ty of each drug to increase the recovery of the 6-TG^r mutants and its ability to promote tumors in vivo. Both phorbol and 4-α-phorbol-12,13-didecanoate (neither of which promotes the growth of skin tumors) gave essentially negative results.

These results, we believe, are applicable for the detection of tumor promoters in Chinese hamster cells in vitro. At present, phenobarbital, anthralin, butylated hydroxytoluene, DDT, and saccharin have blocked metabolic cooperation in the V79 system (*23*). We are now testing suspected promoters of tumors in humans by using Lesch-Nyhan fibroblasts (HGPRT$^-$) cocultivated with normal diploid fibroblasts (HGPRT$^+$).

It would appear that the mechanism through which TPA increases the recovery of 6-TG^r cells in the V79 system is related to a reduction of metabolic cooperation. This could occur through alteration of membrane structure or function or both, which would reduce the amount of cell-to-cell communication. This explanation is consistent with a variety of experimental and theoretical reports. Borek and Sachs (*24*) showed that nontransformed hamster or rat cells can inhibit cell replication of transformed cells. Sivak and Van Duuren (*25*) noted that croton oil, a potent promoter of skin tumors in mice, enhances the recovery of virus-transformed cells that are cocultivated with many nontransformed 3T3 mouse cells. Krieg *et al.* (*26*) showed that TPA is an effective inhibitor of a G1 chalone in mouse skin. A mathematical model was postulated by Bell (*27*) to explain how cancer cells escape regulation by diffusible mitotic inhibitors. He postulated a locally reduced mitotic inhibitor to account for a single transformed cell proliferating until it reaches a "critical mass." Bertram (*28*) and Lloyd *et al.* (*29*) showed that untransformed 10T$^1/_2$ cells can, by cocultivation, suppress the expression of transformed C3H 10T$^1/_2$ cells. Recently, Bertram (*30*) showed that the mechanism by which nontransformed 10T$^1/_2$ cells inhibit the growth of cocultivated malignant cells seems to involve the modulation of cyclic nucleotides in mediating intracellular communication between normal and transformed cells.

Two additional observations should be considered: (i) transformed human cells exhibit reduced contact-mediated communication when compared to normal cells (*9*) and (ii) malignant cells in mice melanomas do not form tumors when mixed with cells of a nonmalignant clone (*5*). Since tumorigenicity has been shown to be a recessive trait (*31*) in all but virally transformed cells, it is likely that tumorigenic cells can be converted into normal cells when regulatory substances from normal cells are transferred by metabolic cooperation. Therefore, we speculate that a possible mechanism for tumor promotion could be the elimination of metabolic cooperation, allowing cells in the initation phase of carcinogenesis to express their transformed phenotype.

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References and Notes

1. J. H. Subak-Sharpe, R. R. Burk, J. D. Pitts, *J. Cell Sci.* **4**, 353 (1969).
2. R. P. Cox *et al.*, *Exp. Cell Res.* **74**, 251 (1972).
3. C. M. Corsaro and B. R. Migeon, *Nature (London)* **268**, 737 (1977).
4. G. Ciment and J. De Vellis, *Science* **202**, 765 (1978).
5. E. W. Newcomb, S. C. Silverstein, S. Silagi, *J. Cell. Physiol.* **95**, 169 (1978).
6. J. C. Fratantoni, C. W. Hall, E. F. Neufeld, *Science* **162**, 570 (1968).
7. U. Wiesmann and E. F. Neufeld, *ibid.* **169**, 72 (1970).
8. L. H. Dickerman and J. A. Tischfield, *Mutat. Res.* **49**, 83 (1978).
9. C. M. Corsaro and B. R. Migeon, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4476 (1977).
10. R. P. Cox, M. R. Krauss, M. E. Balis, J. Dancis, *J. Cell. Physiol.* **84**, 237 (1974).
11. R. Evans and P. Alexander, *Nature (London)* **228**, 620 (1970).
12. W. R. Loewenstein, *Cellular Membranes and Tumor Cell Behavior* (Williams & Wilkins, Baltimore, 1975), p. 239.
13. I. Berenblum, *Cancer Res.* **1**, 44 (1941).
14. H. Witschi, D. Williamson, S. Lock, *J. Natl. Cancer Inst.* **58**, 301 (1977).
15. C. Peraino, F. J. M. Fry, E. Staffeldt, J. P. Christopher, *Cancer Res.* **35**, 2884 (1975).
16. W. H. Hall, *Br. J. Cancer* **2**, 273 (1948).
17. B. S. Reddy, T. Narasawa, J. H. Weisburger, E. L. Wynder, *J. Natl. Cancer Inst.* **56**, 441 (1976).
18. D. Gaudin, R. S. Gregg, K. L. Yielding, *Biochem. Biophys. Res. Commun.* **45**, 630 (1971).
19. G. R. Lankas, C. S. Baxter, R. T. Christian, *J. Toxicol. Environ. Health* **4**, 37 (1978).
20. J. P. Cruse, M. R. Lewin, G. P. Ferulano, C. G. Clar, *Nature (London)* **276**, 822 (1978).
21. J. E. Trosko and C. C. Chang, *Q. Rev. Biol.* **53**, 115 (1978).
22. L. Diamond, T. G. O'Brien, G. Rovera, *Life Sci.* **23**, 1979 (1978).
23. J. E. Trosko *et al.*, in *Short-Term Tests for Chemical Carcinogens*, H. H. Stick, Ed. (Springer-Verlag, New York, in press).
24. C. Borek and L. Sachs, *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1705 (1966).
25. A. Sivak and B. L. Van Duuren, *Science* **157**, 1443 (1967).
26. L. Krieg, I. Kuhlmann, F. Marks, *Cancer Res.* **34**, 3135 (1974).
27. G. I. Bell, *Science* **192**, 569 (1976).
28. J. S. Bertram, *Cancer Res.* **37**, 514 (1977).
29. E. L. Lloyd, M. A. Gemmell, C. B. Henning, *Radiological and Environmental Research Division Annual Report* (Argonne National Laboratory, Argonne, Ill., 1977–1978), part 2, p. 20.
30. J. S. Bertram, *Proc. Am. Assoc. Cancer Res.* **20**, 212 (1979) (abstract).
31. E. J. Stanbridge and J. Wilkinson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1466 (1978).
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Noncycling Tumor Cells Are Sensitive Targets for the Antiproliferative Activity of Human Interferon

