The Effect of Inhibitors of Glycosylation on Interferon Production in Human Lymphoblastoid Cells

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Summary

Interferon production was inhibited in the Namalwa line of human lymphoblastoid cells by treatment with 2-deoxy-D-glucose or D-glucosamine. D-Glucosamine also inhibited protein synthesis and the cells were no longer viable, whereas 2-deoxy-D-glucose allowed protein and RNA synthesis to continue at control rates, and the cells remained fully viable. It is concluded that a glycosylation step is essential for production of lymphoblastoid interferon.

Human fibroblast interferon has been shown to be a glycoprotein by periodic acid-Schiff base staining of the purified material on a polyacrylamide gel (Knight, 1976). On the other hand, the question of whether human leucocyte interferon is a glycoprotein remains open. On chromatography through columns of lectin bound to a solid support, leucocyte interferon behaved as though it contained no carbohydrate moieties (Jankowski et al. 1975). In addition, treatment of leucocyte interferon with glycosidases showed that the preparation did not lose its antiviral activity (Bose et al. 1976), and treatment with sialidase did not alter the isoelectric point or the biological activity of the interferon, implying that sialic acid residues were not present (Mogensen et al. 1974; Morser et al. 1978). However, Stewart and his co-workers (1977) showed that treatment of leucocyte interferon with periodate removed both charge and mol. wt. heterogeneity, presumably because the protein had been deglycosylated. Thus, even if leucocyte interferon does contain carbohydrate, it is clearly not necessary for biological activity.

Inhibitors of glycosylation have been used to show that production of human fibroblast interferon is suppressed in their presence (Havell et al. 1975). It has subsequently been demonstrated that the residual interferon produced under these conditions is altered in some properties because it contains less carbohydrate (Havell et al. 1977a). We have investigated the production of interferon from human lymphoblastoid cells of the Namalwa line in the presence of 2-deoxy-D-glucose or D-glucosamine. These cells produce a mixture of leucocyte and fibroblast interferons, but the major component is similar to leucocyte interferon (Havell et al. 1977b).

Suspension cultures of Namalwa cells were grown in medium RPMI 1640 containing 10% newborn calf serum (P. N. Baker, personal communication). For induction of interferon, the cells were pre-treated for 48 h with 25 μg/ml 5-bromodeoxyuridine (Tovey et al. 1977), then pelleted, and resuspended in 2 ml vol. at 2 x 10⁶ cells/ml in RPMI 1640 containing 2% newborn calf serum. Sendai virus (200 HA/ml) was added and the cultures were incubated for 1 h. Then the cells were pelleted and resuspended at 2 x 10⁶ cell/ml in the same medium containing the appropriate concentration of inhibitor. Both 2-deoxy-D-glucose and D-glucosamine (purchased from Sigma Chemical Co. Ltd, London) were dissolved in medium, sterilized by filtration and stored at -20 °C before use. After 24 h the cells were pelleted, and the supernatant was dialysed overnight at pH 2 and returned to pH 7 before it was assayed for interferon (Atherton & Burke, 1975) on human foreskin.
Short communications

Fig. 1. Production of interferon by Namalwa cells in the presence of (a) different concentrations of 2-deoxy-D-glucose (■—■) and D-glucosamine (●—●) and (b) at different times after induction in the presence of 10 mM-2-deoxy-D-glucose (■—■) or 100 mM-D-glucosamine (●—●) or in the absence of inhibitors (○—○).

fibroblasts (in one particular experiment bovine turbinate cells were used as the assay cell). Results are expressed in units in terms of the human leucocyte research reference standard preparation 69/19. When intracellular interferon was measured, the cells were washed three times with cold phosphate buffered saline, before being lysed by three cycles of freeze-thawing. Neutralization assays were carried out by incubating the sample with antiserum prepared against either leucocyte or fibroblast interferon for 2 h at 37 °C and then assaying the remaining interferon (A. Meager, personal communication).

3H-uridine (26 Ci/mmol) and 35S-methionine (650 Ci/mmol) were purchased from Radiochemical Centre Ltd, Amersham. Their incorporation was measured by adding them to two duplicate 5 ml cultures at a concentration of 100 μCi/ml. The determination of trichloracetic acid (TCA)-soluble and insoluble radioactivity was carried out according to P. N. Baker (personal communication). Cell viability was determined by trypan blue staining.

Treatment of Namalwa cells with 2-deoxy-D-glucose resulted in a dose-dependent inhibition of interferon yield (Fig. 1a), except at low concentrations of about 0.1 mM which always led to a small but consistent rise in interferon titre. Unlike findings with fibroblast cells, D-glucosamine only inhibited interferon production at the highest dose used (100 mM) and had no effect at lower concentrations (Havell et al. 1975; Fig. 1a). The amount of intracellular interferon measured 8 h after induction was also decreased in a dose-dependent manner by 2-deoxy-D-glucose, but again was only affected by D-glucosamine at a concentration of 100 mM. This suggests that 2-deoxy-D-glucose does not simply inhibit the release of interferon from the cells.

The titre of extracellular interferon was maximum at 14 h after induction of Namalwa cells (Fig. 1b). The time-course of interferon production in the presence of 10 mM-2-deoxy-D-glucose or 100 mM-D-glucosamine (Fig. 1b) was not significantly different: at each time there was proportionately the same reduction in the interferon titre.
Fig. 2. Incorporation of labelled precursor into Namalwa cells at different times after induction in control cells (O--O) or cells treated with 10 mM-2-deoxy-D-glucose (C--□) or 100 mM-D-glucosamine (►—►). 35S-methionine is shown in (a) and 3H-uridine incorporation into the acid-precipitable fraction (b).

