nal activity and pathological changes in the local dynamics of cerebral glucose utilization and intermediary metabolism. As a result, serious underestimates of the extent of neuronal loss can follow from an evaluation of labeled 2-DG uptake alone. Histological examination should serve to demarcate those areas in which metabolic changes cannot be attributed exclusively to alterations in functional state.

The metabolic and hemodynamic alterations observed in this study together with the histological changes (*6*) in ibotenate lesions resemble those seen during the subacute response to a variety of processes that result in focal brain necrosis. We therefore propose that the attenuated barbiturate response is a general characteristic of damaged brain tissue. In clinical studies where direct inspection of tissue is precluded, the combined use of barbiturates and positron-emitting 2-DG analogs should provide a functional contrast enhancement technique for the detection of recently lesioned areas.

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18. The pattern of 2-DG labeling observed in these animals has also been obtained in eight lesioned rats pretreated with either diethyl ether, muscimol, or Valium. In each case, there is a global decrease in 2-DG uptake with relative preservation of labeling within the lesion. Thus, the effect is not barbiturate-specific and is consistently seen after the administration of agents that suppress neuronal activity and the cerebral metabolic rate.
19. The lack of difference between the distributions of [1-^{14}C]glucose and [6-^{14}C]glucose suggests that there is no appreciable increase in glucose metabolism through the hexose monophosphate shunt in the lesioned striatum. Thus, the less expensive [1-^{14}C]glucose appears suitable for evaluation of glucose metabolism in lesioned as well as normal rats. Glucose labeled in the 1 or 6 position was preferred to [2-^{14}C]glucose, used in earlier studies (*12*), because the former compounds give rise to [acetyl-2-^{14}C]acetylcoenzyme A. Label in this position is retained longest during oxidation via the tricarboxylic acid cycle, thus minimizing loss of $^{14}CO_2$ during the experiment.
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24. The lumped constant (LC) in the 2-DG model appears in the denominator of the operational equation of the method and corrects for differences in transport and phosphorylation kinetics between 2-DG and glucose. In awake normal rats and in barbiturate-anesthetized rats, the value of the LC is 0.48 (*2*). Under conditions in which the rate of blood-brain barrier transport greatly exceeds the rate of glucose utilization, such as hyperglycemia, the value of the LC is reduced to 0.25 (*20, 21*). Thus, assumption of a regionally invariant LC in the present study is expected to result in underestimation of the rate of glucose utilization within the lesion, since transport is assumed intact and glucose phosphorylation reduced.
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Human Brain Tumor–Derived Cell Lines: Growth Rate Reduced by Human Fibroblast Interferon

Abstract. *The biological response modifier human β-interferon had pronounced antigrowth effects on various histologic types of human brain tumor cells but no effects on a nontransformed cell line, MRC-5. The cultures of brain tumor cells showed severe alterations indicative of cell injury and death after exposure to β-interferon for 2 to 6 days. Similar results were obtained with cells freshly explanted from human brain tumors. The results indicate that it may be possible to use fresh, explanted tumor tissue to identify patients who might benefit from therapy with β-interferon.*

It is widely accepted that antiproliferative activity is a property of all three antigenic types of interferon, that is, IFN-α, IFN-β, and IFN-γ (*1*). There is evidence that IFN expresses its antiproliferative activity on target cells both indirectly and directly. Indirect action requires the cooperative action of other elements of the nonspecific defense system, and the clearest example of this is

Table 1. Sensitivity of human brain tumor cell cultures to IFN-β. The cultures received fresh medium with or without IFN-β every 42 to 48 hours as shown. The concentration of IFN-β is expressed as the amount required to reduce the growth rate by 50 percent.

