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# Antiviral Action of Mouse Interferon in Heterologous Cells[1]

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ABSTRACT

BUCKLER, CHARLES E. (National Institute of Allergy and Infectious Diseases, Bethesda, Md.), AND SAMUEL BARON. Antiviral action of mouse interferon in heterologous cells. J. Bacteriol. **91**:231–235. 1966.—The antiviral action of mouse interferon in cell cultures of mouse, hamster, rat, chicken, and monkey origin was investigated. Using a vesicular stomatitis virus (VSV) plaque reduction test, we found that mouse serum interferon, assayed on closely related rat or hamster cells, exerted 5% of its homologous antiviral activity. This activity was characterized as interferon by its temperature of inactivation, trypsin sensitivity, nonsedimentability, stability at *p*H 2, lack of inactivation by antibody to virus, and inability to be washed off cells. In the more distantly related chicken and monkey cells, mouse interferon had less than 0.1% of its homologous activity. Conflicting reports of heterologous activity of chicken and mouse interferon preparations may result in part from the observed action of noninterferon inhibitors of vaccinia virus. These inhibitors, like interferon, are stable at *p*H 2. They are present in mouse serum, mouse lung extracts, and allantoic fluid, and they prevent the development of vaccinia plaques when allowed to remain in contact with cells during virus growth. Unlike interferon the inhibitors are removed by adequate washing of cells prior to virus challenge, and they are not active in the VSV assay system. These findings reemphasize the need for thorough characterization of interferon preparations.

The property of species specificity of interferon was first reported by Tyrrell (30), when he showed that calf and chick interferon failed to induce antiviral activity on heterologous cells. This unusual property of these antiviral proteins, that of exhibiting their strongest activity on homologous cells, has been demonstrated for interferons prepared in many different cell types [reviewed by Isaacs (15)]. However, the barrier against heterologous activity has not always been reported to be absolute (15). Reports of heterologous activity of chick and mouse interferon (8, 29) differ with findings of a strong barrier between these species (3, 21).

The present report extends studies on the specificity of action of mouse serum interferon. The antiviral activity of this interferon on rat, hamster, and monkey cells was studied. A noninterferon inhibitor of vaccinia virus was also investigated. This inhibitor possibly explains the reported heterologous activity between chick and mouse interferon preparations.

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## MATERIALS AND METHODS

*Tissue cultures.* Primary cultures of 9-day-old chick embryos (CE), and near term mouse (ME), rat (RE), or hamster (HE) embryos were used. Cells were grown in Eagle's medium (MEM) with 10% serum and antibiotics. Calf serum was used with CE cultures and horse serum was used with ME, RE, and HE cultures. Primary cultures of rhesus monkey testes cells (MT) were grown in medium 199 (Difco) plus 2% calf serum and antibiotics. Cells were grown to confluent sheets in 60-mm plastic petri dishes in a 5% $CO_2$ incubator at 37 C.

*Viruses.* Vesicular stomatitus virus (VSV), Indiana strain, was obtained from the American Type Culture Collection, and was grown in ME cells. Newcastle disease virus (NDV), Hertz strain, was obtained from A. Isaacs and was grown in the allantoic cavity of 11-day-old chick embryos. Allantoic fluid was harvested after 48 hr of incubation at 36 C. Influenza virus, strain NWS, was prepared in embryonated eggs similar to NDV. Chikungunya virus was grown in the brains of suckling mice. Vaccinia virus, Mill Hill

TABLE 1. *Mouse serum interferon assayed on rat or mouse cells*

| Interferon prepn | Titer (units × $10^2$/ml) on | |
|---|---|---|
| | Rat cells | Mouse cells |
| 5-0564 | 10 | 130 |
| 2-2864 | 0.5 | 16 |
| 6-0064 | 0.5 | 30 |

TABLE 2. *Mouse serum interferon assayed on hamster or mouse cells*

| Interferon prepn | Titer (units × $10^2$/ml) on | |
|---|---|---|
| | Hamster cells | Mouse cells |
| 6-0064 | 1 | 30 |
| 6-2364 | 10 | 200 |
| 6-2864 | 3 | 50 |

strain, was grown on the chorioallantoic membrane of 11-day-old embryonated chicken eggs.

