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Interferon Assay of High Sensitivity (39580)

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There are many conditions where the ability to quantify low levels of interferon is important. For example, in the study of new compounds as interferon inducers, promising groups of compounds might be overlooked because the particular member of the group studied showed only minimal activity. The interrelationship of low levels of interferon and the immune response (1) is another area where a sensitive procedure would be useful. In situations where only very small volumes of biological samples are available with a sensitive assay it would be possible to dilute the small volume sufficiently to have a workable quantity of test solution.

The present report summarizes experiments which have led to the development of an interferon assay method that is significantly more sensitive than the one previously used in this laboratory. Through the use of this assay, interferon has been quantified in mouse and primate serums where standard methods failed to detect any.

Materials and methods. Interferons. Human and mouse standard interferons No. G-023-901-537 and No. G-002-904-511 (NIAID catalog of research reagents, 1975-1977) were used. Serum from mice treated with a low molecular weight inducer *S*,2-aminoethylisothiouronium 2HBr (AET) was used as a source of low titer interferon (E. Lvovsky, H. Levy, D. C. Doherty, and S. Baron, in preparation).

Cell cultures. Human diploid fibroblast strains HFS-1 (Biofluids, Rockville, Md.) and HR-203 (HEM Research, Inc.) and mouse L-929 cells were used for interferon assay. Cell suspensions, at a concentration of 3-5 $\times$ 10^4 in 0.1 ml of Eagle's minimal essential media (MEM) with 10% fetal bovine serum (FBS), were added to each well in 96 well microtiter plates (Falcon Plastics). The cell cultures were incubated in a CO_2 atmosphere at 37°. Procedures for preparing the cell cultures for interferon assays are described in the section of results.

Interferon assays. Serial 0.5 log dilutions of interferon were made in MEM with 2% FBS, and 0.1 ml of each dilution was added to quadruplicate cell cultures. After overnight incubation, the fluid was removed and the cells were challenged with 10^2 $TCID_{50}$/0.1 ml of Indiana strain of vesicular stomatitis virus (VSV) in MEM with 2% FBS. CPE was read 24-36 hr later. The degree of CPE was graded from 0 to 4, the latter being complete destruction of the cell sheet. The dilution of interferon that gave 50% protection (2 + CPE) was taken at the end point. Alternatively, reduction of virus hemagglutinin (HA) yield in interferon-treated cultures was used with Sindbis virus as the challenge for human cells (HFS-1 and HR-203) and GD-7 for mouse cells (L-929).

Results. Four different incubation conditions were used to prepare the cell cultures for assay. After the cells reached confluency at 37°, the first group of cultures (both human and mouse) were kept 24 hr in an incubator at 37°. The third group of confluent culture was aged at 30° for 5 days. The second and the fourth groups were the same cultures as the first and the third, but 24 hr before assay the medium was replaced with fresh MEM – 10% FBS. The cells were further incubated at 37°. Table I summarizes data from the experiments. The first line, incubation for 24 hr at 37° after reaching confluency, is the most common method of growing cells for interferon assay (reference method). Assay of standard human and mouse interferons in these cultures showed that the titer of the reference interferon was in agreement with its nominal value, i.e., 4.2 $\pm$ 0.44 log units for human and 3.9 $\pm$ 0.35 log units for mouse interferon. Changing of the media in these cells did not increase the interferon titers. Some insignificant increase of interferon titers has

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TABLE I. INTERFERON YIELD FOLLOWING DIFFERENT CONDITIONS OF AGING OF CELL CULTURES.

	Aging conditions before interferon assay	Interferon titer (log units/ml)	
		Human interferon	Mouse interferon
1	(a) 37° for 48 hr, Reference method	4.2 ± 0.44	3.9 ± 0.35
2	(a) 37° for 24 hr (b) Change of medium (c) 37° for 24 hr	4.2 ± 0.5	3.9 ± 0.35
3	(a) 37° for 24 hr (b) 30° for 6 days	4.6 ± 0.44	4.1 ± 0.5
4	(a) 37° for 24 hr (b) 30° for 5 days (c) Change medium (d) 37°for 24 hr	5.4 ± 0.44[a]	4.9 ± 0.23[a]

[a] The difference between numbers presented on lines 1 and 4 are highly significant ($P < 0.001$ in Student's t test).

been found in cultures aged at 30° for 6 days: 0.4 log for human and 0.2 log for mouse standard interferons. However, changing of the medium on the cell cultures aged at 30° significantly increased the titers of both human (from 16- to 30-fold) and mouse interferon (up to 10-fold).

The substantial increase in the sensitivity of the interferon assay was an important factor in finding a new class of low molecular weight interferon inducers: mercaptoalkylamines and derived thiophosphates. Table II contains data from an experiment where three samples of serum from mice treated with one such compound, AET, as well as two samples of mouse standard interferon, were assayed in cell cultures using the new technique. With the standard test, no interferon was detected in the sera from AET-treated mice. However, using procedure no. 4 (see Table I), significant levels of interferon were found. Since undiluted serum was toxic to the cells, a 1:10 dilution was used as the lowest concentration. Since no interferon was detected at this dilution using the reference method, one cannot state the exact degree of increase in sensitivity found in this sample.

TABLE II. DETECTION OF INTERFERON BY THE AGING METHOD.

Interferon	Samples number	Interferon titer[a] as measured in L cells incubated under different conditions	
		Reference method	Aging method
Mouse serum interferon induced by ip administration of 2 mg of AET per mouse	1	<1.0[b]	1.0
	2	<1.0	1.9
	3	<1.0	1.7
Normal mouse serum	4	<1.0	<1.0
Reference standard mouse interferon	5	4.2	5.3
	6	4.1	5.1

[a] Log units per milliliter.

[b] For assay of serum or tissue interferons, a starting dilution of 1:10 was used to avoid toxicity.

Discussion. The data presented here summarize minor modifications of commonly used interferon assay procedures which have led to an increase from 16- to 30-fold in the sensitivity in human interferon assays and up to 10-fold in mouse interferon. The effectiveness of aging cells at 37° previously has been reported to increase by 1.6- to 3-fold (2–5). However, the new combination of procedures currently presented, further increases the sensitivity of interferon assay in a simple and reproducible way.

We have used this method to detect interferon-inducing activity in different groups of weak interferon inducers under conditions where the standard method of assay gave dubious results. It is realized, of course, that when one is working with high concentrations of interferon an increase in the sensitivity of an assay has no particular merit. However, even with samples of moderate activity, where the volume of the samples is extremely small a sensitive assay method can serve a useful purpose. This method might also be useful in studying human and animal specimens for minimal concentrations of interferon.

Summary. A method of aging cells is described which allows for an increase in sensitivity of the assay of interferon. After reaching confluency the cell cultures are aged at 30° for 5 days, the growth medium is replenished, and the cells are incubated for an additional 24 hr at 37° before applying interferon. Using cell cultures so treated (diploid cells HFS-1 or HR-203), and mouse L-929 cells), the sensitivity of the interferon assay increase from 16- to 30-fold for human and up to 10-fold for mouse standard interferon.

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