Havell et al. (1975) reported that fibroblast interferon produced in the presence of either 2-deoxy-D-glucose or glucosamine had altered physical characteristics. Namalwa interferon contains both fibroblast-type and leucocyte-type interferons, with the former constituting at least 13% of the total activity (Havell et al. 1977b). The properties of Namalwa interferon synthesized in the presence of 1 mM, 10 mM and 100 mM-2-deoxy-D-glucose were therefore investigated.

First it was shown that the proportion of interferon prepared in the presence of 2-deoxy-D-glucose that was neutralized by anti-fibroblast serum was the same as that in control preparations. This showed that there was no change in the amount of fibroblast interferon present.

Second it was shown that the neutralizing titre of anti-leucocyte interferon against interferon made in the presence of partially inhibitory concentrations of 2-deoxy-D-glucose was two to four times lower than against a control interferon made simultaneously. This small change showed that either the specific activity of the interferon was lower (more inactive interferon present) or that the antigenic site was altered.

Finally the interferon activity on the bovine turbinate cells was measured. The different types of human interferon have different activities on heterologous cells, and on bovine cells the fibroblast type has a lower activity than the leucocyte type (Havell et al. 1977b; Lin et al. 1978). The antiviral activity of the control interferon preparation was tenfold higher on bovine turbinate cells than on human foreskin fibroblasts. The same enhancement of activity on bovine cells was observed when interferon prepared in the presence of 10 mM-2-deoxy-D-glucose or 100 mM-D-glucosamine was tested showing that the proportion of fibroblast interferon was not significantly increased.

In an attempt to localize the effect of the inhibitors, RNA and protein synthesis were measured during the course of induction. Protein synthesis in the presence of 100 mM-D-glucosamine was severely inhibited, but there was no effect on the acid-soluble radioactivity (Fig. 2). On the other hand 10 mM-2-deoxy-glucose affected neither acid-soluble nor acid-insoluble incorporation. RNA synthesis was not affected in cells treated with 10 mM-2-deoxy-D-glucose (Fig. 2b); 3H-uridine labelling cannot be used to measure RNA synthesis.
in the presence of glucosamine, because the formation of UDP-glucosamine alters the sizes of the precursor pools (Scholtissek, 1971). Finally the viability of the cells at the end of the induction period, estimated by trypan blue staining, was 95% cells in the control cultures. This figure was not changed by 2-deoxy-D-glucose at any concentration used, but was reduced to below 20% when the cells had been treated with 100 mM-D-glucosamine.

These experiments show that the formation of interferon by Namalwa cells can be inhibited by 2-deoxy-D-glucose and D-glucosamine. Only the highest concentration of D-glucosamine used affected the interferon yield and at this concentration protein synthesis was severely inhibited and after 24 h the cells were no longer viable. On the other hand, treatment with 10 mM-2-deoxy-D-glucose did not alter the rates of protein or RNA synthesis and the cells remained viable, but the interferon yield was only 10% of that in control cells. This suggests that the effect of the 2-deoxy-D-glucose is specifically on the glycosylation system.

Since Namalwa cells produce interferon of both antigenic types (Havell et al. 1977b) it is possible that the synthesis of only one was affected by 2-deoxy-D-glucose. The experiments with the antiserum rule this out as an explanation for the inhibition of interferon production. The slightly decreased neutralizing titre of anti-leucocyte serum suggests that there is some alteration in the interferon produced in the presence of 2-deoxy-D-glucose. However, the ratio of antiviral activity on bovine and human cells was the same in control preparations and in preparations made in the presence of the inhibitors. This suggests that both types of interferon produced by Namalwa cells are equally affected.

2-Deoxy-D-glucose could inhibit interferon production by preventing the events in virus replication necessary for induction. This is highly unlikely since paramyxovirus replication is only affected at the stage of the production of envelope proteins (Hodes et al. 1975; Scholtissek, 1975), which follows the stage(s) necessary for interferon induction (Johnston & Burke, 1973). The only circumstances in which early events in virus replication are affected is when the virus has previously been grown in glucose-free medium and the infection also carried out in glucose-free medium (Scholtissek et al. 1974). Since all the media used in these experiments contained 2 mg/ml glucose, it is most likely that the effect of the 2-deoxy-D-glucose is not mediated via an effect on virus multiplication.

2-Deoxy-D-glucose is an anti-metabolite of mannose (Kaluza et al. 1973) and the available evidence on its mode of action indicates that it selectively alters the glycosylation of proteins, thus preventing their normal processing (Scholtissek, 1975). Its effect on the formation of lymphoblastoid interferon again suggests that this is glycosylated. In the presence of 2-deoxy-D-glucose, the properties of the interferon should be altered, and the change in the neutralizing titre of anti-leucocyte serum may well be a reflection of this.

It should be noted, however, that interferon which has had the carbohydrate removed by treatment with either glycosidases or periodate retains its biological activity (Bose et al. 1976; Stewart et al. 1977). Therefore it seems possible that the effect of 2-deoxy-D-glucose is to produce an incorrectly glycosylated molecule and thus prevent a step that is essential for the formation of active interferon.

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