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Bluetongue Virus, an Exceptionally Potent Interferon Inducer in Mice

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The attenuated American BT-8 strain of bluetongue virus is 5 to 10 times more potent an interferon inducer than any other viral or nonviral agent reported to date, inducing as much as 600,000 units/ml of plasma by 8 h after intravenous injection.

Agents that contain double-stranded ribonucleic acid (RNA) are reported to be among the best inducers of interferon (1, 3, 11) both in vitro and in vivo, including members of the reovirus family, such as reovirus type 3 (11) and Colorado tick fever (1). One of the numerous serotypes of bluetongue virus (BTV), a member of the orbivirus subgroup of the reovirus family, had been reported to induce only small amounts of interferon in mice or in primary mouse cell cultures (5). During a search for more potent interferon inducers, we found that the attenuated American BT-8 strain of BTV induced unusually high levels of interferon.

BTV, obtained from Colorado Serum Co. (Denver, Colo.) as a vaccine strain (6, 7), was propagated and titrated as previously described (4, P. Jameson and S. E. Grossberg, *Proceedings of a Symposium Workshop of the Tissue Culture Association,* in press). The Dearing strain of reovirus, provided by W. K. Joklik, Duke University, as purified concentrate from cell culture supernatant fluids, was propagated and titrated in L-cell monolayers. The double-stranded RNA phage $\phi 6$ and its host cell *Pseudomonas phaseolicola* (12) were obtained from A. K. Vidaver, University of Nebraska. The CG strain of Newcastle disease virus was propagated in embryonated eggs and titrated by plaque assay in chicken embryo cell monolayers, as described before (9). *Escherichia coli* endotoxin of the Westphal type was obtained from Difco Laboratories (Detroit, Mich.), and polyinosinic acid·polycytidylic acid was obtained from P-L Biochemicals (Milwaukee, Wis.). RNA of *Penicillium chrysogenum* mycophage was provided by W. J. Kleinschmidt (Eli Lilly & Co., Indianapolis, Ind.). Random-bred adult female Swiss albino mice were obtained from Taconic Farms (Germantown, N.Y.).

The maximum interferon yields after injection into mice of a variety of agents are shown in Table 1. Viruses were injected at the highest possible dose available without concentrating the stock viruses, except for one experiment with BTV. The doses of nonviral inducers were injected at levels comparable to those reported from other laboratories, and other than for BTV, the interferon yields achieved with viral and nonviral inducers are similar to those reported by others. Interferon yields obtained with BTV are 5- to 10-fold higher than those obtained by us or others with any other inducer. In the same experiment, injection of BTV at a dose ($10^{6.8}$ plaque-forming units) comparable to that used for reovirus (Table 1) induced 64,000 units/ml. Because prolonged exposure of Newcastle disease virus to ultraviolet light inactivates its interferon-inducing capacity, Newcastle disease virus was only briefly irradiated (13).

The antiviral substance induced by BTV possessed properties characteristic of mouse serum interferon (2) as follows: (i) stable at pH 2 for 2 days at 4°C; (ii) non-dialysable; (iii) non-sedimentable in 1 h at 100,000 × *g*; (iv) relatively labile to heat (6% remaining) at 56°C in 1 h; and (v) destroyed by trypsin (5 mg/ml) in 1 h at 37°C.

The kinetics of interferon production in serum after injection of BTV are shown in Fig. 1. Interferon production increases to a maximum about 8 h after BTV injection. Interferon levels decline slowly thereafter. The time of maximum interferon production in mice corresponds approximately to the early log phase of viral replication in cell cultures. However, the interferon response in mice appears to be independent of the production of infectious viral progeny because totally inactivated BTV induces yields of interferon comparable to those obtained with infectious virus.

It is not presently known what is responsible for the extraordinary interferon-inducing power of BTV. We have found that BTV also induces high titers of interferon in cell cultures of human and animal origin (unpublished data). Because

TABLE 1. *Maximum interferon titers obtained after injection of BTV and other inducers*

Agent	Dose[a] per mouse	Time sample taken (h)[b]	Maximum interferon yield (units/ml)[c]
BTV (concentrated)[d]	$10^{8.3}$ PFU[e]	8	680,000
BTV	$10^{7.5}$ PFU	8	250,000
BTV (UV irradiated)[f]	$10^{7.5}$ PFU (before UV)	8	140,000
NDV (UV irradiated)[g]	10^{8} PFU (before UV)	12	25,000
NDV	10^{8} PFU	12	18,000
Poly(I)·poly(C)[h]	20 μg	2	17,000
Reovirus type 3	$10^{6.9}$ PFU	18, 24	2,500
RNA of *P. chrysogenum* mycophage	$A_{257} = 0.038$[i]	2	1,500
φ6 phage of *P. phaseolicola*	10^{10} PFU	2	780
Endotoxin	100 μg	2	780

[a] Random-bred adult female Swiss albino mice were injected intravenously with 0.2 ml of the inducer.

[b] Samples were collected at 2, 8, and 18 h for all inducers and at 24 h for some agents by retroorbital bleeding into heparinized capillaries; the blood was diluted in 10 volumes of medium containing 2% fetal bovine serum, and blood cells were removed by centrifugation.

[c] Interferon was titrated by a hemagglutinin yield reduction method using GDVII virus in L cell monolayers (10). This assay measures the same number of units in the National Institutes of Health G002-904-511 reference interferon standard as the unitage assigned to it.

[d] BTV was concentrated by polyethylene glycol "precipitation" (8).

[e] PFU, Plaque-forming units.

[f] BTV suspended in protein-free Hanks balanced salt solution was exposed to ultraviolet (UV) light for 10 min at 80 ergs/s per mm^2 to destroy completely its infectivity.

[g] Newcastle disease virus (NDV) in allantoic fluid was exposed to a nonlethal dose of UV light as described by Youngner et al. (13).

[h] Poly(I), Polyinosinic acid; poly(C), polycytidilic acid.

[i] A stock solution of the RNA in saline had an absorbance at 257 nm (A_{257}) of 1.94; it was diluted 1:10 in saline for injection.

complete cycles of BTV replication do not seem necessary, parental double-stranded RNA segments and/or new double-stranded RNA formed by virtue of activity of the virion-bound RNA transcriptase may be responsible. The exceptionally high yield of interferon induced by BTV raises some additional interesting biological questions. (i) Is there a limit to the amount of interferon an organism can produce? (ii) Is the inducing capability of a given inducer dependent upon the number or type of cells it can stimulate or upon the degree to which it stimulates a finite number of cells having the capacity to produce interferon? (iii) Does BTV affect the cellular regulatory mechanism that is responsible for repressing further interferon production? (iv) Is it possible with such an inducer to achieve "toxic" levels of interferon? Given the potency of BTV as inducer, it should be possible to approach such questions.

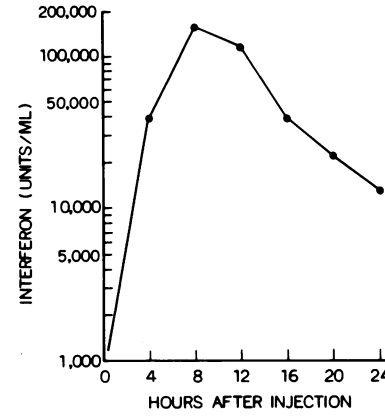

FIG. 1. *Production of interferon measured in mouse plasma after intravenous injection of BTV. The dose was $10^{7.7}$ plaque-forming units per mouse.*

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