Studies on Interferon Induction by the Mouse Mammary Tumor Virus[1] (34449)

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Mouse mammary tumor virus (MTV) has not been reported as possessing hemagglutinin activity nor as being capable of inducing interferon *in vitro* or *in vivo*. MTV shares certain properties common to the myxoviruses, such as gross chemical composition and morphology (1, 2), and therefore might be expected to hemagglutinate and induce interferon. After the initial report of interferon induction with influenza virus (3), other myxoviruses, such as mumps, parainfluenza, fowl plague, and Newcastle disease virus (NDV) (4–7), were shown to be capable of inducing interferon. In this report several approaches have been investigated to determine the capacity of MTV to induce interferon or an interferon-like inhibitor. In addition, MTV was tested for its ability to induce resistance in mice to experimental virus challenge. An attempt was also made to demonstrate hemagglutinin (HA) activity of MTV with erythrocytes from a variety of vertebrates.

Materials and Methods. Interferon assays. The plaque inhibition test described by Wagner (8) was employed, using L929 cells and vesicular stomatatis virus (VS). Most serums, milk, and tissue culture media were treated at pH 2.0 overnight and filtered prior to being assayed for interferon content.

Circulating interferon. Groups of C57BL/Haag (MTV susceptible) female mice and random bred male Swiss mice were injected intravenously (iv) with 0.1 ml of MTV purified by density-gradient centrifugation, containing approximately 10^{10}–10^{12} particles as evidenced by electron microscopy. Serums were obtained from the C57BL mice at 6 and 18 hr and from the Swiss mice at 1, 2, 3, 4, 6, 10, and 18 hr after MTV injection into tail vein. In experiments with Swiss mice, UV-irradiated MTV, as described below, was tested; in addition, blood from RIII tumor-bearing mice was tested.

Milk. In an attempt to investigate a system in which MTV synthesis is maximal, milk obtained from mice at the third to fifth lactation was assayed for interferon content. Electron microscopic examination of such milk reveals high (as much as 10^{12} particles/ml) concentrations of virus. The presence of such quantities suggests very high replicative activity.

Cell culture. A cell line, designated CCL-51/MMT 060562 by the American Type Culture Collection, which actively produces MTV (9, 10) was grown in monolayer cultures. After semiconfluent monolayers were established in 32-oz prescription bottles, the growth medium was decanted and replaced with 40–50 ml of fresh medium and the bottles incubated at 37°. At various time intervals up to 5 days the medium was collected, concentrated 5- to 10-fold by vacuum evaporation and tested for interferon activity.

Induction of host resistance. The capacity of MTV to induce resistance to challenge with Columbia SK virus was determined in male Swiss mice injected iv 24 hr previously with MTV. As controls, NDV, influenza, pneumonia virus of mice (PVM), Statolon, and double-stranded polymer of polyinosinic-polycytidilic acid were inoculated prior to intraperitoneal challenge with 5–10 LD_{50} of Columbia SK virus. All control viruses tested for induction of resistance were UV-irradiated for an interval sufficient to reduce the plaque

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titer of NDV to 0.001%.

Hemagglutinin tests. A variety of erythrocytes was tested with MTV to determine if HA could be observed. Most cells were tested at 4, 25, and 37°, as 0.25% or 0.5% suspensions in 0.85% NaCl or phosphate-buffered saline. MTV was employed as defatted whole milk or milk partially purified by electrophoresis or density-gradient centrifugation. The erythrocytes tested were from the following animals: calf, cat, chick, dog, duck, goat, goose, guinea pig, hamster, horse, human, monkey, mouse, ox, pigeon, rabbit, rat, sheep, pig, and turkey. MTV concentration was calculated to be in excess of 10^8 particles/ml.

Results. In none of these many attempts was it possible to demonstrate induction of interferon by MTV, whether fully infectious or UV-irradiated virus was inoculated. Serums obtained from C57BL mice at 6 and 18 hr contained no demonstrable interferon or interferon-like inhibitor at serum dilutions as low as 1:8. The serums from Swiss mice at 1, 2, 3, 4, 6, 10, and 18 hr also contained no detectable interferon. Some of the samples were tested even at dilutions of 1:2.

The culture fluid, concentrated 5- to 10-fold, from the CCL-51/MMT established cell line, which has been producing MTV (both bioactivity and virions) for more than 5 years, contained no interferon-like inhibitor when tested at dilutions as low as 1:2.

No interferon could be detected in milk samples tested at dilutions of 1:4.

The results of the challenge experiments are presented in Table I. It can be seen that NDV and influenza virus, as well as PVM, which resembles the myxoviruses, afforded protection to Columbia SK-infected mice. Statolon and the double-stranded polymer of polyinosinic-polycytidilic acid also protected. MTV-UV at concentrations containing from 10^6 to 10^{10} virus particles/injection conferred no resistance to challenge. Tests employing nonirradiated MTV also failed to protect.

No specific hemagglutinin activity for MTV could be demonstrated with the erythrocytes from the 20 species tested under the conditions used.

