APPLIED MICROBIOLOGY, May 1971, p. 959-961

Vol. 21, No. 5
Printed in U.S.A.

Assay of Chick Interferons by the Inhibition of Viral Ribonucleic Acid Synthesis

MICHAEL McWILLIAMS,[1] MARTIN S. FINKELSTEIN,[2] PATTON T. ALLEN,[3] AND DAVID J. GIRON

Infectious Diseases Branch, Biosciences Division, USAF School of Aerospace Medicine, Brooks Air Force Base, Texas 78235

Received for publication 18 December 1970

A method for assaying chick interferons by their inhibition of viral ribonucleic acid synthesis was devised and evaluated. The technique yielded results faster and had more flexibility than other methods with similar sensitivity and reproducibility.

The technique for assaying mouse interferons based on the inhibition of viral ribonucleic acid (RNA) synthesis (1) has been adapted for assaying chick interferons with the Indiana serotype of vesicular stomatitis (VS) virus as the challenge agent. The method, abbreviated $INAS_{50}$ for 50% inhibition of nucleic acid synthesis, was compared to a 50% plaque reduction (PR_{50}) technique by using VS virus in terms of reproducibility, sensitivity, and usefulness.

Several preparations of chicken interferon were used. Some were prepared in embyronated eggs by the method of Lampson et al. (5) with influenza A2 (Jap 305/75) virus. Others were prepared in chicken tracheal organ cultures (Finkelstein, McWilliams, and Huizenga, *in preparation*) or chick embryo fibroblast (CEF) monolayer cultures by treatment for 18 hr with double-stranded RNA composed of paired polyriboinosinic acid and polyribocytidylic acid (poly rI/rC; Microbiological Associates, Bethesda, Md.) at concentrations of 50 or 100 μg/ml. Poly rI/rC was eliminated from the interferon preparations by treatment with ribonuclease A-RAF (Worthington Biochemical Corp., Freehold, N.J.) as described previously (2). Other interferon preparations were produced in CEF cultures inoculated with Sindbis virus [>1 plaque-forming unit (PFR)/cell]. The fluid was taken at 24 hr postinoculation. Virus was eliminated by ultraviolet irradiation as previously described (1).

The materials thus obtained were characterized as interferons by (i) stability at *p*H 2.0, (ii) sensitivity to trypsin (0.25 μg/ml for 1 hr at 25 C), (iii) nonsedimentation at 100,000 × *g* for 1 hr, (iv) activity against several different viruses, and (v) lack of activity in L-cell cultures.

The $INAS_{50}$ interferon assay procedure used was as follows. (i) Confluent CEF monolayers in 35-mm polystyrene dishes (Falcon Plastic, Oxnard, Calif.) were treated overnight with serially diluted interferon in medium 199 (1 ml per culture). Control cultures were treated with medium 199 containing no interferon. Two to five cultures were used for each dilution of the specimen and for each infected and uninfected control. (ii) Fluids were removed and the cultures were challenged with 0.2 ml of VS virus at an input multiplicity of approximately 10 PFU per cell. Uninfected controls were treated with medium 199. (iii) After virus adsorption for 45 min at 37 C, 1 ml of medium 199 containing 1% fetal calf serum (FCS) and 2.5 μg of actinomycin D (Calbiochem, Los Angeles, Calif.) was added and each culture was incubated for an additional 1.5 hr. (iv) The actinomycin D medium was then discarded, and 1 ml of medium 199 containing 1% FCS and 0.5 μCi of ^{3}H-uridine (25 Ci/mmole; New England Nuclear, Boston, Mass.) was added. (v) Cells and medium were harvested 18 hr after the time of virus addition, and the trichloroacetic acid-insoluble material was assayed as previously described (1). The selection of 18 hr postinoculation as the time of harvest was based on the observation that ^{3}H-uridine incorporation into viral RNA approached a maximal level at this time (Fig. 1). Additional controls were included in most assays to test for toxic effects of the interferon specimens. Two or more cultures treated overnight with the lowest dilution of each interferon specimen and several untreated control cultures were neither infected with VS virus nor treated with actinomycin D. No interferon speci-

[1] Present address: Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, Calif. 95616.

[2] Present address: Department of Medicine, New York University School of Medicine, New York, N.Y. 10016.

[3] Present address: M. D. Anderson Hospital and Tumor Institute, University of Texas at Houston, Houston, Tex. 77025.

mens assayed to date have caused reduction in ^{3}H-uridine incorporation in such uninfected cultures.

The use of actinomycin D at 2.5 μg/ml was adopted after examining its effect on several parameters. Under assay conditions, the drug reduced cellular RNA synthesis by >95%. The drug did not significantly reduce yields of infectious virus. Average yields were (i) control cultures, 2.1 × 10^5 PFU/ml (n = 3); (ii) actinomycin D-treated cultures, 1.8 × 10^5 PFU/ml (n = 3). The amount of ^{3}H-uridine incorporated was similar whether the actinomycin D was added before or after virus challenge, but treatment with the drug after virus challenge was generally more convenient.

Interferon titers were estimated graphically by plotting the average counts per minute versus the reciprocal of dilution on log-log paper. Regression curves were then drawn by eye. The amount of radioactivity in viral RNA was taken as the counts per minute in infected cultures minus the counts per minute in actinomycin D-treated uninfected controls. The interferon titer was taken as the dilution estimated to permit the synthesis of 50% as much viral RNA as was made in virus controls not treated with interferon (1).