Experiment No.	Cell line	Duration of treatment (days)	Concentration of IFN-β (U/ml)
	Cultures received IFN-β on days 2 and 4		
56-6	A172	6	~ 30
56-6	MRC-5	6	> 3000
	Cultures received IFN-β on days 2 and 4, and medium alone on days 6 and 8		
56-16	A172	10	~ 30
56-16	A382	10	~ 30
56-16	MRC-5	10	> 3000
56-36	A172	10	~ 30
56-36	A382	10	< 30
56-50	MRC-5	10	> 3000
56-69	SK-N-SH	10	< 300 > 30
56-69	U87-MG	10	< 30
	Cultures received IFN-β on days 0, 2, 4, and 6, and medium alone on days 8 and 11		
56-75	SK-N-SH	13	30

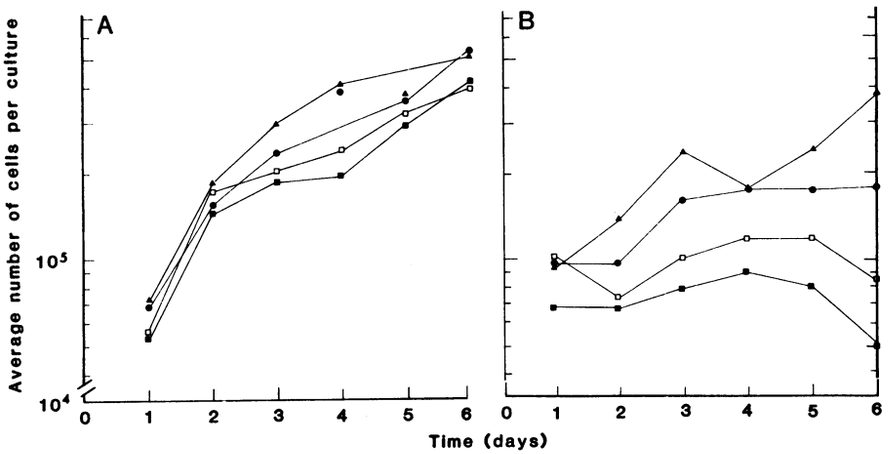

Fig. 1. The effect of IFN-β on the growth rate of (A) MRC-5 fetal lung cells and (B) A172 glioblastoma cells. Groups of 48 cultures of MRC-5 cells (1.8×10^5 cells per dish) and A172 (1.5×10^5 cells per dish) were planted on day 0. Six hours later, 36 cultures in each group were fed with fresh medium containing IFN-β (30, 300, or 3000 U/ml); 12 cultures in each group received only the medium. All cultures again received fresh medium with or without IFN-β at 48 and 96 hours after planting. Duplicate cultures from each set were counted daily and the growth rate was expressed as the percentage of growth of the untreated control cultures on day 6. Symbols: ▲, no interferon; ●, 30 U of interferon per milliliter; □, 300 U/ml; and ■, 3000 U/ml.

