Inhibition of cell division by interferons

The relationship between changes in utilization of thymidine for DNA synthesis and control of proliferation in Daudi cells

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Inhibition of the proliferation of Daudi cells by exposure to human lymphoblastoid interferons is associated with an early and marked decrease in the incorporation into DNA of exogenous [^3H]thymidine when cells are incubated with trace amounts of this precursor. In contrast, incorporation of exogenous deoxyadenosine into DNA is unchanged under the same conditions. Interferon treatment results in a lowering of thymidine kinase activity, an effect which may be largely responsible for the inhibition of incorporation of labelled thymidine into DNA. At higher concentrations of exogenous thymidine, which minimize the contribution of intracellular sources to the dTTP pool, the inhibition of thymidine incorporation is abolished. Under conditions in which exogenous thymidine is rigorously excluded from the medium or, conversely, in which cells are entirely dependent on exogenous thymidine for growth, the magnitude of the inhibition of cell proliferation by interferons is the same as under normal culture conditions. We conclude that, even though cell growth is impaired, the rate of DNA synthesis is not grossly inhibited up to 48 h after commencement of interferon treatment. Furthermore, changes in neither the utilization of exogenous thymidine nor the synthesis of nucleotides *de novo* are responsible for the effect on cell proliferation.

The interferons are recognized as an important group of regulatory molecules, capable of affecting a number of cell functions in addition to the much studied inhibition of viral replication (Balkwill, 1979; Stewart, 1979; Taylor-Papadimitriou, 1980). One of the most interesting and potentially important of their effects is the inhibition of growth and proliferation of a variety of cell types. This inhibition is initially due to a reversible cytostatic effect, with a progressive increase in the duration of several phases of the cell cycle following interferon treatment (Fuse & Kuwata, 1977; Matarese & Rossi, 1977; Balkwill & Taylor-Papadimitriou, 1978; Balkwill *et al.*, 1978; Lundgren *et al.*, 1979; Creasey *et al.*, 1980; Panniers & Clemens, 1981). There is little information, however, as to the biochemical basis of such growth inhibition and its relationship, if any, to the mechanisms of the antiviral activities characteristic of these molecules.

In the absence of viral infection, no changes in the overall rates of cellular RNA and protein synthesis are usually noted in interferon-treated cells (Tovey *et al.*, 1975), although the synthesis of specific proteins may be modulated (Gupta *et al.*, 1979). In many cell types, however, interferons inhibit the incorporation of exogenous [^3H]thymidine into DNA (Fuse & Kuwata, 1977, 1978; Brouty-Boye & Tovey, 1978; Lundgren *et al.*, 1979). This has previously been considered to reflect a slower rate of DNA synthesis in interferon-treated cells and has been used as an assay for the growth-inhibitory effects of interferons. Some reports, however, have suggested that interferons may affect steps in the incorporation of exogenous [^3H]thymidine, other than DNA synthesis itself (Fuse & Kuwata, 1978; Brouty-Boye & Tovey, 1978), and we have shown that the inhibition of proliferation of the Daudi line of human lymphoblastoid cells by human interferons is accompanied by decreased rates of membrane transport and phosphorylation of thymidine. This is apparently due to changes *in vivo* in the activity of thymidine kinase (Gewert *et al.*, 1981). These effects will therefore contribute to the overall

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inhibition by interferons of the incorporation of the radioactive precursor and do not necessarily reflect changes in the rate of DNA synthesis in interferon-treated cells. In the present paper, we have investigated possible relationships between the effects of interferons on Daudi-cell growth and on the incorporation of exogenous thymidine. We present evidence which suggests that inhibition of thymidine incorporation does not reflect any major inhibition of DNA synthesis and that this effect is not responsible for, or even necessarily related to, the impairment of cell proliferation.

Materials and methods

Interferons

A partially purified human lymphoblastoid interferon preparation (specific activity 6×10^5 International Reference Units/mg of protein) was kindly provided by Dr. K. H. Fantes and Dr. M. D. Johnston (Wellcome Research Laboratories, Beckenham, Kent, U.K.) and was handled as described previously (Gewert *et al.*, 1981). Most of the experiments described here were performed with this material, but identical results were also obtained with more highly purified interferons, prepared by affinity chromatography on a monoclonal-antibody column (Secher & Burke, 1980). All preparations contained several distinct species of interferon- α (Allen & Fantes, 1980). Interferon concentrations are expressed in this paper in International Reference Units/ml (Ref. U/ml).