TABLE I. The Effect of MTV and Interferon Inducers on the Survival of Columbia SK Virus-Infected Mice.[a]

Inducer	Dose[b]	Survivors /total	% Survivors
None	—	0/10	0
None	—	1/10	10
MTV	10^{-1}, iv	0/10	0
MTV	10^{-2}, iv	1/10	10
MTV	10^{-3}, iv	2/10	20
MTV	10^{-4}, iv	1/10	10
MTV	10^{-5}, iv	1/10	10
PVM	1×10^5 PFU, iv	4/10	40
NDV	2×10^8 PFU, iv	8/10	80
Inf. A_2/Jap. 305/57	1×10^8 PFU, iv	7/10	70
Poly I:C	10 μg, ip	7/10	70
Statolon	5 mg, ip	10/10	100

[a] For PVM, NDV, Inf. A_2/Jap. 305/57, the PFU titer reflects the titer prior to the UV-irradiation. All inducers of interferon were administered 24 hr prior to ip challenge with approximately 5–10 LD_{50} of Columbia SK virus.

[b] For MTV, five serial 10-fold dilutions of purified virus (estimated to contain 10^{11}–10^{12} particles /ml prior to dilution was used).

Discussion. These findings indicate that MTV, which has a morphologic and gross chemical composition similar to myxoviruses, was not capable of inducing detectable amounts of interferon in cells chronically infected with MTV nor were detectable quantities of circulating interferon found in random-bred Swiss mice or C57BL mice inoculated with up to 10^{12} virus particles. Intravenous injection of various concentrations of MTV did not confer resistance in mice to subsequent challenge with Columbia SK virus. The absence of resistance is noteworthy since mice infected with Columbia SK virus are protected by a wide variety of interferon inducers of both viral and nonviral origins.

It is known that detectable MTV replication in animals does not take place for several weeks after inoculation[2] and that the yield

[2] Charney, J., Pullinger, B. D., and Moore, D. H., The development of an infectivity assay for mouse mammary tumor virus. *J. Natl. Cancer Inst.* (accepted for publication).

is low[3] in the cell cultures (9, 10). Little replicative form of the virus could, therefore, be expected in the mice injected iv with MTV or in the chronically infected CCL-51/MMT cells. If double-stranded viral RNA is required for interferon synthesis, as suggested by Tytell *et al.* (11) and Skehel and Burke (12), the slow replication of the MTV could explain the absence of interferon in mice, as well as in the chronically infected cells employed in this investigation.

Although these results do not exclude the possibility that MTV will be shown capable of producing interferon or hemagglutinating erythrocytes under different conditions or of inducing interferon in other systems, they do indicate that MTV does not exhibit the qualitative HA activity or interferon-inducing capacity of most classical myxoviruses.

Summary. Mammary tumor virus (MTV) did not induce detectable circulating interferon in female C57BL or male Swiss mice upon intravenous injection nor did it induce resistance in mice to subsequent challenge with Columbia SK virus. Numerous other viral and nonviral inducers of interferon did protect mice from challenge with Columbia SK virus. Neither milk obtained from mice during their third to fifth lactation, which contained very high concentrations of MTV (10^{12} particles by electron microscopy), nor blood from tumor-bearing RIII mice, contained any detectable interferon or interferon-like inhibitor. MTV did not exhibit any specific hemagglutinin (HA) activity with the erythrocytes from 20 species of vertebrates. The data indicate that MTV, which shares certain properties with the myxoviruses, lacks the HA activity and interferon-inducing capacity frequently associated with many myxoviruses.

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[3] Lasfargues, E. Y., Kramarsky, B., Lasfargues, J. C., Pillsbury, N., Sarkar, N. H., and Moore, D. H. Stimulation of MTV production in a mouse mammary tumor cell line. *Cancer Res.* (accepted for publication).

Note added in proof: P. C. Hageman and E. DeMaeyer have reported that C57BL and BALB/c mice inoculated iv with MTV and bled at 4, 8 and 24 hours showed circulating interferon at 8 hours in 2 of 3 experiments. (personal communication)

1. Lyons, M. J., and Moore, D. H., J. Natl. Cancer Inst. **35**, 549 (1965).

2. Almeida, J. D., Waterson, A. P., and Drewe, J. A., J. Hyg. Camb. **65**, 467 (1967).

3. Isaacs, A., and Lindenmann, J., Proc. Roy. Soc. Ser. B. **147**, 258 (1957).

4. Henle, W., Henle, G., Deinhardt, F., and Bergs, V. V., J. Exptl. Med. **110**, 525 (1959).

5. Cahny, C., Compt. Rind. **250**, 3903 (1960).

6. Burke, D. C., and Isaacs, A., Brit. J. Exptl. Pathol. **39**, 78 (1958a).

7. Burke, D. C., and Isaacs, A. Brit. J. Exptl. Pathol. **39**, 452 (1958b).

8. Wagner, R. R., Bacteriol. Rev. **24**, 151 (1966).

9. Lyons, M. J., Lasfargues, E. Y., and Came, P. E., Nature **212**, 100 (1966).

10. Sykes, J. A., Whitescarver, J., and Briggs, L., J. Natl. Cancer Inst. **41**, 1315 (1968).

11. Tytell, A. A., Lampson, G. P., Field, A. K., and Hilleman, M. R., Proc. Natl. Acad. Sci. U.S. **58**, 1719 (1967).

12. Skehel, J. J., and Burke, D. C., J. Gen. Virol. **3**, 191 (1968).

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