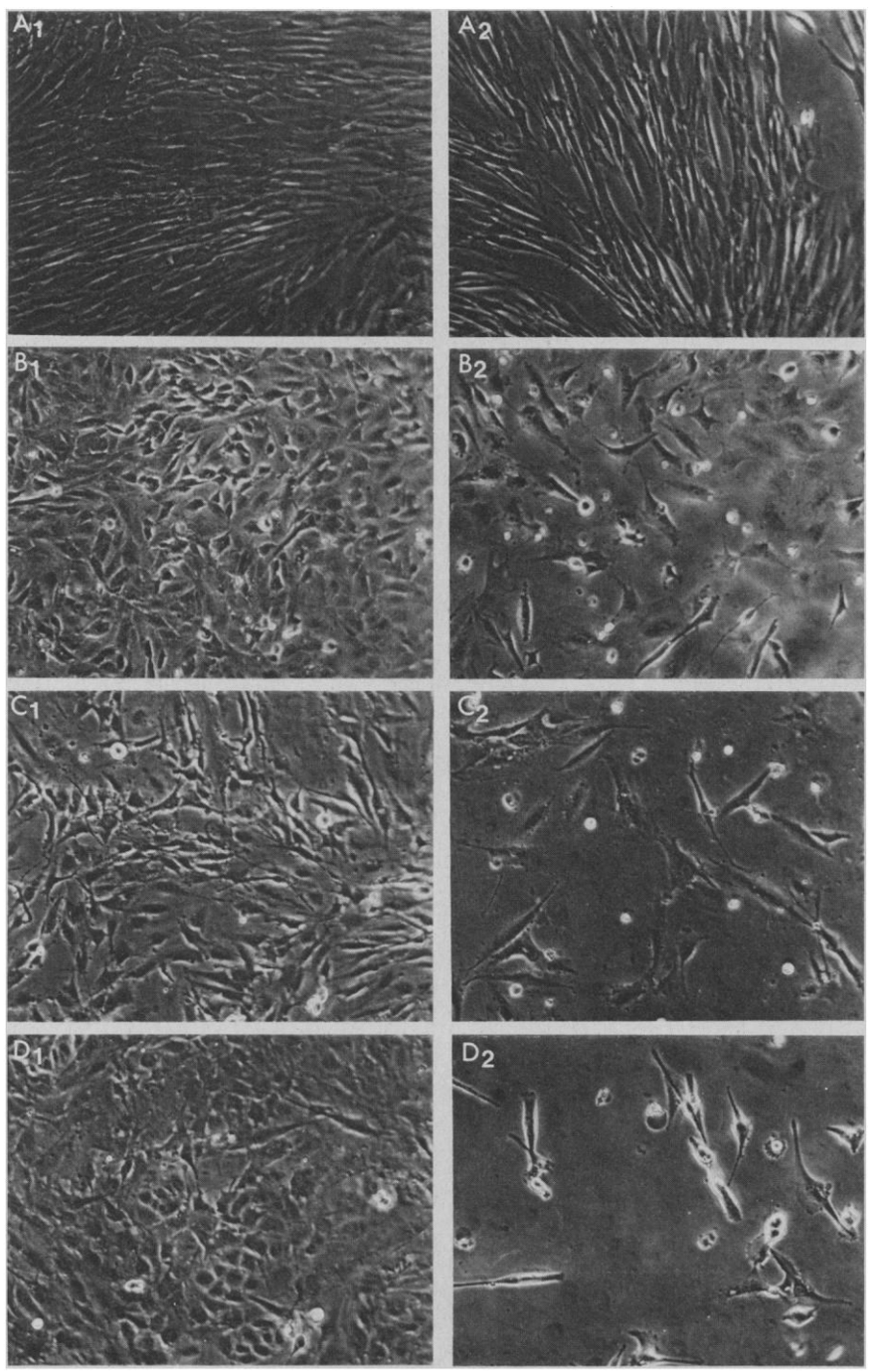

Fig. 2. Morphological changes in brain tumor cell lines after exposure to IFN-β (3000 U/ml). The experimental design was as described in the legend to Fig. 1. (A) MRC-5 cells were incubated for 7 days without (A_1) or with (A_2) IFN-β. (B) SK-N-SH cells were incubated for 7 days without (B_1) or with (B_2) IFN-β. (C) A382 cells were incubated for 5 days without (C_1) or with (C_2) IFN-β. (D) A172 cells were incubated for 5 days without (D_1) or with (D_2) IFN-β (×100).

the activation of natural killer cells (*2*). Evidence for the direct action of IFN on human tumor cells has been reported during the past 5 years (*3*). In several studies, Burkitt lymphoma cell lines were treated with human IFN-α (leukocyte) and human IFN-β (fibroblast) (*3*, *4*). Although natural killer cells and Burkitt lymphoma cells are classified as lymphoid cells, these cells of clearly diverse function do not share a common pathway for sensitivity to IFN, as far as is known. Indeed, we do not know the mechanism of action of the antiproliferative effect of IFN in any cell system (*5*).

Despite our lack of knowledge about the mechanism of the direct or indirect action of IFN, investigations of the efficacy of IFN as an antitumor agent have continued. Interest in the potential usefulness of IFN in the treatment of brain neoplasia is especially intense (*6*) because of problems associated with brain surgery and irradiation. In the present study we examined the effect of graded doses of purified human IFN-β on the growth of three brain tumor cell lines and a prototypic normal human cell line (MRC-5).