Cell culture

Human lymphoblastoid (Daudi) cells were propagated at 37°C in stationary suspension culture in RPMI 1640 medium supplemented with 10% (v/v) foetal-calf serum (Flow Laboratories), 10 µg of streptomycin/ml and 10 µg of kanamycin/ml and buffered with 20 mM-Mops (4-morpholinepropane-sulphonic acid) (sodium salt) (pH 7.2). Other additions were as indicated in the text. Cells were maintained at a density below 12×10^5 /ml. For interferon treatment, batches of exponentially growing cells at about 2×10^5 /ml were split into two and the interferon preparation was added to one half. Except where indicated, the cells were then incubated for 48 h in the presence or absence of interferons before being used in an experiment.

Incorporation of [3 H]thymidine and [3 H]deoxyadenosine into DNA

Control and interferon-treated cells were labelled for various times with [3 H]thymidine or [3 H]deoxyadenosine. Duplicate 300 µl samples of cell

suspensions were processed for measurement of radioactivity in acid-insoluble material as described previously (Gewert *et al.*, 1981), except that for [3 H]deoxyadenosine incorporation a 1 h incubation at 37°C in 0.1 M-NaOH was included before precipitation with trichloroacetic acid to ensure hydrolysis of labelled RNA. This is an essential step, since substantial incorporation of radioactivity into RNA is otherwise observed, owing to breakdown of [3 H]deoxyadenosine by adenosine deaminase and purine nucleoside phosphorylase and consequent rapid labelling of the ATP pool (Hunting *et al.*, 1981; Snyder & Lukey, 1982).

Assays of thymidine kinase activity in cell extracts

Cell extracts for the assay of thymidine kinase activity *in vitro* were prepared from 1 litre of control and interferon-treated (80 Ref. U/ml; 48 h) cells. Exponentially growing cells were rapidly chilled with 0.5 vol. of crushed ice and harvested by centrifugation (500 g, 4°C, 20 min). The cell pellets were washed once in cold phosphate-buffered saline (140 mM - NaCl / 2.7 mM - KCl / 6.5 mM - Na₂HPO₄ / 1.5 mM - KH₂PO₄, pH 7.2) and resuspended in twice the packed cell volume of Tris buffer (10 mM- β -mercaptoethanol/10 mM-Tris/HCl, pH 8.0). Nonidet P-40 was added to a final concentration of 0.2% (v/v) and the suspension kept on ice for 15 min with regular vortex mixing. Glycerol was added to a final concentration of 10% (v/v) and the lysates were centrifuged at 10000 g for 10 min. The supernatants were stored at -180°C in 50 µl batches.

Thymidine kinase activities were assayed by determining the phosphorylation of the labelled nucleoside to mono-, di- and tri-phosphates. The following composition of reaction mixture was found to be optimal: 5 mM-Tris/HCl buffer, pH 8.1; 8 mM-MgCl₂; 5 mM-ATP; 10 mM-NaF; 10 mM- β -mercaptoethanol. [3 H]Thymidine (4 Ci/mmol) was added to the assay mixture in the concentration range of 6–100 µM. After preincubation of the reaction mixture for 5 min at 37°C, the enzymic reaction was started by the addition of 10 µl of cell extract in a final volume of 50 µl. Nucleoside phosphorylation was assayed as described by Munch-Petersen & Tyrsted (1977). Duplicate 3 µl samples were spotted on 2.5 cm discs of DEAE-cellulose paper (Whatman DE81) and non-phosphorylated nucleoside was eluted by washing the discs three times for 5 min each in cold 5 mM-ammonium formate buffer (pH 4.0; 20 ml/disc) and then in water and ethanol successively. The discs were dried, added to 5 ml of liquid-scintillation fluid (4 g of 2,5-diphenyloxazole/litre of toluene) and the radioactivity was determined. The enzymic reaction was linear with time for at least 30 min after the addition of cell extract, and the reaction rate was linearly related to the amount of extract used in the incubation.