Abstract. *Resting Burkitt's lymphoma cells (Daudi) in culture are more sensitive targets for the antiproliferative activity of purified human fibroblast interferon than cells that are rapidly multiplying. Thus, interferon may be of significant clinical value in neoplasms involving stem cells and, after chemotherapy, in suppressing the re-emergence of tumors.*

Interferons (IF's), produced by different cell types, are glycoproteins with antiproliferative as well as antiviral activities. They are being tested as therapeutic agents in a variety of malignant tumors and viral diseases (*1*). Although IF's affect a wide range of tumor cell types, little is known about the events in the mitotic cycle that are targets of IF action. Better insight into the action of IF's could result in greater suppression of tumor cell proliferation and possibly effective combinations with available chemotherapeutic drugs. This would be very useful for clinical trials, which are now limited by both the expense and the amount of IF's available.

Lymphoblastoid cell lines, established from Burkitt's lymphoma, have been used to study the IF effect on cell division and the virus growth cycle (*1, 2*). Simultaneous measurements of IF effects on expression of virus genes (Epstein-Barr virus) and cell growth show that the kinetics of appearance of the antiproliferative and antiviral effects are parallel, illustrating the close functional similarities between them. Moreover, these two properties of IF's are always coordinately influenced, as shown by the gradient of IF sensitivity observed among certain lymphoid tumor lines (*2*). Both biological effects reside in the same glycoprotein (*1, 3*).

The antiviral effect of IF's is enhanced in noncycling human cells (*4*). In this report we demonstrate that the antiproliferative activity of purified human fibroblast IF (HFIF) on Daudi lymphoblastoid cells is most pronounced in resting or nondividing cells. Our experiments imply that intracellular events, in conjunction with the extracellular IF concentration, are critical determinants of the magnitude of antiproliferative response. They suggest a strategy for the

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combined use of IF's and chemotherapeutic agents to achieve maximal tumor cytoreduction and containment of the residual tumor cells.

Experiments were designed to determine whether resting and actively cycling Daudi cells are equally susceptible to an IF-induced blockage of division. The HFIF was added at 0 and 24 hours after plating (resting cells, Fig. 1, A and B), 50 hours (initiation of cell division, Fig. 1C), and 70 hours (logarithmic growth, Fig. 1D). All experiments were performed with cells that had been plated simultaneously from one batch of suspension culture, ensuring that conditions for cell growth were identical.

The antiproliferative effect was maximal when HFIF was added immediately after cell seeding in microplates (Fig. 1A); this allowed 48 hours of action before the cells entered exponential growth. The effect appeared to be approximately proportional to the logarithm of the HFIF dose applied. It was qualitatively independent of cell concentration, but slight quantitative differences were noted. Even at its highest concentration, 3000 reference units per milliliter (see legend of Fig. 1), HFIF was not directly toxic to cells, since there was no decline in viability.

