Interferon and Cell Division
VII. Inhibitory Effect of Highly Purified Interferon Preparations on the Multiplication of Leukemia L 1210 Cells (36945)

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Interferon preparations inhibit the multiplication of murine tumor and normal cells in culture (1–7). The evidence that interferon is the responsible factor is based on the constant correlation between the antiviral and anticell division activities of a given preparation (1, 4, 6), and the finding that cell lines resistant to the antiviral activity of interferon are also resistant to the effect of interferon on cell multiplication (4, 6). However, since the interferon preparations used were semipurified it might still be argued that the effect of interferon on cell multiplication was due to a factor closely related, but distinct from interferon. Indeed Borecky and coworkers (8) reported "that the cell inhibitory and the virus inhibitory (interferon) activities can be separated" by electrophoresis in polyacrylamide gel. We report herein inhibition of L 1210 cell multiplication by five highly purified interferon preparations prepared by different techniques in 3 laboratories. Moreover quantitative comparisons of the antiviral activity of a given interferon

TABLE I.

Laboratory origin of Interferon	Code Reference	Production and purification of mouse interferon	Ref.	Interferon sp act (units/mg protein)
G. Bodo 1	(B-1)	L_{929} cells + NDV, concd by ZN acetate, chromatography on SE Sephadex C-25	(10,11)	2×10^6
G. Bodo 2	(B-2)	L cells + NDV, concd by ZN acetate, 3 chromatographic steps on SP-Sephadex C-25, DEAE-cellulose, and CM Sephadex C-25	(10,11)	1×10^7
K. Paucker 1	(P-1)	L cells + NDV, concd by ultrafiltration, chromatography on SE-Sephadex, electrophoresis on polyacrylamide gel pH 4.3, "acid" pool	(12–14)	$\geqq 1.1 \times 10^7$
K. Paucker 2	(P-2)	L cells + NDV, concd by ultrafiltration, chromatography on SE-Sephadex, electrophoresis on polyacrylamide gel pH 4.3, "alkaline" pool	(12–14)	$\geqq 1.6 \times 10^7$
W. Stewart	(S)	L cells + MM virus, selective precipitation by ammonium sulfate at pH 2, concd by ultrafiltration, chromatography on CM Sephadex	(15,16)	1×10^7

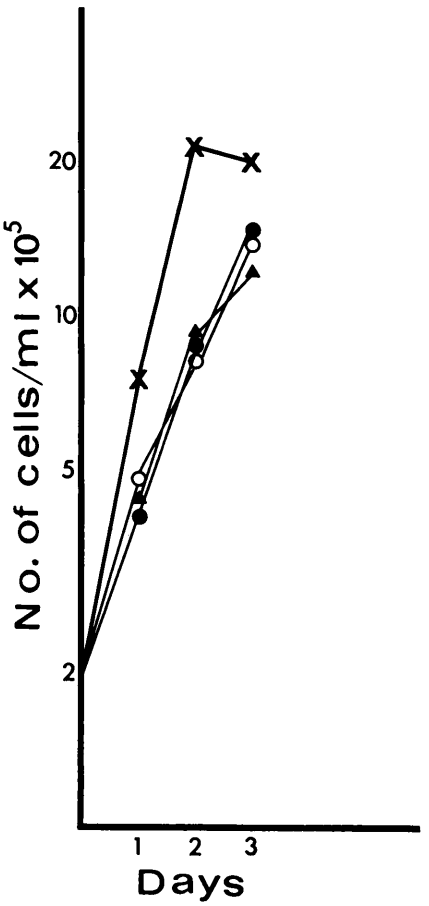

FIG. 1. Effect of highly purified interferon preparations on the multiplication of murine leukemia L 1210 cells in nonagitated suspension cultures: (x) untreated cells; (▲) interferon B-2, 640 units/0.2 ml; (○) interferon P-1, 640 units/0.2 ml; (●) interferon P-2, 640 units/0.2 ml.

preparation and the inhibitory activity on cell multiplication using a recently developed sensitive assay (9) adds strong evidence that interferon itself is indeed responsible for both effects.

Materials and Methods. The source of interferon preparations, the techniques used in their purification and their specific activities (expressed in N.I.H. international reference units) are listed in Table I. The antiviral activity and the inhibitory activity on cell multiplication of each interferon preparation were undertaken concomitantly in the same laboratory. For convenience the inhibitory effect of interferon on cell multiplication will be referred to as the anticellular effect, although it is understood that the "antiviral effect of interferon" is also cell mediated. The antiviral activity was assayed by determining the inhibition of vesicular stomatitis virus (VSV) plaques on monolayer cultures of L cells using standard techniques. The techniques used in determining the anticellular effect of interferon either by daily cell counts of mouse leukemia L 1210 cells in nonagitated suspension cultures (3–5), or by determining the 50% reduction of L 1210 cell colony formation in agarose (9), have also been previously described.