Results

Effect of interferon treatment on the incorporation of trace amounts of [³H]deoxyribonucleosides

The Daudi line of human lymphoblastoid cells shows an unusually high sensitivity towards the growth-inhibitory effects of human interferons (Adams *et al.*, 1975; Hilfenhaus *et al.*, 1976). These cells are also sensitive to the effects of interferons on the incorporation of trace amounts of exogenous [³H]thymidine. In cells preincubated in the presence of interferons at 100 Ref. U/ml for 48 h, the incorporation of this precursor into acid-insoluble material is strongly inhibited (Fig. 1). In contrast, in the same cell population the incorporation of trace amounts of [³H]deoxyadenosine into DNA is barely affected by interferon treatment (Fig. 1). The experiment shown is typical of numerous similar assays performed in our laboratory and shows that the extent of the effect of interferon on the labelling of DNA is dependent on the nature of the precursor. The results in Fig. 1 suggest that the rate of DNA synthesis itself may not be significantly altered by interferon treatment, and that the inhibition of [³H]thymidine incorporation into DNA is the result of an inhibitory effect on one or more of the metabolic steps involved in the conversion of this nucleoside into the immediate precursor of DNA synthesis (dTTP). We have shown previously (Gewert *et al.*, 1981) that the rate of phosphorylation of exogenous thymidine, which is the rate-limiting step in the intracellular accumulation of labelled dTTP (Plagemann *et al.*, 1978), is indeed inhibited in intact cells after interferon treatment. This effect is also seen when thymidine kinase activity is assayed in cell extracts (Fig. 2). The inhibition observed is not as marked as in whole cells, but, as noted previously (Gewert *et al.*, 1981), is entirely attributable to a lower $V_{\max}$ for the reaction.

Effect of precursor concentration on the incorporation of [³H]thymidine

In an attempt to overcome possible effects of interferon treatment on thymidine metabolism and changes in the specific radioactivity of intracellular dTTP pools, we have measured the incorporation of [³H]thymidine at extracellular nucleoside concentrations up to 100 μ M. Such conditions flood the pools and minimize the contribution of endogenously synthesized dTTP (Cleaver, 1967; Adams, 1969; Marz *et al.*, 1977). Fig. 3 shows an experiment, typical of many that we have performed, in which the extent of inhibition of [³H]thymidine incorporation in interferon-treated cells is lower in the presence of increasing concentrations of extracellular thymidine. At 100 μ M-thymidine, there is virtually no inhibition of the incorporation of this

Fig. 1. Effects of interferon treatment on the incorporation of trace amounts of [³H]deoxynucleosides into DNA in Daudi cells

Exponentially growing cells were incubated in the absence (●) or presence (○) of the interferon preparation (100 Ref. U/ml) for 48 h. The cells were counted (controls, 11.0×10^5 /ml; interferon-treated, 4.7×10^5 /ml) and samples of cell suspensions were incubated at 37°C with either (a) [³H]thymidine (2 μ Ci/ml; 19.3 Ci/mmol) or (b) [³H]deoxyadenosine (2 μ Ci/ml; 40 Ci/mmol). At regular intervals, 0.3 ml samples of cell suspension were either (a) precipitated directly in ice-cold 5% (w/v) trichloroacetic acid or (b) added to 0.3 ml of 0.2 M-NaOH, incubated at 37°C for 1 h and then precipitated with excess trichloroacetic acid. The radioactivity incorporated into acid-insoluble material was determined as described in the Materials and methods section.

precursor in interferon-treated cells compared with controls. This result is not due to any inhibition of DNA synthesis by thymidine in the controls, since such concentrations of extracellular thymidine do not substantially alter the labelling of DNA with trace amounts of [³H]deoxyadenosine (results not shown). Furthermore, the rate of thymidine in-

corporation at saturating concentration (approx. 40 pmol/h per 10^5 cells) is sufficiently high to account for the rate at which DNA is replicated in

Fig. 2. Inhibition of thymidine kinase activity by interferon treatment

Cells were incubated in the presence or absence of interferons for 48 h and extracts prepared as described in the Materials and methods section. These extracts were then incubated with [3 H]-thymidine at concentrations from 6 to 100 μ M. At 5 min intervals samples were transferred to DEAE-cellulose paper discs, which were then washed and counted for bound radioactivity. A double-reciprocal plot of the data is presented: $1/v$ is calculated from the slopes of the linear time-courses, and $1/[S]$ is the reciprocal of the [3 H]thymidine concentration. The kinetic parameters obtained are: control cells ($\bullet$), $K_m = 14.3 \mu$ M, $V_{max} = 11.1$ pmol/ A_{260} unit per h; interferon-treated cells ($\circ$), $K_m = 14.3 \mu$ M, $V_{max} = 7.4$ pmol/ A_{260} unit per h.