*Interferon.* Mouse serum interferon was produced in mice as previously described (2). Chicken interferon was produced by inoculating 11-day-old embryonated eggs with $10^{6.0}$ 50% egg infectious doses of NWS influenza (19). After 48 hr of incubation at 36 C, the allantoic fluid was harvested, and virus protein and some of the extraneous protein were denatured by the addition of perchloric acid to 0.15 M. After 4 to 6 hr at 4 C, the inactive precipitate was removed by centrifugation. The supernatant fluid, containing the interferon, was collected and adjusted to *p*H 7 by the addition of 10 N NaOH.

*Interferon assays.* Interferon was assayed in a VSV plaque reduction system as previously described (2). A 1-ml amount of interferon was incubated with cells for 3 hr before virus challenge. Control plates were incubated with control medium or with normal mouse serum collected and treated in a similar manner as mouse interferon. Titers are expressed as units per milliliter, and were determined as the reciprocal of the dilution which reduced the plaque count to 50% of that of the control. The titer of a given mouse interferon preparation, tested in a homologous system, was observed to increase or decrease threefold from assay to assay.

*Vaccinia virus assays.* In certain experiments, a liquid overlay vaccinia plaque system (20) was used to determine antiviral activity. Modifications of this procedure are described in Results.

*Characterization of antiviral activity as interferon.* The properties of the viral inhibitory activity of mouse interferon on RE and HE cells were determined as described previously (2).

## RESULTS

Table 1 shows titers obtained for several mouse interferon preparations, containing different amounts of interferon, which were assayed on either RE or ME cultures. A definite but reduced antiviral activity of mouse interferon on rat cells was observed. The ratio of heterologous activity to homologous activity varied from 1:13 to 1:60. This variation in the ratio of heterologous to homologous activity is within that expected from assay to assay and, therefore, a ratio of 1:30 seems a reasonable average.

TABLE 3. *Characterization of antiviral activities of mouse serum interferon on mouse, hamster, and rat cells**

| Treatment | Activity on cells of | | |
|---|---|---|---|
| | Mouse | Hamster | Rat |
| *p*H 2 | + | + | + |
| Heating at 44 C | + | + | + |
| Heating at 56 C | ± | ± | ± |
| Heating 60 C | 0 | 0 | 0 |
| Wash cells five times | + | + | + |
| Trypsin | 0 | 0 | 0 |
| 110,000 × *g*, 3 hr | + | + | + |
| Antibody to NDV | + | + | + |
| Encephalomyocarditis virus | + | NT | NT |
| Chikungunya virus | NT | NT | + |

* The results were obtained from several experiments in which 30 hamster units, 1 rat unit, or 10 homologous units per ml of mouse serum interferon were treated as described. Assays for residual antiviral activity were performed, and the results were compared with the activity of an untreated control; + indicates no significant reduction of activity; 0 indicates complete loss of antiviral activity; NT indicates not tested; ± indicates partial reduction of activity.

Mouse interferon preparations assayed on HE cells were also found to have heterologous antiviral activity (Table 2). As with RE assays, the variations in the ratios of heterologous to homologous activity (1:16 to 1:30) were within the expected interassay variation, and an average ratio of 1:20 was found. As can be seen from the homologous titers of various mouse interferon preparations shown in Tables 1 and 2, amounts produced in mouse serum may vary over a large range. However, the heterologous activity tended to parallel the homologous activity.

To determine that the antiviral activity of mouse interferon on RE and HE cells was produced by interferon, mouse interferon preparations were treated as shown in Table 3, and were assayed on ME, RE, or HE cells. Since all mouse serum interferon preparations were treated with acid to destroy residual NDV, the heterologous activity was stable at *p*H 2. Heating at 44 C for 1 hr did not reduce activity, whereas heating at 56 C for 1 hr partially reduced both the homologous

TABLE 4. *Comparative interferon assays using either vaccinia virus-fluid or VSV-agar systems*

| Material tested | Dilution | Percentage of control plaques (chick assay) | |
|---|---|---|---|
| | | Vaccinia | VSV |
| Allantoic fluid interferon | $10^{-2}$ | 7 | 20 |
| | $10^{-2.5}$ | 35 | 41 |
| | $10^{-3}$ | 60 | 59 |
| Mouse serum interferon | $10^{-1}$ | 2 | 80 |
| Normal mouse serum | $10^{-1}$ | 3 | 80 |

