INTERFERON ASSAY ANOMALY VARIATION OF INTERFERON RESPONSE WITH CELL TYPE AND SIALIC ACID CONTENT

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ABSTRACT

Human interferon derived from leukocytes and cultured fibroblast sources has been compared by assay in two human cell systems. The ratio of the activities of the two interferon preparations differed markedly in two assay systems. Neuraminidase treatment had no effect on the activity of either interferon in the more sensitive system but reduced the activity of the fibroblast material in the second system so that the resulting ratio of activities approximated that seen in the first assay system.

INTRODUCTION

Although variations in the sensitivity of human cells to human interferon are known to exist (1,2) and it has furthermore been observed that some non-human cells may be more sensitive to interferon than the human system (3), a human interferon standard (B 69/19) (4) is generally employed with the assumption that at least some relative activities can be measured. Recently, differences in the dose-response curves seen in different assay cells have been observed (5). We have compared the ratio of activity of preparations of human interferon from leukocyte and fibroblast sources on two different human cells and have found that the ratio of activities as conventionally determined is a function of the cells used to measure this activity. More particularly, we have shown that the residual activity of human fibroblast interferon after neuraminidase treatment is also dependent on the...
Purified human leukocyte interferon (P-IF) and concentrated human leukocyte interferon (C-IF), sendai virus induced, was kindly provided by Dr. Kari Cantell, State Serum Institute, Helsinki, Finland. Human fibroblast interferon (P-IF), poly I-poly C induced, was supplied by Dr. T. Cartwright, Searle Laboratories, High Wycombe, England. The FS-7 human fibroblast cell strain and the vesicular stomatitis virus (VSV) were the gifts of Dr. Jan Vilcek, New York University Medical School, New York City; the U-amnion cell line was generously provided by Dr. S. Baron, NIH, Bethesda. Interferon titres were measured by the inhibition of cytopathic effect (CPE) following VSV challenge (6). The FS-7 and the U cell assays differ only in the virus dose. The FS-7 cells are infected at .07 pfu per cell; the U cells at 10 pfu per cell and the latter read at 24 rather than 48 hours post challenge. We have found that this procedure gives the same results as the lower dose/longer time method in these cells. Neuraminidase was purchased from Schwarz/Mann, lot #8Z2199. The enzyme was free of protease activity in an assay sensitive to 1.5 international units of standard protease/ml (7). Samples for neuraminidase treatment were dialyzed against 0.15 M NaCl, 0.05M sodium acetate-acetic acid buffer, 20 mM CaCl₂ pH 5.5 (8). Reactions were carried out at 37°, 4 hours, in the presence of 5 μl chloroform with a total of 35 international units (IU) neuraminidase per mg protein added in two parts, at 0 and 2 hours. Incubated controls were treated identically except for the enzyme addition.

The results of assaying the interferons from two different sources on each of the two cell lines are presented in Table I. The P-IF standard is an internal standard made by diluting P-IF with 10 mg/ml bovine serum albumin (BSA) in phosphate buffered saline (PBS) pH 7.2, aliquoting and freezing at -70° until use. The difference in sensitivity of the two cell lines to interferon was not unexpected. It is, however, also clear that the ratio of activities of the two preparations differ markedly when assayed in the two different cell lines. The titre of fibroblast interferon is 10 fold lower measured against a leukocyte standard when the assay is done in FS-7 cells despite the fact that this more sensitive system yields apparent titres that are as much as 1000 x higher. The variations in absolute titre in the FS-7 system are attributed to a characteristic increase in sensitivity of these cells as a function of higher passage number (J. Vilcek, personal communication) (9).

In Table II, we present the results of studies on the effect of neuraminidase on this phenomenon. Samples incubated in the absence of neuraminidase are used as a control. It will be