Results and Discussion. As shown in Fig. 1, three different highly purified interferon preparations each diluted to contain 640 interferon units/0.2 ml inhibited the multiplication of L 1210 cells in nonagitated suspension cultures.

Figure 2 presents the results of the determination of the antiviral activity of each interferon preparation expressed as the inhibition of the number of VSV plaques and the determination of the anticellular effect expressed as the inhibition of L 1210 cell colony formation by interferon dilutions incorporated in the agarose nutrient base (9). In each instance, highly purified interferon preparations inhibited L 1210 cell colony formation. The dose–response curves of the anticellular and antiviral activities of a given preparation were found to be parallel indicating a close relationship between the two actions of interferon. The anticellular titer of the interferon (as determined by the 50% reduction in colony formation) was somewhat lower than the antiviral titer (as determined by the 50% reduction in viral plaques).

Two other types of experiment further demonstrated the parallelism between the antiviral and anticellular activity of interferon. Figure 3 shows that the kinetics of the appearance of the anticel'ular activity of interferon paralleled the kinetics of the appearance of the antiviral activity. Figure 4 shows that the curves of thermal inactivation of the anticellular and antiviral activities of a semipurified cell culture preparation were also parallel.

It seems worth emphasizing the marked

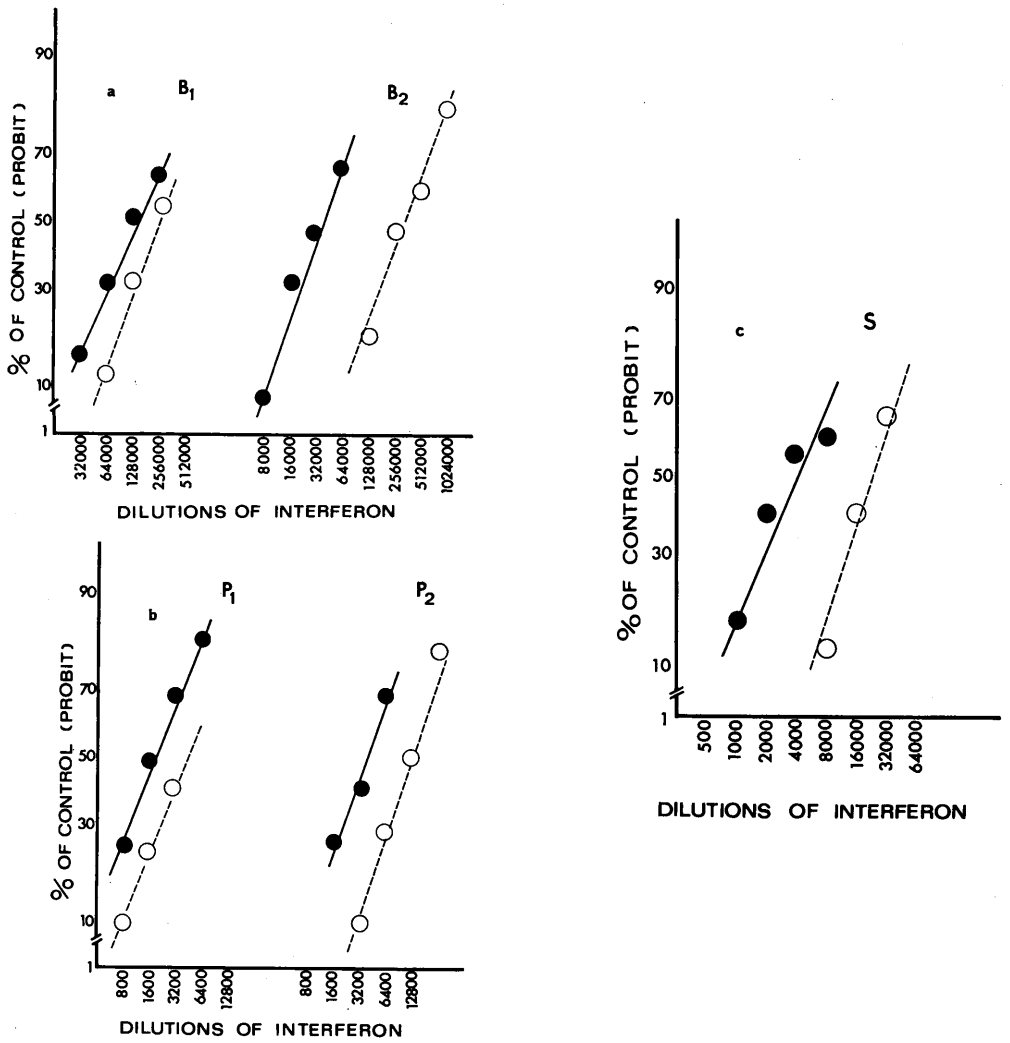

FIG. 2. Determination of the activity of five highly purified interferon preparations by inhibition of vesicular stomatitis virus plaques in L cells (○—), or by inhibition of L 1210 cell colony formation in agarose (●—); see Table I for identification of interferons B-1, B-2, P-1, P-2, and S.