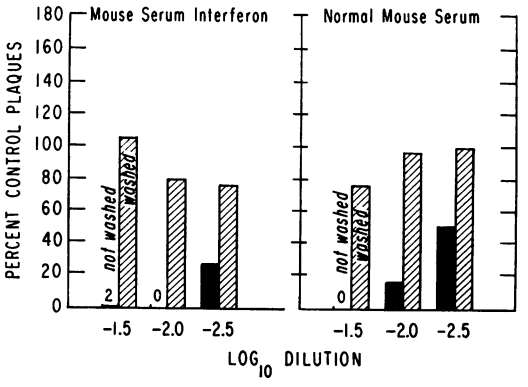


FIG. 1. *Effect of washing on the inhibition of vaccinia virus plaque formation on chick embryo cells after treatment with mouse serum.*

and heterologous activity. Thorough washing of the cells after reaction with interferon and prior to VSV challenge has no effect on activity. The heterologous activity, like the homologous activity, was found to be trypsin-sensitive, nonsedimentable, and not neutralized by antibody to NDV. The mouse interferon was effective against Chikungunya virus in rat cells and against encephalomyocarditis virus in mouse cells. Thus, the heterologous antiviral activity had characteristics consistent with interferon.

Mouse serum interferon showed no significant activity on monkey cells. In these experiments, MT plates were treated with 600 units of mouse interferon. No significant reduction in plaques on these treated plates was observed. Hamster serum interferon and monkey serum interferon were produced and were shown to be active in homologous systems (Baron et al., J. Immunol., *in press*). Attempts to produce rat serum were unsuccessful; however, rat cells are known to be sensitive to their homologous interferon (5).

Since heterologous activity of mouse interferon on chick cells has been reported from some laboratories (8, 29) but not from others (3, 21), the assay system used to show heterologous activity on chick cells was examined in an attempt to find some explanation for the divergent results. Dilutions of chick allantoic fluid interferon (2 ml per plate) were assayed on CE cells with the use of either a vaccinia virus or VSV challenge. The results are shown in Table 4. Also included were plates treated with 2 ml of a 1:10 dilution of mouse interferon (containing 200 units of mouse interferon per ml) and plates treated with 2 ml of a 1:10 dilution of normal control mouse serum. As may be seen in Table 4, both vaccinia and VSV were equally inhibited by the chicken interferon preparation. In this assay on CE cells, vaccinia virus was strongly inhibited by heterologous mouse interferon and normal control mouse serum, whereas VSV was not significantly inhibited by either of these preparations.

A major difference between the two assay techniques is that with the vaccinia virus assay system interferon is allowed to remain in contact with cells during the entire period of viral adsorption and plaque development. Extracellular inhibitors of vaccinia virus, therefore, have an opportunity to react with the challenge virus. To test for the influence of such inhibitors, a modification of the vaccinia virus assay system was employed. Amounts of 1 ml of dilutions of various interferon preparations or suitable control fluids were incubated with CE cells for 3 hr at 37 C. Controlled experiments had determined that the maximal antiviral effect of interferon was obtained by this time. The plates were then divided into two groups. To one group an additional 1 ml of Eagle's medium was added and then 0.2 ml of challenge virus. The other group of plates were decanted and washed five times with 3 ml of Earle's balanced salt solution (BSS). After washing, the plates were refed with 2 ml of Eagle's medium and 0.2 ml of virus challenge was added.

Figure 1 shows the results obtained when mouse interferon or normal control mouse serum was assayed. With assays using unwashed cells, both mouse interferon and normal control mouse serum inhibited vaccinia plaques. Washing removed this inhibition, indicating that the effect was not due to interferon. When 20% mouse lung extracts, from mice previously inoculated intranasally with either yeast nucleic acid or Earle's BSS (Takano et al., Federation Proc. **23**:507, 1964; Neal et al., Federation Proc. **23**:507, 1964), were assayed as described, similar results were obtained as with mouse serum (Fig. 2). The vaccinia inhibitory activity was removed by thorough washing prior to challenge. These same prepara-

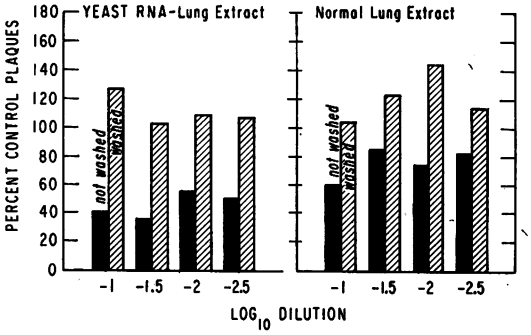


FIG. 2. *Effect of washing on the inhibition of vaccinia virus plaque formation on chick embryo cells after treatment with mouse lung extracts.*