control Daudi cells during exponential growth (1.21 μ g of DNA/day per 10^5 cells). A similar loss of the interferon-induced inhibition of incorporation at higher thymidine concentrations is also observed when this experiment is conducted in the presence of 2 μ M-fluorodeoxyuridine (Table 1). This agent blocks synthesis of dTMP by thymidylate syn-

Table 1. Rates of thymidine incorporation into DNA in control and interferon-treated cells in the presence of 5-fluorodeoxyuridine

Exponentially growing Daudi cells were incubated in the absence or presence of the interferon preparation (100 Ref. U/ml) for 48 h. The cells were counted (controls, 11.5×10^5 /ml; interferon-treated, 5.8×10^5 /ml) and then preincubated for 15 min with 2 μ M-5-fluorodeoxyuridine, to inhibit synthesis of thymidine nucleotides *de novo*. Samples of each cell suspension were then incubated with [3 H]thymidine (2 or 10 μ Ci/ml) together with unlabelled thymidine at the indicated concentrations. Duplicate samples were then taken after 60 min for measurement of radioactivity incorporated into DNA.

Extracellular thymidine (μ M)	Rate of incorporation of [3 H]thymidine (pmol/h per 10^5 cells)		Inhibition by interferon treatment (%)
	Control	Interferon-treated	
0.1	0.85	0.23	73
0.6	2.57	1.12	56
5	3.95	4.37	0
25	9.79	9.38	4
50	16.6	24.2	0
100	43.0	36.4	15

Fig. 3. Effect of precursor concentration on the incorporation of [3 H]thymidine into DNA in control and interferon-treated cells

Exponentially growing Daudi cells were incubated in the absence ($\bullet$) or presence ($\circ$) of the interferon preparation (100 Ref. U/ml) for 48 h. After cell counting, samples of control and interferon-treated cell suspensions were incubated with [3 H]thymidine (28 or 0.5 Ci/mmol) at concentrations of (a) 4 μ M, (b) 30 μ M, or (c) 100 μ M. Samples were taken at the times indicated for measurement of radioactivity incorporated into acid-insoluble material.

thase (Santi *et al.*, 1974) so that DNA synthesis becomes dependent on extracellular thymidine and salvage-pathway activity (Baumunk & Friedman, 1971; Stimac *et al.*, 1977). The fact that similar rates of thymidine incorporation are observed in the presence and absence of fluorodeoxyuridine when thymidine is present at $100\mu\text{M}$ (compare Table 1 and Fig. 3) confirms that synthesis of dTTP *de novo* is quantitatively unimportant in the latter conditions. Our conclusion from these experiments is that there can be very little impairment in the rate of DNA synthesis at 48 h of interferon treatment, in spite of the inhibition of cell proliferation at this time.

Effect of time of exposure to interferons on thymidine incorporation and cell growth rate

We have identified conditions (Fig. 3) under which the effect of interferon treatment on thymidine incorporation into DNA can be dissociated from inhibition of DNA synthesis. Comparison of the time-courses of the impairment in DNA labelling by this precursor and of the inhibition of cell proliferation (Fig. 4) further shows that the former

Fig. 4. Differential time-courses for inhibition of thymidine incorporation and cell growth rate by interferon treatment

Exponentially growing cells (initial density $2 \times 10^5/\text{ml}$) were incubated with and without the interferon preparation (100 Ref. U/ml) for up to 3 days. At the times indicated cell numbers were determined in a haemocytometer and incorporation of $[^3\text{H}]$ thymidine into DNA was measured as described in Fig. 1. Inhibitions of cell growth rate (●) were calculated from the slopes of plots of $\log(\text{cell number})$ against time for control and interferon-treated cultures; inhibitions of thymidine incorporation (O) are corrected for differences in cell numbers as in Fig. 1.

effect develops earlier after exposure to interferons than does the inhibition of cell growth rate. This may be partly due to the different sensitivities of the two assays; also, the time at which cell growth inhibition is first observed does vary from one experiment to another (compare, for example, Figs. 4 and 5). However, it is clear that in the presence of 100 Ref. U of interferons/ml the rate of cell proliferation becomes progressively more impaired over 48 h, whereas thymidine incorporation shows near-maximum inhibition (80–90%) within 30 h (Fig. 4).

