An Immunoradiometric Assay of Serum Interferon using a Monoclonal Antibody

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SUMMARY

An immunoradiometric assay for human interferon-α (HuIFN-α) has been adapted for the assay of low concentrations of HuIFN-α in human serum. The sensitivity of the assay is 5 to 10 IU/ml and the coefficient of variation less than 10%. The assay was shown to compare well with a biological (antiviral) assay in the measurement of serum interferon following intramuscular injection of HuIFN-α in nine volunteers. Serum interferon was also measured in the serum from 250 normal human donors. Two donors appeared to have detectable levels of HuIFN-α.

The production and isolation of a monoclonal antibody specific for human interferon-α (HuIFN-α) was recently described by Secher & Burke (1980). This antibody has been used to purify HuIFN-α by immunoabsorption chromatography and, after labelling with 125I, an immunoradiometric assay for HuIFN-α was developed (Secher, 1981). In this labelled antibody sandwich method, sheep anti-interferon antibody was attached to polystyrene tubes, beads or microtitre trays. This anchored HuIFN-α present in the sample, and bound HuIFN-α was detected by the addition of 125I-labelled monoclonal anti-HuIFN-α and measurement of the counts bound to the solid phase. The assay to be described was modified for measurement of low concentrations of HuIFN-α in serum.

Polyclonal immunoglobulin, for coating to plastic, was purified from a sheep antiserum to HuIFN-α (titre 450 000 IU/ml; Mogensen et al., 1975, a gift from Dr K. Cantell). An equal volume of saturated ammonium sulphate was added to 10 ml sheep antiserum, and after 16 h at 4 °C the precipitated proteins were dissolved in 0·0175 M-sodium phosphate pH 7, and dialysed against the same buffer. The protein solution was then passed through a DEAE-cellulose column (Whatman, DE52; 6 × 2 cm) equilibrated and eluted with the same buffer. Fractions were analysed by electrophoresis on cellulose acetate (Beckman, Microzone) and those containing immunoglobulin (Ig) pooled and dialysed against phosphate-buffered saline (PBS). The yield of Ig was 13 mg per ml of sheep serum. HuIFN-α standards used were either the MRC 69/19 reference standard, or a laboratory standard NK2-Sepharose-purified interferon which had been calibrated with reference to the MRC 69/19 standard. Thus, the concentrations of IFN-α referred to in this paper are expressed in international units (IU)/ml. Assay standards were prepared in pooled normal human serum from which a standard calibration curve was constructed for each batch of specimens under test. 125I-labelled NK2 (monoclonal anti-HuIFN-α) was prepared from the serum and ascitic fluid of mice carrying NK2 tumours. The monoclonal antibody was purified by ammonium sulphate precipitation (50% saturation, as above) and DEAE ion-exchange chromatography. Labelling with 125I was by the chloramine T method (Greenwood et al., 1963; Jensenius & Williams, 1974), and followed by desalting on a Sephadex G-50 (fine) column. The labelled IgG had a specific activity of about 2 Ci/μmol or about 1 atom 125I per molecule of IgG.

The preferred procedure for the assay was as follows: 6·4 mm diam. (1/4 inch) etched polystyrene beads (manufactured by the Precision Plastic Ball Co., Chicago, Ill., U.S.A. and bought from Northumbria Biologicals, Northumberland, U.K.) were used as the solid
Fig. 1. Calibration and variability of the assay in normal human serum. Interferon standards diluted in pooled normal human serum were incubated with beads coated with sheep anti-interferon at 20 µg/ml or at 100 µg/ml as described in the text. Washing and incubation with ¹²⁵I-labelled NK2 (100000 ct/min/200 µl) were as described in the text. Ten replicates were performed for each point. (a) Calibration of assay in the range 0 to 50 IU/ml and comparison of coating beads at 20 µg/ml (▲) or at 100 µg/ml (●). The bars indicate ±1 standard deviation (S.D.) from the mean. (b) Coefficient of variation at different interferon concentrations in the range 0 to 500 IU/ml. The coefficient of variation (s.o./mean) was calculated from the same results as those used for (a) but includes the data obtained in the range 50 to 500 IU/ml. Beads were coated with sheep anti-interferon at 100 µg/ml (▲) or at 20 µg/ml (●).
Short communications

30

\[ \begin{align*}
\text{Time post-injection (h)} & \\
4 & 8 & 12 & 16 & 20 & 24 & 4 & 8 & 12 & 16 & 20 & 24
\end{align*} \]

Fig. 2. Comparison of (a) antiviral and (b) immunoradiometric assay for interferon in individual serum samples from a single volunteer after injection with NK2-IFN (△), PIF (○) or placebo (■). For further details of the injection protocol and antiviral assay see text and Scott et al. (1981).

the ct/min bound by the 20 μg/ml beads were much more widely scattered than the 100 μg/ml beads, as shown in Fig. 1 (b), and so the higher concentration was routinely used.

