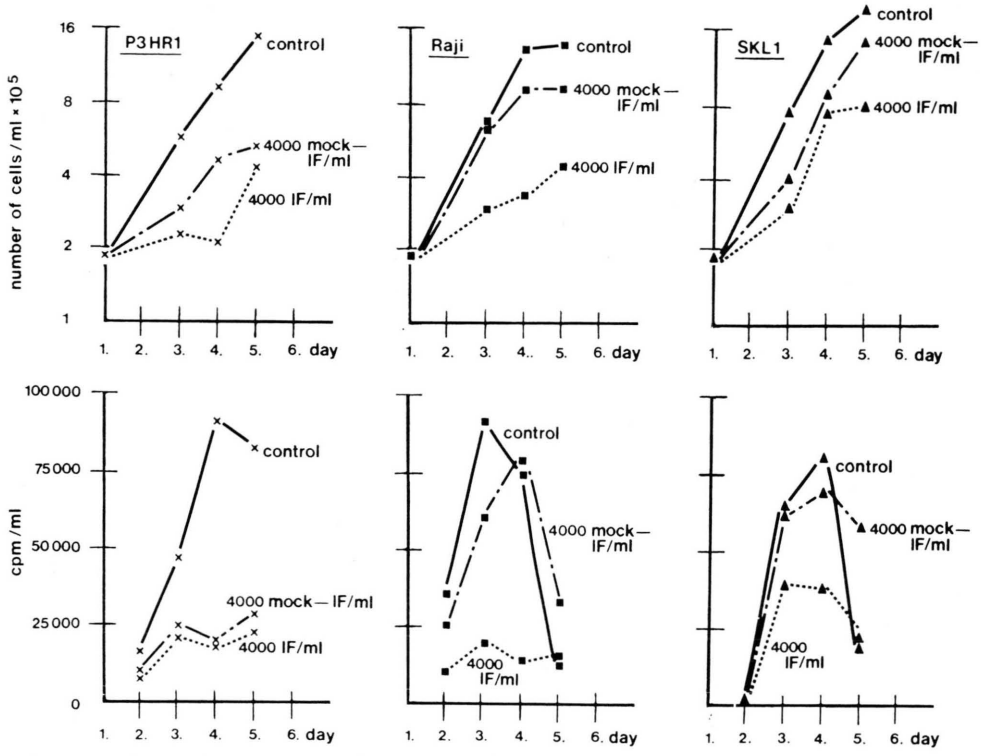

Fig. 3. Growth curves of control (———), mock interferon- (— - — -), and interferon (HIF 3)-treated (- - - - -) cultures of the same cell lines as in Fig. 1. For details refer to Fig. 1.

significantly lower. No influence could be observed on the cell line RPMI 1788 originally isolated from a healthy donor. The number of the studied lymphoblastoid cell lines is too small to permit any interpretation of the differences between the cell lines.

Surprisingly, mock interferon had an inhibiting effect on the proliferation of the three interferon sensitive cell lines, which was comparable to the activity of the interferon preparations in the case of P3HR1. J. A. Green *et al.* [13, 14] reported on a factor with similar properties which inhibited the proliferation of established human cell lines such as HeLa. The antiviral activity, however, had not been tested. This factor was isolated from the supernatants of both phytohemagglutinin-stimulated and non-stimulated primary human leukocytes. By contrast I. Gresser [15] found that mock interferon preparations did not inhibit cell proliferation. He therefore concluded that the antiviral and anticellular activities of mouse interferon preparations are likely to be properties of the same molecule, namely interferon. However, Gresser's and our results cannot be directly compared since his interferon preparation was of murine origin and had been synthesized by cells of different organs.

In our opinion, two possible explanations could be given concerning the antiviral activity of the interferon and mock interferon preparations:

1. Leukocytes incubated *in vitro* at 37 °C synthesize an anticellular substance, which is produced in increasing amounts during interferon induction. This substance could be one of the cell growth inhibitors called chalones [16]. In this case, the anticellular substance would have no antiviral property, and the interferon would be without anticellular activity.
2. The anticellular substance in the mock interferon preparation is the same as postulated above. However, under the conditions of interferon induction, another substance with anticellular activity would be induced, which could be identical with interferon. Therefore two substances with cell growth inhibitory properties would exist in the interferon preparation, one of which could display antiviral and anticellular activity.

The physico-chemical similarities of the anticellular substance and interferon are based on the following comparisons. Both are stable at pH 2 at 4 °C for four days. They are sensitive to tryptic digestion. They have a molecular weight in the range between 5,000 and 40,000 daltons.

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