inhibitory activity of interferon on cell multiplication since in some experiments a potent preparation could be diluted 256,000-fold in agarose and still inhibit by 50% the formation of L 1210 cell colonies. At this dilution there were less than 4 ng of interferon protein in the agarose. (The exact amount can not be determined since even purified interferon is not pure).

The highly purified interferon preparations used in these experiments are to the best of our knowledge among the purest, if not the purest mouse interferon preparations available, exhibiting a specific activity of 10^7 units/mg protein. Since these different preparations inhibited the multiplication of L 1210 cells in suspension cultures and in agarose, and their activity closely paralleled the antiviral activity, it seems reasonable to conclude that interferon itself can inhibit cell multiplication.

Summary. Highly purified mouse interferon preparations prepared by different techniques in different laboratories inhibited the multiplication of mouse leukemia L 1210 cells. The dose–response curves of the antiviral

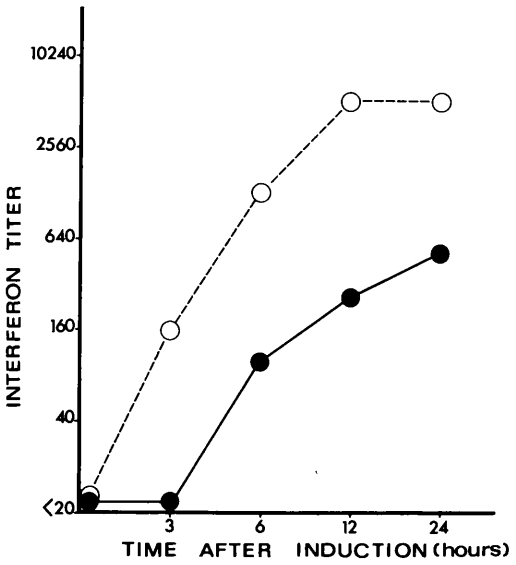

FIG. 3. Monolayer cultures of L cells were inoculated with ultraviolet irradiated NDV to induce the production of interferon. At the times indicated aliquots of the cell culture medium were harvested and treated at pH 2 for 6 days. The antiviral (○) and anticellular activities (●) of these aliquots were determined.

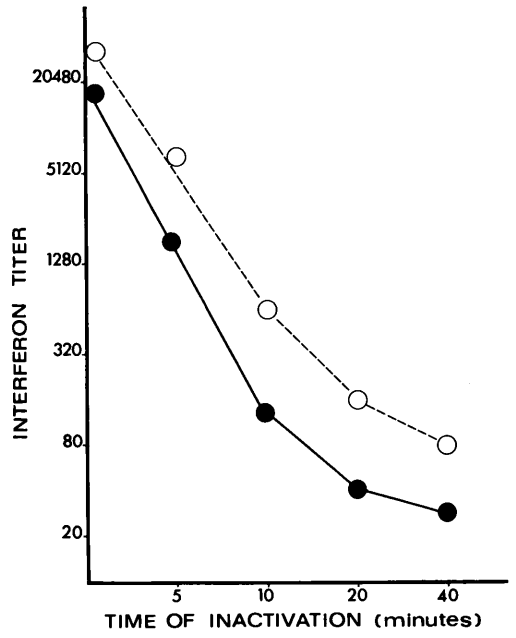

FIG. 4. Effect of incubation at 60° on the antiviral activity (○) and on the anticellular activity (●) of a semipurified mouse cell culture interferon preparation.

activity of interferon and the anticell multiplication activity using a sensitive assay were parallel. Likewise, the kinetics of the appearance of the factor responsible for both activities and the curves of thermal inactivation of this factor were also parallel. It seems reasonable to conclude that interferon itself can inhibit cell multiplication.

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