INFECTION AND IMMUNITY, Apr. 1974, p. 762-763

Vol. 9, No. 4
Printed in U.S.A.

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Susceptibility of Fertilized Mouse Eggs to Minute Virus of Mice

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Received for publication 19 October 1973

Fertilized two-celled mouse eggs deprived of their zona pellucida were susceptible to infection with minute virus of mice. The virus had no deleterious effect on development of embryos cultivated in vitro.

The effect of parvoviruses on fetuses of different animals has been studied, and the role of these viruses in inducing abortions and stillbirths in some domestic animals has been discussed (1, 4, 6). However, no information is available on the effect of parvoviruses on early stages of embryonic development. There are only few reports on the action of some viruses on mammalian eggs (2, 3, 8). Infection with minute virus of mice (MVM), a parvovirus, is highly prevalent in mouse colonies (7). Although the probable mechanism of MVM transmission is by urinary or fecal excretion, or both, vertical transmission of this virus cannot be excluded (5). This report describes the effect of MVM on preimplantation mouse embryos in vitro as a model for studying the action of parvoviruses on embryos at early stages of development.

Four- to six-week-old Swiss ICR and strain A virgin mice were superovulated (8) by intraperitoneal injection of 0.4 IU of serum gonadotrophin per g of body weight followed by the same dose of chorionic gonadotrophin 48 h later. The mice were mated after the second hormone injection and sacrificed after 42 to 48 h. Two-celled embryos were obtained by puncturing the wall of the oviduct. They were picked up in a short, finely drawn capillary pipette operated by a rubber tube held in the mouth. The medium of Whitten and Biggers (WBM) (9) with the addition of phenol red as pH indicator was used throughout. Zona pellucida was removed by digestion with 0.25% Pronase in phosphate-buffered saline containing 1% polyvinylpyrrolidone (2) and washed three times.

MVM was purified in CsCl density gradients and diluted to contain 10^6 mean tissue culture infective doses per ml in WBM. Eggs with and without zona pellucida (about 50 to 200) were infected with the virus for 2 h at 37 C. After virus adsorption, the eggs were washed three times in WBM, exposed to rabbit anti-MVM serum (hemagglutination inhibition titer 1:4,000 [vol/vol], diluted to contain 50 to 80 hemagglutination inhibition units) for 30 min, and washed three times again. In several experiments, an equal number of infected embryos with or without zona were frozen immediately after exposure to the antiserum, and these were labeled as "zero-hour harvests." The infected and control embryos were placed in small glass petri dishes containing WBM and incubated under 5% CO_2 at 37 C for 3 to 4 days. Morphological examination indicated that the cells used consisted of two-celled embryos exclusively.

Three to four days after infection and cultivation in vitro, the embryos were frozen and thawed once, and sonified three times. The homogenates were placed on cover-slip cultures of primary and secondary rat embryo fibroblast (REF) cells, and the virus was assayed by the direct fluorescent-antibody technique 48 to 72 h later.

No virus was detected in noninfected control cultures, and the virus was not detected in zero-hour harvests. Similarly, virus could not be recovered from embryos infected with intact zona pellucida. However, MVM could be demonstrated in embryos denuded of their zona pellucida 72 to 96 h postinfection, as evidenced by the presence of specific fluorescence. Infection with MVM had no deleterious effect on the development of embryos in culture (Table 1), even after they were incubated in concentrated

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TABLE 1. *Effect of MVM infection on two-celled fertilized mouse eggs*[a]

Infecting material	No. of embryos	No. (and percentage) of embryos observed 3 days after infection at different stages of development					
		2-Celled	4-Celled	6- to 8-Celled	Morula	Blastula	Degenerated
MVM	200	14 (7%)	13 (6.5%)	36 (18%)	41 (20.5%)	75 (37.5%)	21 (10.5%)
	100	9 (9%)	13 (13%)	6 (6%)	17 (17%)	43 (43%)	12 (12%)
	60	3 (5%)	7 (11.7%)	2 (3.3%)	12 (20%)	29 (48.3%)	7 (11.7%)
	140	5 (3.6%)		5 (3.6%)	40 (28.5%)	75 (53.6%)	15 (10.7%)
	100	2 (2%)	5 (5%)	6 (6%)	32 (32%)	47 (47%)	8 (8%)
None	118	12 (10.2%)	13 (11%)	8 (6.8%)	19 (16%)	50 (42.4%)	16 (13.6%)

[a] All embryos were infected without zona pellucida.

viral suspension for 3 to 4 days or when they were infected at the morula stage. In both the infected eggs and the controls, the numbers and percentage of eggs dividing and degenerating were comparable. The virus recovered from infected eggs could not be quantified because neither cytopathic effect nor specific fluorescence was detected at 10^{-1} dilution of embryo homogenates. It is presumed that the number of cells was small (60 to 200 embryos) and the virus failed to multiply in quantitative amounts.

Although the virus was isolated from infected eggs treated with MVM antiserum, one cannot exclude completely the possibility that the virus adhering to the egg surface, despite antibody treatment, may have been the source of infection of REF cells. It is also not certain whether the parental or progeny virus caused infection of REF cells. Because zero-hour harvests were uniformly negative for virus isolation, it is probable that the progeny virus caused infection of these cells.

It is reasonable to assume from these data that fertilized mouse eggs deprived of their zona pellucida are susceptible to infection with MVM. Mouse ova with intact zona, however, cannot be infected in vitro with MVM, a finding consistent with the results of oncogenic virus infection of mouse eggs (8). The results differ from other studies in which the zona was not a barrier to Mengovirus infection (2, 3) and in which the virus was harmful to fertilized mouse embryos. MVM had no discernible effect on fetuses after parenteral inoculation of pregnant mice (5), and it is shown here that the virus is not deleterious to early development of mouse embryos.

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