Interferon Induction by Colorado Tick Fever Virus: A Double-Stranded RNA Virus[1] (36093)

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Since the discovery of interferon, numerous studies have been directed toward elucidating the mechanism of its synthesis. One approach to this problem has been to subject the viral inducers to various physiochemical stresses and then to test the effect on the interferon response. Two of these stresses, ultraviolet radiation (UV) and heat, have produced conflicting results. UV-irradiated influenza (1), Newcastle disease virus (2, 3), mumps, Sendai (4), vaccinia (5), adenovirus (6), and fowl plague (7) can induce or release interferon while UV-irradiated Mayaro (8), Sindbis (9), Western equine encephalitis (WEE) (10), and tick-borne encephalitis (11) cannot. Mild heat inactivation (37° or less) does not destroy the interferon inducing ability of WEE and Sindbis (12), Chickungunya (13), and Semliki forest virus (14) while inactivation at higher temperatures abolishes this ability (9, 10, 15).

The recent report by Green (16) that Colorado tick fever virus (CTF) was a double-stranded RNA virus has provided the opportunity to compare the interferon inducing ability of an infectious and an inactivated double-stranded RNA virus. This approach avoids the controversy of whether or not an inactivated single-stranded RNA virus forms a multistranded RNA molecule following cell penetration (7, 17).

Materials and Methods. Cell cultures. The cell lines used in this study were the mouse fibroblast, L-929, and a continuous line of African green monkey kidney, BSC-1. Both were cultivated on Eagle's minimum essential medium (MEM) supplemented with 5–7% fetal bovine serum (FBS).

Virus. The virus employed in this study was one of seven strains originally isolated from human cases of Colorado tick fever by the State Department of Public Health, Berkeley, Calif. The CTF-5 strain was used throughout this study. Pools of virus were prepared using confluent BSC-1 cell monolayers in 16 oz prescription bottles. Virus was harvested 72 hr after inoculation. At this time, fetal bovine serum was added to a final concentration of 35% prior to three cycles of freeze-thawing. Cell debris was removed by centrifugation and the lysates were stored at −70°. A single pool of CTF-5 virus was used for all experiments.

Viral assays. CTF-5 virus was found to plaque on BSC-1 cells. Assays were routinely performed on BSC-1 monolayers in 60 mm petri dishes incubated at 37° in a 5% CO_2 atmosphere. Monolayers were inoculated with 0.2 ml of viral suspension and allowed to adsorb for 1–2 hr at 37°. The monolayers were then overlayed with a 5 ml mixture of 1.5% washed agar, MEM with 5 %FBS, and 250 μg/ml of DEAE-dextran. On day 4, 5 ml of neutral red 1:10,000 in Earle's salt solution was added and counts were made 24 hr thereafter.

Viral inactivation. For UV inactivation, 5 ml of CTF-5 virus in MEM with 35% FBS was exposed to UV radiation in 60 mm petri dishes at a dosage rate of 22 ergs/mm²/sec. The virus was constantly agitated by means of a mechanical shaker. Under these conditions, it required 30 min of exposure to abolish detectable infectivity in a suspension of

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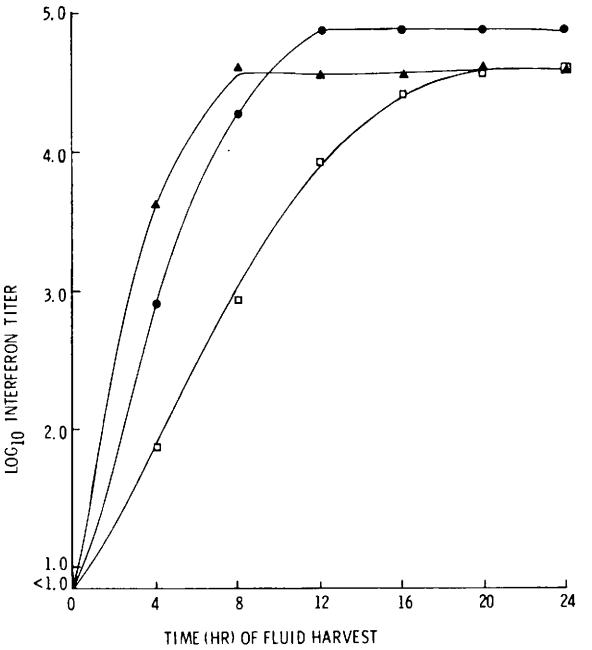