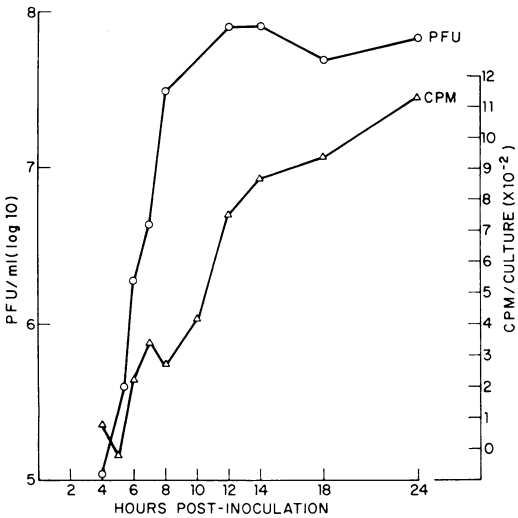

FIG. 1. *Replication and RNA synthesis by VS virus in CEF cell cultures. Cultures inoculated with VS virus (~10 PFU/cell in 0.2 ml) were incubated for 45 min. A 1-ml amount of modified Eagle's medium (1) containing 5% FCS and 2.5 μg of actinomycin D per ml was added, and cultures were incubated for 2 hr. Appropriate control cultures without virus, or without actinomycin D, or without both were also prepared. All cultures were washed twice with Hanks solution containing 1 mg of bovine serum albumin per ml. All cultures were nourished with 2 ml of medium containing 2% FCS; those used in the assay of RNA synthesis received medium also containing 1 μCi of ^{3}H-uridine per ml. Virus titers and trichloroacetic acid-insoluble radioactivity were measured at the times indicated employing duplicate cultures for each value. The counts per minute (CPM) shown were corrected to reflect only viral RNA by employing uninfected controls at each time interval.*

TABLE 1. *Reproducibility of INAS$_{50}$ assay method as indicated by repeated assay on different dates*

Titers obtained			
Prepn A	Prepn B	Prepn C	Prepn D
23	35	80	20
22	26	30	15
20	25	26	12
20	15	25	12
20	12	22	11
10	9		
6			
17.3[a]	20.3	36.6	14.0
±6.02[b]	±9.16	±21.85	±3.29

[a] Average.
[b] Standard deviation.

TABLE 2. *Comparison of titers obtained in concomitant assays with the INAS$_{50}$ and PR$_{50}$ techniques*

Interferon prepn	Expt	INAS$_{50}$ titer	PR$_{50}$ titer
A	1	10	5
B	2	6	10
C	3	15	16
D	3	26	28
D	4	12	23

To test the reproducibility of the INAS$_{50}$ assay method, four interferon preparations were assayed on several different occasions with different batches of cell cultures (Table 1). The reproducibility was similar to that reported for other methods (1, 3, 4, 6).

As shown in Table 2, the INAS$_{50}$ and PR$_{50}$ (VS virus) methods for assaying chick interferon were approximately equal in sensitivity.

The INAS$_{50}$ method has several useful advantages over the PR$_{50}$ technique. (i) Assays may be completed 36 hr earlier. (ii) Incomplete monolayers or cultures otherwise not suitable for plaque formation may be used. (In several parallel experiments, cultures which failed to give satisfactory PR$_{50}$ assays due to partial monolayer destruction, poor plaque definition, etc., nevertheless yielded perfectly satisfactory results by the INAS$_{50}$ technique.) (iii) Smaller volumes of

interferon are required since smaller culture dishes may be used. (The method could be modified to use microtiter or tube cultures if desired.)

Since completion of these studies, a report by Miller et al. has appeared in which a method somewhat similar to ours was used to test for antiviral substances, including interferon, in rabbit kidney cell cultures infected with VS virus (7).

The excellent technical assistance of Kenneth Flexer and James Connell is gratefully acknowledged.

LITERATURE CITED

1. Allen, P. T., and D. J. Giron. 1970. Rapid sensitive assay for interferons based on the inhibition of MM virus nucleic acid synthesis. Appl. Microbiol. 20:317–322.
2. Finkelstein, M. S., G. H. Bausek, and T. C. Merigan. 1968. Interferon inducers *in vitro*: difference in sensitivity to inhibitors of RNA and protein synthesis. Science 161:465–468.
3. Finter, N. B. 1964. Quantitative heamadsorption, a new assay technique. I. Assay of interferon. Virology 24:589–597.
4. Finter, N. B. 1967. Dye uptake methods for assessing viral cytopathogenicity and their application to interferon assays. J. Gen. Virol. 5:419–427.
5. Lampson, G. P., A. A. Tytell, M. M. Nemes, and M. R. Hilleman. 1963. Purification and characterization of chick embryo interferon. Proc. Soc. Exp. Biol. Med. 112:468–478.
6. Lindenmann, J., and G. E. Gifford. 1963. Studies on vaccinia plaque formation and its inhibition by interferon. III. A simplified plaque inhibition assay of interferon. Virology 19:302–309.
7. Miller, P. A., H. L. Lindsay, M. Cormier, B. R. Mayberry, and P. W. Trown. 1970. Rapid semi-automated procedures for assaying antiviral activity. *In* Second Conf. Antiviral Substances. Ann. N.Y. Acad. Sci. 173:151–159.