Relationship of Daudi-cell growth and interferon activity to the availability of exogenous deoxy-nucleosides

We have investigated the possibility that the subsequent inhibition of cell growth by interferons may be mediated by the early effects on utilization of thymidine described above. However, this nucleoside is not a component of the culture medium, and Daudi cells do not require a supply of exogenous thymidine for growth. The dTTP necessary for DNA replication is derived predominantly through synthesis *de novo*. We have observed that the growth of Daudi cells is normal in medium supplemented with extensively dialysed serum, and moreover the anti-proliferative effect of interferon treatment is equally manifest in cells grown in medium supplemented with dialysed serum (Fig. 5a). It is thus unlikely that the effect of interferons on cell proliferation is mediated solely via inhibition of the uptake of thymidine.

In contrast with the normal situation, cell growth can be made dependent on the extracellular supply of thymidine when the synthesis of nucleotides *de novo* is inhibited by aminopterin (results not shown). We have tested the effect of interferons on the growth of Daudi cells cultured in the presence of this drug and supplied with exogenous thymidine, together with hypoxanthine and adenosine (as a source of purine nucleotides). The growth rate of control cells under these conditions is slightly slower than in standard medium, with doubling times of 29.5 h and 24.9 h respectively, but the effect of interferons is similar in both media (Fig. 5b). This indicates that the interferon-mediated inhibition of cell division does not require the synthesis of nucleotides *de novo*. Inhibition of the latter process cannot therefore be an obligatory part of the mechanism by which cell proliferation is impaired.

Finally, because the inhibition by interferons of $[^3\text{H}]$ thymidine incorporation could be overcome when the exogenous precursor concentration was raised to $100\mu\text{M}$ (Fig. 3), we examined the effect of added deoxynucleosides, and thymidine in particular, on the long-term growth of interferon-treated cells. The effect of interferons was tested on Daudi cells propagated in RPMI 1640 medium supple-

Fig. 5. Interferon-mediated inhibition of cell growth in the presence and absence of deoxynucleosides

(a) Daudi cells were maintained in RPMI 1640 medium supplemented with 15% (v/v) foetal-calf serum which was undialysed (●, ○) or extensively dialysed (48 h against 200 vol. of RPMI 1640 medium; ■, □). Cultures were divided, and one half of each was treated with interferons at 85 Ref. U/ml (○, □); the other half of each was left as the control (●, ■). (b) Daudi cells were propagated in standard RPMI 1640 medium or in HAAT medium containing hypoxanthine (50 μM), aminopterin (0.2 μM) adenosine (80 μM) and thymidine (100 μM). Cultures of exponentially growing cells in standard (●, ○) or HAAT medium (■, □) were divided, and one half of each was treated with interferons at 100 Ref. U/ml (○, □). (c) Daudi cells were maintained in RPMI

medium with all four deoxyribonucleosides at concentrations up to 100 μM . Under these conditions, the inhibition of cell growth by interferons was, if anything, greater than with cells grown in the absence of added deoxynucleosides (Fig. 5c). Similar results were also obtained when each deoxynucleoside was tested individually (results not shown). It would appear, then, that, although the interferon-mediated inhibition of [^3H]thymidine incorporation can be overcome by the addition of excess precursor, the inhibition of cell growth is not alleviated under these conditions. This confirms that uptake or utilization of thymidine cannot be the rate-limiting step in the overall effect of interferons on cell proliferation.

Discussion

We have previously reported (Gewert *et al.*, 1981) that lymphoblastoid interferons inhibit the intracellular accumulation of thymidine and its metabolites in Daudi cells, and we proposed that changes in the incorporation of exogenous tracer [^3H]thymidine are therefore not a true reflection of the effects of interferons on DNA synthesis. This proposal is confirmed in the present study, since the rates of incorporation of thymidine (at high concentrations) or of deoxyadenosine are not altered significantly under conditions of slower cell growth in the presence of interferons, at least within 48 h of treatment. This is not to say that the process of DNA replication is normal, however, since we have observed that in interferon-treated cells changes occur in the stability of newly synthesized DNA sequences and in their processing into mature replicated strands (G. Moore, D. R. Gewert, V. J. Tilleray & M. J. Clemens, unpublished work). These changes result in eventual degradation of the DNA, and account for a paradoxically high rate of DNA synthesis in cells which are proliferating more slowly than normal.

