

## NOTES

### Viral Contamination of a Subline of *Toxoplasma gondii* RH

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**A subline of *Toxoplasma gondii* RH was determined to be contaminated with a viral agent, apparently lymphocytic choriomeningitis virus.**

In 1983 Grimwood et al. (5) reported that when *Toxoplasma gondii* RH was propagated in BHK-21 clone 13 cell cultures, adult mice exposed to the used *T. gondii* medium (UTM) exhibited transient signs (body weight loss and hepatosplenomegaly) and thymic atrophy resembling in many respects those of experimental toxoplasmosis. In addition, mouse pregnancies were jeopardized (resorption, abortion, and fetal abnormalities) by maternal exposure to UTM. Filtration (0.22- and 0.45- $\mu$ m pore sizes) and other attempts to implicate a microbial contaminant in the cultures gave negative results. The signs observed in mice were therefore attributed to a substance(s) called toxofactor, which appeared to be produced during trophozoite proliferation.

More recent information indicates that a subline of *T. gondii* RH originally isolated by Sabin 45 years ago (14) and propagated in this center for the past 15 years is contaminated with a viral agent(s). I do not know when or how the contamination occurred, but it appears to have been present when the experiments reported earlier (5) were undertaken. On the basis of new serologic and electron microscopic evidence the contaminant appears to be lymphocytic choriomeningitis virus (LCMV).

Another strain, *T. gondii* BK, was earlier found to be contaminated with a virus (2) that proved to be LCMV (9) and that was implicated (9) in lymphocytic choriomeningitis of a laboratory worker (15). Another *T. gondii* strain reported to be contaminated with LCMV (19) was never satisfactorily so proven (9).

The sera examined for antiviral antibodies were obtained during a continuation of the previously reported (5) study, this time with adult male Nya:Nylar mice (rather than females, as used earlier). The control groups were uninjected mice and mice injected with used control medium (UCM), which contained water-lysed soluble components from non-*T. gondii*-infected cell cultures. The protocols were not intended to detect prior viral contamination or to prevent cross-contamination between test and control groups.

Each group consisted of 20 mice that were bled (5) 8 weeks after the beginning of the experiments (injection of UTM or UCM). The UTM and UCM were produced and administered intraperitoneally as previously described (5), except that the *T. gondii* trophozoites, obtained from mice, were inoculated into cell culture flasks containing human embryonic lung monolayers and minimal essential medium (5) without serum, tryptose phosphate broth, or antibiotics. The UCM was prepared from similar, but non-*T. gondii*-infected cultures.

Two samples from each group were submitted to AnMed Laboratories Inc. (New Hyde Park, N.Y.) to be tested for antiviral antibodies. Each sample was a pool of sera from two mice.

Only the UTM-injected mice had indirect fluorescent antibodies to LCMV. All sera were positive by enzyme-linked immunosorbent assay for anti-mouse hepatitis virus antibodies. Both sera of the UTM-treated mice plus one each of the UCM-treated and noninjected controls were positive for indirect fluorescent antibodies to minute virus of mice. None of the sera tested was positive for other antibodies (pneumonia virus of mice, reovirus type 3, K virus, Theiler's virus, mouse adenovirus, ectromelia virus, or polyoma virus). All were negative for antibodies to *Mycoplasma* spp.

The presence of LCMV antibodies in sera from UTM-treated mice was confirmed in this center by a complement fixation test. Sera from the same mice tested by AnMed Laboratories plus three more pooled sera from each group were tested for LCMV antibodies. All sera from UTM-treated mice were positive (titers, 64 to 512), and all control sera were negative (titers, <4).

Since the mice were not bled before experimentation, I cannot determine at what point the antiviral antibodies were raised. In this and all prior experiments (5) the controls, housed simultaneously in the same room as the treated groups, never exhibited the adult or fetal signs observed in the UTM-treated groups. Furthermore, none of the adult controls was protected from exhibiting those signs when challenged with UTM.

The viral contamination was further confirmed by transmission electron microscopy. Human embryonic lung cell culture monolayers, as described above, were incubated (5 days at 37°C) with 10 ml of UTM. This volume was 10 to 50 times larger than when UTM, with trophozoites still present, was used as the inoculum for cell culture propagation of *T. gondii*. The monolayers were then prepared for transmission electron microscopy as previously described (6).

Virions were observed budding from the surface of the cells. The virions were spherical, 110 to 130 nm in diameter, and composed of a bilaminar membrane enclosing ribosome-like particles, with distinct spikes on the surface (Fig. 1). These virions were indistinguishable morphologically from arenaviruses, of which LCMV is a member (12). No viral particles were observed in human embryonic lung cells incubated with UCM.