As the time of HFIF addition was progressively postponed, there was an increasing loss of antiproliferative effect (Fig. 1, B to D). This suggested that the antiproliferative effect was linked to intracellular events that varied as the cells passed through a resting phase to an actively cycling phase.

After HFIF was added to Daudi cells about to enter active cycling, more than 24 hours were required before an apparent effect on cell multiplication could be discerned (Fig. 1C). A similar lag phase was observed in suspension cultures of mouse leukemia (*5*) and other mammalian cells treated with IF (*6*). This lag phase is consistent with the buildup of intracellular effector molecules induced by IF (*7*). Figure 1C also shows a marked reduction in the overall magnitude of the IF effect compared to that observed when identical concentrations were applied to noncycling cells. For example, addition of both 300 and 3000 U/ml to resting cells (Fig. 1A) resulted in 10- to 18-fold greater inhibition of cell division (Fig. 1, C and D, and Table 1). The antiproliferative effect was further reduced when HFIF was added to cells during exponential growth (Fig. 1D); the growth curves for the treated cells now deviated little from the control curve. Thus, when HFIF was added to actively dividing cells, the antiproliferative effect fell more than tenfold compared to that on nondividing cells. It appears that the departure of Daudi cells from a noncycling mode, or a state analogous to the G_0 phase, is the important determinant in explaining our results.

Table 1. Population doublings of Daudi cells between 50 and 100 hours after treatment with purified HFIF. For a starting cell concentration of 1×10^5 cells per well, the number of population doublings was calculated for the control and HFIF-treated cells from the cell counts in Fig. 1. Results are expressed as percentages of the population doublings observed in untreated cultures during the same time interval.

Time of HFIF addition (hours)	HFIF (U/ml)			
	0	30	300	3000
0	100	65	5	2
24	100	84	36	22
50	100	95	53	28
70	100	95	54	36

In separate experiments, we and others (*6*) have demonstrated that exposure of cells to purified HFIF for as little as 60 minutes is as effective as exposure for 5 to 8 days in interfering with the mitotic cycle. Thus, the antiproliferative effect, like the antiviral effect of IF, can be triggered by short exposure times. This implies that the apparently lower stability of HFIF (compared to human leukocyte IF) may not be a major factor influencing its antiproliferative function in vivo (*8*). Indeed, brief exposures to HFIF in vivo—for example, every 2 to 4 days—might have a pronounced antiproliferative effect. In the experiments reported here, the HFIF levels fell to approximately 20 percent of the original level by 75 hours after addition (data not shown). Treatment of cells with purified HFIF may render them more sensitive to normal control mechanisms that limit cell cycling as the population density increases (*6*). With HFIF treatment the cell's cytoskeleton enlarges and its plasma membrane may become more rigid (*6, 9*). Decreased proliferation of cells is normally accompanied by a stepdown in macromolecular synthesis but the factors that regulate cell division at high population densities are not fully defined. When cells are at or near G_0, HFIF may effectively augment the normal buildup of modulators, such as oligoadenylic acid triphosphate (2-5A) (*10*), that results in the observed stepdown in cellular macromolecular synthesis. When cells enter a logarithmic phase of growth, with its attendant increase in macromolecular synthesis, our experiments suggest that the IF-induced effector molecules (such as 2-5A) are either less abundant or much less effective.

At a practical level, these experiments imply that a stronger effect of IF's on human tumors in vivo might be obtained by combining them with drugs that impair cell cycling activity and result in a population enriched in resting tumor cells. The superiority of chemotherapy and IF over either drug alone has been demonstrated in the mouse (*11*). A tumor is a mixture of cells, some of which are momentarily quiescent while others are in an active mitotic cycle; in such a situation, IF and a chemotherapeutic drug might be expected to have a synergistic effect when used in an appropriate sequence. It would be required that the