FIG. 1. Production of interferon by L cell monolayers exposed to infectious and UV-inactivated Colorado tick fever virus: L cell monolayers were exposed to CTF at an moi of 3. The virus was removed after 1 hr and replaced with 5 ml of MEM with 2% FBS. Individual monolayers were harvested by freezing at the times indicated. Interferon assays were performed after dialysis at pH 2.3; infectious virus (□); 30 min UV-exposed virus (●); 60 min UV-exposed virus (▲).

4.4×10^7 infective particles/ml.

Heat inactivation was accomplished using a 60° water bath. Undiluted viral suspensions in capped plastic tubes were heated for 15 min. This exposure was sufficient to abolish detectable viral infectivity. For pH inactivation, the virus suspension was dialyzed against a citric acid buffer for 24 hr at 4°. No infectivity could be detected after dialysis at pH 3.0, 4.0, or 5.0.

Interferon preparation. L cell monolayers containing 8×10^6 cells were exposed to CTF-5 virus at a multiplicity of infection of 3. After 1 hr at 37°, the cell sheets were washed with 8 ml of Earle's salt solution and 5 ml of MEM with 2% FBS was added to each bottle. Individual monolayers were harvested at the indicated times by freezing at —20° in order to suspend interferon production. All samples were dialyzed for 24-48 hr against a KCL–HCl buffer, pH 2.3, and then for 24 hr against phosphate buffered saline, pH 7.2.

In vivo induction of interferon was accomplished by intravenous (iv) injection of 0.5 ml of viral suspension ($1–2 \times 10^6$ plaque forming units) into 6 week old (20–25 g) NAMRU specific pathogen-free mice. Whole blood was collected by heart puncture and the serum was separated by centrifugation. Ten mice were used for each determination.

Interferon assay. Interferon assays were performed by the method of Campbell and Colter (18). Briefly, L cell monolayers in 3 oz prescription bottles were exposed to 5 ml of serially diluted interferon samples for 24 hr. The interferon suspension was removed and the monolayers were challenged with 50–70 plaque forming units of vesicular stomatitis virus. A methylcellulose overlay (19) was applied after 1 hr and the plaques were enumerated 3 days later. The interferon titer was expressed as the reciprocal of the dilution which reduced the plaque count to 50% of that of the controls.

Results. Although CTF-5 virus could be

TABLE I. Interferon Response in Mice Using Infectious and UV-Inactivated Colorado Tick Fever Virus as the Inducer.

Postinjection (hr)	Infectious CTF	UV exposure (min): 30	60	120
2	470[a]	3800	6800	15,100
4	51,000	65,700	62,000	33,800
6	82,100	36,000	40,400	14,000
8	27,600	30,000	22,000	8400
12	6500	—	—	—

[a] Interferon units defined as the reciprocal of the dilution which reduced the plaque count of vesicular stomatitis virus to 50% of that of the controls.

propagated on BSC-1 monolayers, all attempts to grow the virus on L cells met with no success. The virus produced extensive cytopathic effects on the initial passage, but subsequent passages yielded little if any progeny. Fluid from the L cell lysates were tested for an interferon-like substance and a titer of greater than 20,000 interferon units was obtained from a 24 hr infected monolayer.

This material was characterized as interferon by the following criteria: (a) nondialyzable; (b) stable at pH 2.3; (c) did not sediment upon centrifugation at 105,000g for 3 hr; (d) pronase treatment (100 μg/ml for 1 hr at 37°) reduced the liter to 10% of control values; (e) showed species specificity by failing to exhibit any antiviral activity on exposure to monkey kidney cells.