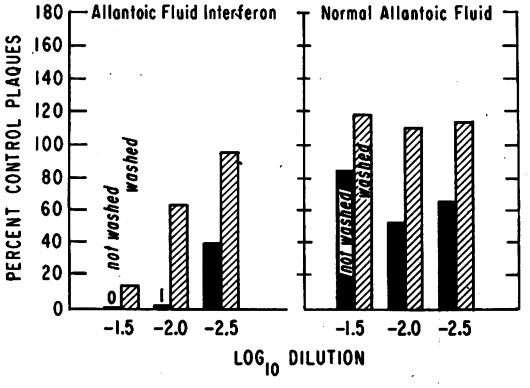


FIG. 3. *Effect of washing on the inhibition of vaccinia virus plaque formation on chick embryo cells after treatment with chicken allantoic fluid interferon or normal allantoic fluid.*

tions had previously been tested in a homologous ME-VSV assay system and were found to contain less than 0.3 units of interferon per ml. Additional control experiments showed that thorough washing of CE, ME, RE, or HE plates prior to VSV challenge has no effect on the number of plaques produced, indicating the absence of noninterferon inhibitors of VSV.

In a homologous CE assay, noninterferon vaccinia inhibitors were also demonstrated by comparison of the inhibition of vaccinia virus plaque formation on washed and unwashed plates (Fig. 3). With a vaccinia virus assay on plates washed to remove noninterferon inhibitors, it was found that vaccinia virus gave a 50% reduction end point at 30 units per ml for preparations which had a VSV titer of 300 units per ml. Vaccinia virus, therefore, is significantly less sensitive to interferon than is VSV. The noninterferon inhibitor of vaccinia virus, like interferon, is stable at *p*H 2. Unlike interferon, it is present in normal allantoic fluid.

## DISCUSSION

The present study demonstrated that mouse serum interferon exhibits antiviral activity on RE and HE cells but not on MT cells. This antiviral substance has similar properties in heterologous and homologous assays. Previously reported heterologous activity between mouse and hamster interferons was not completely characterized (11). That the extent of the genetic relationship between two species may determine the ability of their interferon to exhibit heterologous activity is suggested by the reported heterologous activity between human and monkey systems (4, 10, 16, 24, 28) and by the present report of the activity of mouse interferon on hamster and rat cells.

One important aspect of any interferon assay is establishing that the antiviral activity observed is actually due to interferon. Heterologous activity of mouse and chick interferon has been reported when a vaccinia interferon assay system was used (1, 8, 29). These observations are in contrast to work presented by others (3, 21), who found that large amounts of chick or mouse interferon exhibited no heterologous activity. As demonstrated in the present report, noninterferon vaccinia virus inhibitors could account for the reported heterologous activity of chick and mouse interferons.

The many reports of species specificity of the antiviral action of interferon (6, 7, 10, 11, 14, 17, 18, 23, 25, 26, 27, 30, 31, 32) support the view that the reaction of interferon with cells is unusual among biological events. Examples of other substances whose actions also have some degree of species specificity are somatotropins (22) and pheromones (33). A lower sensitivity to interferon of the heterologous cells, however, could erroneously indicate species specificity (16). To minimize this possibility, the same challenge virus was used for comparative assays in this report and the hamster and monkey testes cells were shown to be sensitive to their homologous interferon.

Several laboratories have reported that crude interferon preparations produced in calf kidney cells exert antiviral activity on human and monkey cells (27, 28). The rather common occurrence of noninterferon inhibitors (9, 13) necessitates a complete characterization of the heterologous antiviral activity before acceptance as interferon. Similar controls would be necessary to confirm the single report of heterologous activity of interferons produced in several mammalian cell types (24) and the report of activity of mouse cell-produced interferon on human kidney cells (12).

The use of purified interferon preparations

would eliminate many of the possible objections to future studies of species specificity with crude interferon. When the most purified interferons available at this time have been utilized, a strong species specificity of action has been demonstrated (19, 21). However, a full biological characterization of heterologous antiviral activity is still necessary with such preparations to rule out undetected, noninterferon antiviral substances.

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