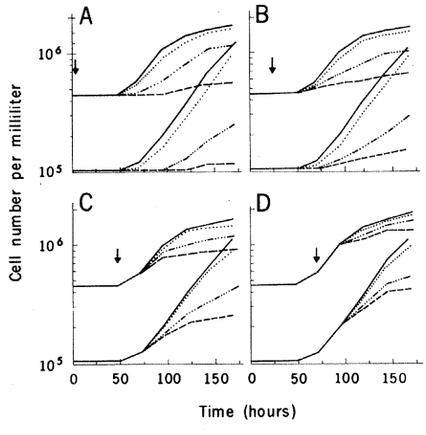

Fig. 1. Effect of purified HFIF on proliferation of Daudi cells. The HFIF was produced in diploid fibroblasts by a superinduction procedure (*13*) and purified by sequential affinity chromatography on concanavalin A–agarose and phenyl-Sepharose CL-4B (*14*). After elution from phenyl-Sepharose, HFIF with a specific activity of 2×10^7 U/mg was obtained, and human serum albumin (3 mg/ml) was added to enhance stability. After dialysis against phosphate-buffered saline, the HFIF was freeze-dried and stored at 4°C. The HFIF concentrations are expressed in units of a National Institutes of Health reference standard (G-023-901-527). Daudi cells (obtained from J. Fogh of Sloan-Kettering Institute) were grown in RPMI medium 1640 supplemented with 1 m*M* glutamine, 15 percent fetal calf serum, penicillin, and streptomycin. From a nonagitated suspension culture of cells grown for 8 days to a plateau phase of growth, plates (Linbro FB-24-16-TC) were seeded with a cell suspension (1×10^5 or 4.5×10^5 cells per well) in a final volume of 1.0 ml per well. The plates were incubated at 37°C in a humidified 5 percent CO_2 atmosphere in air; purified HFIF (50 μl diluted in cell culture medium) was added (triplicate wells) at the times indicated by arrows. Culture medium (50 μl) was added to controls. Cell counts at 24-hour intervals were determined with a Coulter counter. Standard errors (not shown) were less than ± 2 percent. Curves are for controls (—) and for cells treated with HFIF at 30 U/ml (.....), 300 U/ml (— ····· —), and 3000 U/ml (— — —).

chemotherapeutic drug selected have a mode of action compatible with IF induction of its various intracellular mediators (*12*). Also, IF may prove of particular value in controlling neoplasms involving stem cells such as leukemias of myeloid origin.

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References and Notes

1. I. Gresser and M. G. Tovey, *Biochim. Biophys. Acta* **516**, 231 (1978); T. C. Merigan, K. Sikora, J. H. Breeden, R. Levy, S. A. Rosenberg, *N. Engl. J. Med.* **299**, 1449 (1978); J. Gutterman, unpublished data; W. A. Carter, S. S. Leong, J. S. Horoszewicz, in *Antiviral Mechanisms and the Control of Neoplasia*, P. Chandra, Ed. (Plenum, New York, 1978), pp. 633-674.
2. A. Adams, H. Strander, K. Cantell, *J. Gen. Virol.* **28**, 207 (1975).
3. E. Knight, Jr., *Nature (London)* **262**, 302 (1976); R. F. Buffett, M. Ito, A. M. Cairo, W. A. Carter, *J. Natl. Cancer Inst.* **60**, 243 (1978).
4. D. H. Carver and P. I. Marcus, *Virology* **32**, 247 (1967); T. G. Rossman and J. Vilček, *J. Virol.* **4**, 7 (1969); W. A. Carter, *Johns Hopkins Med. J.* **130**, 166 (1972).
5. M. Tovey, D. Brouty-Boýe, I. Gresser, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2265 (1975).
6. L. M. Pfeffer, J. S. Murphy, I. Tamm, *Exp. Cell Res.*, in press.
7. B. LeBleu, G. C. Sen, S. Shaila, B. Cabrer, P. Lengyel, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3107 (1976).
8. W. E. Stewart, II, P. DeSooner, J. G. Edy, K. Paucker, K. Berg, C. A. Oghurn, *J. Gen. Virol.* **26**, 327 (1975).
9. I. Tamm and P. B. Sehgal, *Am. J. Med.* **66**, 3 (1979).
10. B. R. G. Williams and I. M. Kerr, *Nature (London)* **276**, 88 (1978).
11. M. A. Chirigos and J. W. Pearson, *J. Natl. Cancer Inst.* **51**, 1367 (1973).
12. P. J. Farrell, G. C. Sen, M. F. Dubois, L. Ratner, E. Slattery, P. Lengyel, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5893 (1978).
13. E. A. Havell and J. Vilček, *Antimicrob. Agents Chemother. (1961-70)* **2**, 476 (1972); J. S. Horoszewicz, S. S. Leong, M. Ito, L. A. Di Berardino, W. A. Carter, *Infec. Immun.* **19**, 720 (1978).
14. M. W. Davey, E. Sulkowski, W. A. Carter, *Biochemistry* **15**, 704 (1976); J. S. Horoszewicz and S. S. Leong, *Cancer Treat. Rep.* **62**, 203 (1978).
15. Supported by PHS grants CA-14801 and CA-15502.