The data presented here indicate the absence of any clear relationship between the effects of interferons on the utilization of exogenous [^3H]thymidine and the inhibition of cell growth, since normal cell proliferation does not require the uptake of thymidine and exogenous nucleosides do not

1640 medium supplemented with deoxycytidine (50 μM), deoxyguanosine (50 μM), deoxyadenosine (50 μM) and thymidine (100 μM). Exponentially growing cell cultures were divided and incubated in the absence (●) or presence (○) of interferons (100 Ref. U/ml). Cell numbers were determined at daily intervals by counting in a haemocytometer. The doubling times (t_D), obtained from semi-log plots of the data, are indicated on the growth curves.

overcome the effect of interferons. This lack of correlation between the two phenomena suggests, not surprisingly, that the activity of the thymidine-transport system or of thymidine kinase does not limit the growth of either control or interferon-treated cells. What is perhaps more interesting is that interferon treatment clearly does not inhibit cell proliferation through any effect on the synthesis of nucleotides *de novo* in Daudi cells, since the effects are still observed when 'de novo' pathways are blocked with aminopterin and growth is dependent on exogenous nucleosides (Fig. 5b).

The relationship between thymidine kinase activity and thymidine incorporation into DNA is a complex one, as Plagemann's laboratory has pointed out (Marz *et al.*, 1977). Published data suggest the compartmentation of dTTP pools in some mammalian cells (Baumunk & Friedman, 1971; Kuebbing & Werner, 1975), although in other cases (Nicander & Reichard, 1983) it is dCTP pools rather than dTTP pools that show kinetic evidence of compartmentation. Our own observations with Daudi cells suggest the presence of more than one pool of dTTP (and perhaps of thymidine kinase), since the $V_{\max}$ of overall thymidine phosphorylation in intact control cells is only 18 pmol/h per 10^5 cells (Gewert *et al.*, 1981). This is insufficient to account for the rate of thymidine incorporation into DNA at high concentrations of this precursor (40 pmol/h per 10^5 cells; Fig. 3 and Table 1). Such a result can only be explained by postulating a relatively large (perhaps cytoplasmic) pool of dTTP, which is labelled only slowly in the presence of excess thymidine, and a much smaller, rapidly turning-over, pool which is functionally associated with the DNA-replication process in the nucleus. The latter would be consistent with the close physical association observed between nuclear thymidine kinase activity and several other enzymes involved in DNA replication (Reddy & Pardee, 1980). There are many reports correlating thymidine kinase activity with cell growth (reviewed by Kit, 1976), and this enzyme is particularly high in activity during the S phase of the cell cycle (Bello, 1974). However, in spite of the decreased thymidine kinase activity in Daudi cells after 48 h of interferon treatment (Fig. 2), we have observed only a minor decrease in the proportion of cells in S phase at this time (D. R. Gewert & G. Moore, unpublished work). Thus the explanation proposed by Leanderson *et al.* (1982), that enzymes involved in DNA synthesis are inhibited by interferon because cells accumulate in the G_0 phase of the cell cycle, is untenable in this case.

In view of the large number of different cell types which exhibit a slower rate of [3 H]thymidine incorporation under conditions of interferon-mediated cell growth inhibition (Hilfenhaus *et al.*,

1976; Fuse & Kuwata, 1977; Bourgeade & Chany, 1979; Lin *et al.*, 1980), it is important to consider whether changes in nucleoside uptake and metabolism are also involved in these cases. Tovey *et al.* (1975) have previously reported rapid changes of [3 H]thymidine uptake in response to interferons in mouse L1210 cells. Fuse & Kuwata (1978) and Lundblad & Lundgren (1982) have noted effects of interferons on intracellular thymidine kinase activities in human RSA cells and in human glioma cells respectively. In these particular cases changes in DNA labelling with exogenous [3 H]thymidine are unlikely to reflect changes in DNA synthesis after interferon treatment. If similar alterations in nucleoside uptake in response to interferon occur in other cell types, then effects on the incorporation of exogenous radiolabelled nucleosides cannot be reliably used as the principal measure of the growth-inhibitory activity of interferons.

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