# Tissue Specificity of Interferon Prepared in Various Tissue Cultures. (27476)

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The lack of cellular specificity exhibited by interferons (ITF) produced in various mammalian tissue cultures (TC) and assayed in homologous and heterologous systems has been reported(1). The present communication reports additional evidence relating to the specificity of ITF and reveals an important role for the dose of challenge virus in detecting ITF activity. A greater sensitivity of monkey kidney cell derived from African green monkeys over that of cells from Rhesus monkeys in assaying ITF is also shown.

*Materials and methods. Cell cultures.* All tissue culture cells with the exception of dog kidney (DK) contained in 100 ml milk dilution bottles or tubes, were obtained from a commercial source.‡ Continuous passage DK was originally obtained from A. H. Fieldsteel, Univ. of California Med. School, San Francisco. These cells were used only for preparation of ITF. The following 7 types of primary tissue culture cells were used in preparing and assaying ITF: Rhesus monkey kidney (MK(Rh)), African green monkey kidney (MK(A.G.)), human embryonic kidney (HEK), bovine embryonic kidney (BEK), calf kidney (CaK), rabbit kidney (RK), and hamster embryo (HAE). Another continuous passage TC cells from porcine kidney (PK) and suspended pieces of chorio-allantoic membrane (CAM)(2) were also used. Before use, cultures were incubated at 36°C in Medium 199(3) containing 5% calf serum, 200 units of penicillin/ml, 100 μg of streptomycin/ml and 50 units of nystatin/ml. Tube cultures were incubated in a roller-drum revolving at 12 RPH.

*Virus.* The Melbourne (MEL) Strain of influenza A virus was employed in this study for production of ITF and as a challenge virus for treated cells. The seed used represented the 60th passage in the allantoic cavity of 11- to 12-day-old chick embryos. The hemagglutinating (HA) potency of the virus was between 1:2048 and 1:4096/0.5 ml and egg infections titer ($EID_{50}$) was $10^{9.2}$/0.2 ml. To prepare ultraviolet (UV) irradiated virus, the virus was partially purified by the chicken RBC adsorption-elution technic(2). Fifteen to 20 ml of the eluted virus was placed in 150 mm clear-plastic Petri dishes for UV inactivation. Irradiation was carried out on a gently rocking platform having a 1-inch excursion with the Petri dish being located 4 inches from the UV source.§ The virus was irradiated for 30 seconds, transferred to a fresh plate, and again irradiated for a similar time. After irradiation, the viral preparation retained its HA potency but contained less than 1 $EID_{50}$/0.2 ml. All UV virus preparations were stored at 6-10°C prior to use.

*Preparation of interferon.* ITF was prepared in monolayers of 8 different mammalian TC cells propagated in 100-ml milk dilution bottles. The cultures were washed 3 times with Medium 199 before inoculation with 5 ml of UV virus. After 3 hours incubation at 36°C, the fluids were discarded and after 3 more washes the cultures were overlaid with 15 ml of Medium 199. ITF was detected in the fluid phase of the cultures after 18 to 24 hours of additional incubation at 36°C. Preparation of ITF from CAM was done according to the method of Lindemann *et al.* (2). Control fluids were obtained from uninoculated TC or CAM cultures. All fluids were stored at –20°C prior to assay.

*Assay of interferon.* ITF was assayed in 2-4 roller tubes containing mammalian cells or suspended pieces of CAM according to the

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TABLE I. Activity of Interferon Preparations in Homologous and Heterologous TC Cells.

| Source of ITF | TC system used for ITF assay | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MK(Rh) | HEK | BEK | CaK | RK | HAE* | PK* | CAM |
| MK(Rh) | — | + | + | — | + | — | — | — |
| HEK | — | + | + | + | + | — | — | — |
| BEK | — | — | + | + | + | — | — | — |
| DK | — | — | + | + | + | — | — | — |
| CaK | — | — | — | + | + | — | — | — |
| RK | — | — | — | — | + | — | — | — |
| HAE* | — | — | + | — | + | — | — | — |
| PK* | — | — | — | + | — | — | — | — |
| CAM | — | — | — | — | — | — | — | + |

* TC cells did not support growth of influenza virus.

(+) 4-fold or greater reduction in virus HA potency after challenge with $10^{6.5}$ $EID_{50}$ influenza A virus, strain MEL.

(—) < 4-fold reduction.

technic of Lindemann *et al.*(2). Washed cultures were overlaid with 1 ml of ITF or its corresponding control fluid and incubated overnight in a roller-drum at 36°C. The fluids were then discarded and the culture challenged with 1 ml of $10^{6.5}$ $EID_{50}$ or decimal dilutions of MEL virus. After one additional hour at 36°C, the cultures were again washed 3 times, placed in 1 ml of fresh Medium 199 and reincubated. Earle's Solution (4) was used as diluent for ITF assay in CAM cultures. All fluids were tested 48 hours after virus inoculation by determining the HA potency(5). A 4-fold, or greater, reduction in HA potency of ITF treated cultures was considered significant activity.