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Cortical Plasticity in Monocularly Deprived Immobilized Kittens Depends on Eye Movement

Abstract. *A marked reduction of binocular cells in striate cortex is found if 4-week-old kittens are visually stimulated monocularly while anesthetized and held in a stereotaxic apparatus. If the kittens are paralyzed and artificially respirated, changes are not found unless an eye is moved mechanically. It appears that eye movement and visual stimulation are necessary conditions for deactivation of binocular connections, but neither is sufficient to induce such changes alone.*

A relatively brief period of monocular deprivation can cause marked changes in the visual cortex of a 4-week-old kitten (*1*). Study of this phenomenon, which could reflect cellular mechanisms similar to those involved in learning and memory, would be greatly facilitated if the consequences of the visual deprivation were measured as they developed. However, attempts to produce alterations in the visual cortex while recording from single neurons have been largely unsuccessful (*2*). Since monocular occlusion for 8 hours causes physiological changes if an animal is alert (*3*), but not if it is paralyzed and anesthetized, one or both of the latter conditions must interfere with the process that deactivates some afferent pathways.

We tested the hypothesis that the cortical effects of brief periods of monocular occlusion are prevented by paralysis or anesthesia or both. Two groups of normally reared 4-week-old kittens were held stereotaxically while they viewed a visual stimulus monocularly for 12 hours. Then, using standard procedures, we studied a sample of cells in their visual cortex. One group of kittens breathed a mixture of nitrous oxide and oxygen during the stimulation but remained unparalyzed. Results from this group showed a significant numerical reduction of binocularly activated cells but not of neurons responsive through the occluded eye. The second group was prepared in the same way as the first group, but the kittens were paralyzed during the exposure to visual stimuli. In this case, no breakdown of binocularity was found. We conclude that some aspect of the paralysis somehow interferes with the effects caused by monocular deprivation.

In the first experiment, ten kittens were anesthetized with halothane; anesthesia was maintained by intravenous infusion of methohexital sodium while a tracheal tube was inserted and EEG electrodes were attached to the skull. The kittens were then positioned loosely in a stereotaxic apparatus. All wound areas were infiltrated with a local anesthetic (zyljectin). Five of the kittens were paralyzed with gallamine triethiodide (10 mg/kg per hour) and were given a mixture of N_2O (75 percent) and O_2 (25 percent) through a respirator. The other five kittens were not paralyzed and were given the gas mixture through a reservoir and a one-way valve arranged so that room air could not enter. (Cursory observation indicated that under the latter condition, irregular eye movements of moderate scope and slow velocity occurred.) All ten kittens were given a very small amount of methohexital sodium (0.75 mg/kg per hour, intravenously) in a mixture of lactated Ringer's solution. Contact lenses were placed on both eyes but the left lens was opaque, occluding the entire palpebral aperture.

Visual stimuli were presented in one of two forms (Fig. 1). The first was a cathode-ray tube (CRT) display of a bright (75 cd/m^2) horizontal grating high in contrast and low in spatial frequency (0.5 cycle per degree), whose bars drifted continuously downward at 1 Hz. The second display was a projected high-contrast pattern of random elements that rotated and moved up and down at about 5° per second. The temperature, expired CO_2, EKG, and EEG of all the kittens were monitored. Visual stimulation was begun when there were no signs of barbiturate anesthesia (spindles) in the EEG.

After presentation of the visual stimuli, we prepared the animals according to standard procedures (*1*) for the study of single neurons. A small section of skull over the hemisphere contralateral to the occluded eye was removed and the dura mater excised. The kittens that had breathed through the reservoir-and-valve apparatus were paralyzed and artificially ventilated. A tungsten-in-glass microelectrode was inserted into the brain to measure action potentials from individual cells. These potentials were amplified, displayed, and fed into audio monitors. The receptive fields of sampled cells were located within an estimated 10° of the area centralis. Subjective estimates were made of absolute and relative response strengths for each eye. Using the estimate for the latter, we assigned ocular dominance ratings (Fig. 1) to indicate whether a cell was monocularly or binocularly activated. It is important to note that for all kittens studied, we decided jointly the ocular dominance category of each cell.

In normal 4-week-old kittens, as in adults, most cells are binocularly activated (*1*) (Fig. 1b). However, with only 8 hours of monocular occlusion while kittens are kept alert (*3*), there is a substantial reduction in the number of binocularly activated cells and a shift to the unblocked eye (see Fig. 1a). In this case, only 36 percent of the cortical cells tested were binocularly activated; 70 percent were activated by the nonoccluded eye. Results for the experiment in which anesthetized kittens viewed a grating monocularly for 12 hours indicate that

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