In a typical experiment, 1.0×10^5 to 3.0×10^5 cells were seeded in 10-cm^2 petri dishes, fed with Dulbecco's minimal essential medium (DMEM) supplemented with 10 percent fetal bovine serum (FBS), and incubated in a humidified atmosphere of 5 percent CO_2 at 37°C. Purified human IFN-β (HEM Research, Inc.) was obtained in vials, each containing 5×10^4 international refer-

ence units per milliliter (specific activity, 5×10^6 to 5×10^7 reference units per milligram of protein).

Medium containing IFN-β was added to the cultures as shown in Table 1. Because of the relatively long period between feedings, it was important to determine that biologically active IFN-β was present throughout the treatment period. We estimated the thermostability of the IFN-β by measuring the antiviral activity of the growth medium containing IFN-β after it had been incubated at 37°C for 72 hours. Portions of medium containing the IFN-β were removed at intervals and frozen at −70°C until assayed for antiviral activity. A laboratory reference interferon was used for calibration. The half-life of the IFN-β was found to be 23 to 25 hours. Although the antiviral activity of IFN-β is functionally separable from its antiproliferative activity, the two activities are properties of the same molecule (*7*).

Cell counts of duplicate cultures were obtained daily during the test periods. As shown in Fig. 1, the rate of growth of A172 cells was reduced in cultures fed with medium containing IFN-β at concentrations of 30 to 3000 U/ml. In contrast, the highest concentration of IFN-β used, 3000 U/ml, did not reduce the rate of growth of MRC-5 cells.

Our study incorporated some of the features described by Slimmer *et al.* (*8*) who also assayed the antiproliferative activity of human interferon on a human cell line derived from a brain tumor. We also examined the effect of IFN-β on cell cultures derived from an astrocytoma (A382), a neuroblastoma (SK-N-SH), and another glioblastoma (U87-MG). Our results are summarized in Table 1.

Compared with its effects on the normal diploid cell line, IFN-β had a dramatic morphological effect on each of the brain tumor cell cultures (Fig. 2). These cultures underwent various severe alterations indicative of cell injury and death including pycnosis, loss of doubling potential, and cytolysis (compare Fig. 2A with Fig. 2B, C, and D).

The brain tumor cultures were generally at least 100 times more sensitive to the antiproliferative activity of purified IFN-β than the MRC-5 cell cultures. This differential sensitivity is particularly important in view of the fact that MRC-5 cells are highly sensitive to the antiviral activity of IFN-β (*9*). Taken together, these experiments establish that the differential antigrowth activity of IFN-β, previously noted with certain other human tumor cell lines [bladder cancer in particular (*10*)], also extends to human brain tumor cells. The three cell lines selected for the present study were derived from brain tumors that collectively represent more than 50 percent of the clinical spectrum of brain neoplasia.

We have obtained similar results with cells explanted from brain tumors, and have found that IFN-β causes major damage to the tumor cell population while having a negligible effect on normal brain tissue. These cultures derived from biopsy specimens showed the same differential pattern of response to IFN-β as the three cell lines derived from metastatic sites.

Results of our clinical trials in selected patients with brain tumors indicate that IFN-β (*10*) has pronounced antitumor effects in vivo [see also the results of ongoing clinical studies in Europe (*11*) and Japan (*12*)]. Studies have also shown that rhesus monkeys are very tolerant to the intrathecal administration of IFN-β (*13*) and show no local or systemic side effects, suggesting that this preparation may be suitable for long-term application, especially in patients with otherwise incurable malignancies within the cranial vault. Taken together, these observations indicate the feasibility of using fresh explanted tumor tissue from the central nervous system to identify patients who might benefit from IFN-β therapy. This type of approach may also provide insight into the properties of human brain tumor cells exposed for long periods to IFN-β. There are continuing constraints in the production of IFN-β for clinical evaluation because of the biolability of the product when it is synthesized without its natural glycoid moiety, as when it is produced by *Escherichia coli*. Nonetheless our data support the view that careful expansion of clinical application of IFN-β to brain tumors may be especially valuable since the side effects often attendant with conventional approaches may well be bypassed while a pronounced antiproliferative effect on the malignant cell population is maintained.

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