HuIFN-α levels in the sera of nine trial patients given partially purified HuIFN-α (PIF) and a preparation purified by passage twice through an NK2 monoclonal anti-HuIFN-α affinity column (NK2-IFN) were compared. Three male and six female volunteers (mean age 37 years, range 25 to 54 years) were given one injection of the two different interferon preparations at an interval of 4 days, and a control injection of PBS with 2 mg/ml human serum albumin according to a random code. The mean dose of PIF given was $1.3 \times 10^6$ IU/ml per m$^2$ body surface area, and $1.2 \times 10^6$ IU/ml per m$^2$ body surface area of NK2-IFN as measured by the immunoradiometric assay (Scott et al., 1981). HuIFN-α concentrations in the sera of the trial patients were measured both by the immunoradiometric assay and by an antiviral assay in which HuIFN-α inhibits the synthesis of Semliki Forest virus RNA in embryonic bovine tracheal cells (Atherton & Burke, 1975; Hayes et al., 1979).

The time courses obtained were similar with both assays, and gave peak levels of PIF and NK2-IFN at 2 h post-injection, with a subsequent fall to low levels by 24 h post-injection. The data from one volunteer are shown in Fig. 2 (a, b).

Serum samples from 50 male and 50 female blood donors, 50 males and females attending a genitourinary clinic, and 50 pregnant females attending an antenatal clinic were assayed for HuIFN-α. The distribution of HuIFN-α (expressed as ct/min bound) in this population is illustrated in Fig. 3. About 98% of the population fell within 2 standard deviations (S.D.) of the mean, and had HuIFN-α levels not more than $\pm 6$ IU/ml from the pool of normal sera.

We believe that this pooled normal human serum does not contain HuIFN-α, since the ct/min bound were no lower if the serum was passed through an NK2-Sepharose column prior to the assay or if animal sera were used (data not shown). Two normal individuals appeared to have high levels of HuIFN-α, one male blood donor had a concentration of 16·5 IU/ml (greater than 5 S.D. from the mean), and one female blood donor a concentration of 20 IU/ml. The male blood donor gave no history of recent illness or infection prior to the donation when serum was taken. A second serum was obtained 6 months later and found to contain 46 IU/ml HuIFN-α. This healthy male therefore appears to have a high concentration of circulating HuIFN-α. The female donor also had no history of infection or
chronic illness. Hooks et al. (1979), using an antiviral assay, found levels of immune interferon or HuIFN-γ of 16 IU/ml and above in the sera of patients with autoimmune disease, but found no interferon in normal sera.

The above results confirm that in direct comparison with a biological assay the immunoradiometric assay is a reliable assay for the measurement of low levels of HuIFN-α in serum. The biological assays in use are highly labour intensive and demand much operational time. They are influenced in a non-specific way by normal human serum, and before assay, samples have to be diluted 1/10 or 1/20, which decreases the effective sensitivity of the assay. The immunoradiometric assay is easy to perform, more reproducible and quicker than the biological assay. Many samples may be assayed together at one time and the method is semi-automated so that operational time is relatively short; results may be obtained within 24 h.

It is possible that the immunoradiometric assay, as with other immunoassays, might sometimes detect molecular species that have lost their biological activity, but retain the (NK2) antigenic site, and that rare serum components other than interferon could act as a 'bridge' between the coated plastic and the radioactively labelled antibody, but we have no evidence for either of these so far. Furthermore, although the NK2 antibody recognizes most of the HuIFN-α species tested (Secher & Burke, 1980; D. S. Secher et al., unpublished results), the assay may be more sensitive for some species than for others. This is a limitation also shared by biological assays where there may be a differential effect of different HuIFN-α species depending on the target cell and virus chosen (Yelverton et al., 1981).

HuIFN-α now being produced on a large scale by genetic engineering in Escherichia coli (Nagata et al., 1980; Goeddel et al., 1981) and from continuous cell lines (Finter & Fantes, 1980) is being used for therapeutic trials in serious virus infections and in malignant conditions. Already, many trials are under way, and it will be necessary to monitor the pharmacokinetics in large numbers of patients given doses of HuIFN-α. For this the immunoradiometric assay provides a valuable alternative to existing methods.

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Short communications

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1Public Health Laboratory, Addenbrooke's Hospital
Hills Road, Cambridge CB2 2QO, U.K.

2Clinical Research Centre, Watford Road
Harrow, Middlesex HA1 3UJ, U.K.

3Medical Research Council Laboratory of Molecular Biology
Hills Road, Cambridge CB2 2QH, U.K.

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