Preliminary experiments with L cell monolayers exposed to infectious CTF-5 virus indicated that the peak of interferon synthesis was attained by 24 hr and no further increase was observed with an additional 8 hr of incubation. Figure 1 shows the production of interferon in the first 24 hr following exposure of L cell monolayers to infectious and UV-inactivated CTF-5 virus. Significant differences are seen when UV-irradiated virus was used as the inducer. Virus exposed to 30 min of UV was able to increase the rate of interferon synthesis, as well as the total amount of interferon. The virus exposed to 60 min of UV increased the rate of synthesis to the extent that the same amount of interferon was produced in 8 hr as was produced in 24 hr by the infectious virus. The results (not shown) of virus exposed to 120 min of UV did not differ significantly from that of the 60 min exposure except that final yields were less.

Heat-inactivated virus (15 min at 60°) and virus inactivated at pH 2.3 failed to induce any detectable (<10 units) interferon over a 32 hr test period.

A similar series of experiments was performed using 6 week old mice. Following iv injection of $1–2 \times 10^6$ plaque forming units of CTF-5 virus, interferon levels in the mouse serum was found to peak at 6 hr (Table I). When UV-inactivated CTF-5 virus was used as the inducer, the pattern observed with L cells was repeated. A comparison of the 2 hr values (Table I) reveals an increase in the rate of appearance of interferon with an increase of UV dosage administered to the interferon inducer. A 30-fold increase was realized using a 120 min exposure time.

As with L cells, viral samples totally inactivated by heating failed to stimulate interferon production or release in mice. However, a sample containing a small amount of residual infectivity (85 plaque forming units/ml) was still able to induce 9300 units of interferon in 4 hr. Inactivation by acid dialysis at pH 3.0, 4.0, and 5.0 rendered CTF-5 virus unable to evoke any interferon response.

Discussion. To evoke an interferon response with a viral inducer, there are two steps generally accepted as prerequisites to this event. The virus must adsorb to and penetrate the cell and it must also uncoat (20). To block either of these processes would result in little, if any, interferon response. In understanding the data presented herein, these events must be kept in mind.

That a double-stranded RNA molecule can in itself act as an excellent interferon inducer has been shown in numerous instances (21–23). Therefore, it is reasonable to assume that after the CTF-5 viral particle uncoats, it is in the necessary physical form to trigger the interferon response. There is no need to form a multistranded RNA molecule if this is a necessary step in other viral systems.

A possible explanation for the effect of UV radiation on the ability of CTF-5 virus to induce interferon is that in abolishing the infectivity of the viral particle, the UV radiation could also suppress the inhibition of host cell RNA and protein synthesis as observed with certain myxoviruses (7) and picornaviruses (24). With fowl plague and Newcastle disease virus (3, 7), the infectious virus yields no interferon on chick embryo cells, but UV-irradiated virus does. CTF-5 virus differs from these systems in that the infectious virus is also an excellent inducer of interferon. Irradiation adds little to the total amount of interferon, but significantly increases the rate of synthesis. For this reason, an alternative explanation seems more plausible.

Any treatment which could decrease the time necessary for penetration or uncoating would increase the rate of appearance of interferon. Although the primary cause of loss of infectivity due to UV-irradiation is damage to the nucleic acid, the capsid proteins also undergo some changes (25). A weakening of the intermolecular bonding of the capsid proteins should facilitate the uncoating process, thus hastening the release of RNA and the triggering of the interferon response. Apparently this is what is seen with UV-irradiated CTF-5 virus.

The case of heat and pH inactivation can be explained along these same lines. The damage caused by heating CTF-5 virus at 60 is presumably to the capsid proteins since double-stranded CTF RNA can be obtained in extraction at 60° (16). The denatured proteins could block interferon induction in either adsorption or uncoating. Receptor complementarity could be lost, resulting in failure of adsorption and penetration. Denaturation could also prevent uncoating or cause the particle to uncoat in such a way as to lead to nucleic acid degradation. Heat inactivation at 37° should not severely damage capsid protein. The viral particle should be able to penetrate and uncoat with resultant interferon induction as seen with Semliki forest virus (14), Sindbis, and WEE (12).

Summary. L cell monolayers and mice were exposed to infectious and to inactivated Colorado tick fever virus. Ultraviolet irradiation of the virus was able to increase the rate of appearance of interferon in both *in vitro* and *in vivo*. Heat- and pH-inactivated virus failed to evoke an interferon response in both systems. It is proposed that UV irradiation permits faster release of the CTF virus RNA while heat and pH inactivation prevent this release.

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