*Results.* A summary of the activity of ITF prepared in various TC systems and assayed in homologous and heterologous cells is shown in Table I. These data confirm earlier observations(6-8) in that there exists a definite host relationship of ITFs produced in cells of various vertebrate species. Under conditions of the test, ITF produced in CAM was active only when assayed in CAM and mammalian ITF was active only in mammalian TC. ITF produced in HEK cultures had the greatest heterologous activity while RK ITF displayed activity in homologous cells only. However, RK cells were most sensitive to the action of mammalian ITFs exhibiting activity to all but PK preparations. It was interesting to observe that although ITF was produced in PK and HAE TC, these cells were not susceptible to the influenza virus.

The unexpected inactivity of MK(Rh) ITF in a homologous system was further investigated to determine if activity is affected by the dose of viral challenge or if sensitivity may be increased by using kidney cells from another species of monkey. Accordingly, ITF was prepared in MK(Rh) and MK (A.G.) TC. For comparative purposes, ITF was also prepared in RK cells. Each preparation was assayed in homologous and heterologous TC systems using several challenge virus levels as shown in Table II. As can be seen, ITF prepared in MK(Rh) TC was active in the homologous test system with a viral challenge of 30 or less $TCID_{50}$. However, MK(A.G.) and RK TC were more sensitive to MK (Rh) ITF at higher challenge levels. Conversely, MK(A.G.) ITF was assayed

TABLE II. Effect of Challenge Dose of Influenza Virus (MEL) on Detecting Activity of Interferon Prepared in 3 TC Systems.

| Source of ITF | Viral challenge ($TCID_{50}$) | TC system used for ITF assay | | |
|---|---|---|---|---|
| | | MK(Rh) | MK(A.G.) | RK |
| | | (Fold reduction in HA potency) | | |
| MK(Rh) | 30,000 | 1 | 16 | * |
| | 3,000 | 1 | 128 | * |
| | 300 | 1 | 128 | 4 |
| | 30 | 8 | 128 | 16 |
| | 3 | 32 | 64 | 16 |
| MK(A.G.) | 300 | 4 | 1 | 8 |
| | 30 | 16 | 2 | ND |
| | 3 | 32 | 32 | ND |
| RK | 3,000 | 1 | 1 | * |
| | 300 | 1 | 2 | 4 |
| | 30 | 1 | 4 | 16 |
| | 3 | 1 | 8 | 32 |

* No growth of challenge virus. ND = Not done.

with more sensitivity in MK(Rh) cells. It was also interesting to observe that MK(Rh) cells were incapable of picking up any activity of heterologous RK ITF compared to MK(A.G.) cells which detected significant quantities of ITF with challenges of 30 or less $TCID_{50}$.

*Discussion.* These results extend previous observations(6-8) concerning species specificity of CAM and mammalian ITFs for various types of TC cells. Although ITF from CAM showed no activity in mammalian cells and vice versa, certain mammalian ITFs were shown to have definite heterologous activities. As reported by Sutton *et al.*(1), certain TC cells were found to be more sensitive than others in detecting mammalian ITFs. In this respect, RK cells were the most sensitive to heterologous ITF when influenza virus was used as challenge. Our findings indicate that the observed cellular sensitivities to ITF may depend, in part, on the degree of cellular resistance to virus infection. Also, we have shown that cellular sensitivity is increased by using 30 or less $TCID_{50}$ of challenge virus. RK ITF, which was active only in homologous cells, was shown to be active in MK (A.G.) cells when tested under these conditions. It is also possible that other ITF preparations may be active in heterologous TC systems when low levels of challenge virus are routinely used.

The observation that MK ITF is much less active in homologous than heterologous cells, raises important questions as to the effectiveness of ITF in various *in vivo* systems. First, failure to obtain positive results *in vivo* even with homologous ITF may be due to the lack of solid protection of susceptible cells; and second, ITF-induced cellular resistance may be overcome by overwhelming dose of challenge virus. Production of ITF in cells, which do not support growth of a particular challenge virus, suggests that natural resistance of these cells to virus infection in part may be due to early rapid build-up of ITF in these infected cells prior to assembly of complete viral particles.

*Summary.* The activity of ITFs prepared in 8 mammalian cell cultures and CAM culture was determined in homologous and heterologous systems. The data indicate that HEK ITF exhibits the greatest heterologous activity under the conditions of the test. Primary RK cells were the most sensitive for detection of heterologous ITF from mammalian source. Sensitivity of the assay method is increased greatly by maintaining a viral challenge of 30 or less $TCID_{50}$. Under this condition, MK(Rh) ITF could be assayed in homologous as well as heterologous cells as could RK ITF. The production of ITF in HAE and PK cultures is, to our knowledge, the first reported case of production in cells not susceptible to influenza virus infection.

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