The serologic data and electron micrographs strongly suggest that LCMV is the viral contaminant in this *T. gondii* RH subline. However, I cannot state unequivocally that

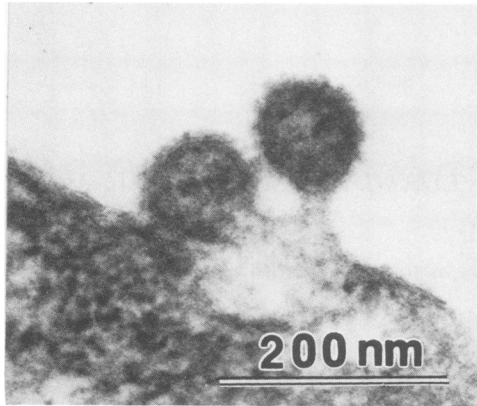


FIG. 1. Electron micrograph of virions budding from the surface of a human embryonic lung cell. Short spikes are seen on the viral surface. Ribosomelike structures are enclosed within the virion bilaminar membrane.

LCMV is the only viral contaminant or that the viral agent(s) in UTM is responsible for any or all of the reported effects in mice (5).

A number of viruses pathogenic to animals are potential teratogens (7). Although LCMV has been implicated as responsible for some cases of human congenital hydrocephaly (16), Mims (11) reported only fetal deaths in mice when the mothers were exposed on day 8 of gestation, i.e., during organogenesis. Other evidence, however, indicates that LCMV can damage animal fetuses when the mothers are experimentally exposed (1, 3).

The serologic data further indicate that both test and control male mice were exposed to mouse hepatitis virus and minute virus of mice, either before or during experimentation. Both viruses are potentially neurotropic in mice (8, 20). Their potential presence should therefore be taken into account in any reinterpretation of the observed damage to the fetal central nervous system (5).

Viral or other microbial agents in animals or established cell lines (13, 17, 18) used to propagate *T. gondii* could easily be a source of contamination in subsequent passages. Not only our findings (5) but other reports will need to be reexamined. Lutz-Ostertag and Senaud (10), for instance, produced gross abnormalities of the chicken embryo central nervous system with an antigen preparation called toxoplasmin (4), derived from *T. gondii* trophozoites. The 0.25% Formalin typically used as a preservative (4) of toxoplasmin does not completely inactivate LCMV (21). If LCMV was a contaminant in the *T. gondii* strain from which their preparation was obtained, it could have been present as live virus in the antigen preparation to which the chicken embryos were exposed.

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#### LITERATURE CITED

1. Ackermann, R., S. S. Kalter, R. L. Heberling, B. McCullough, J. Eichberg, and A. R. Rodriguez. 1979. Fetal infection of the baboon (*Papio cynocephalus*) with lymphocytic choriomeningitis virus. *Arch. Virol.* **60**:311-323.
2. Berger, J., and G. Schoop. 1967. Über ein an den Toxoplasma-Stamm BK (Winsser, vanThiel, 1948) gekoppeltes infektiöses Agens. *Z. Med. Mikrobiol. Immunol.* **153**:269-283.
3. Del Cerro, M., D. Grover, A. A. Monjan, C. Pfau, and J. Dematte. 1984. Congenital retinitis in the rat following maternal exposure to lymphocytic choriomeningitis virus. *Exp. Eye Res.* **38**:313-324.
4. Frenkel, J. K. 1948. Dermal hypersensitivity to toxoplasma antigens (toxoplasmins). *Proc. Soc. Exp. Biol. Med.* **68**:634-639.
5. Grimwood, B., G. O'Connor, and H. A. Gaafar. 1983. Toxofactor associated with *Toxoplasma gondii* infection is toxic and teratogenic to mice. *Infect. Immun.* **42**:1126-1135.
6. Jansing R., and W. A. Samsonoff. 1984. Effect of epidermal growth factor on cultured adult rat hepatocytes. *Tissue Cell* **16**:157-166.
7. Johnson, R. T. 1978. Teratogenic effects of viruses, p. 369-389. *In* P. J. Vinken and G. W. Bruyn (ed.), *Handbook of clinical neurology*, vol. 34, part 2. North-Holland Publishing Co., Amsterdam.
8. Kilham, L., and G. Margolis. 1970. Pathogenicity of minute virus of mice (MVM) for rats, mice, and hamsters. *Proc. Soc. Exp. Biol. Med.* **133**:1447-1452.
9. Lehmann-Grube, F. 1971. Lymphocytic choriomeningitis virus. *Virol. Monogr.* **10**:1-173.
10. Lutz-Ostertag, Y., and J. Senaud. 1966. Action de l'extrait de toxoplasme (toxoplasmine) sur le developpement embryonnaire du poulet. *Arch. Anat. Microsc. Morphol. Exp.* **55**:363-386.
11. Mims, C. A. 1969. Effect on the fetus of maternal infection with lymphocytic choriomeningitis (LCM) virus. *J. Infect. Dis.* **120**:582-597.
12. Murphy, F. A., and S. G. Whitfield. 1975. Morphology and morphogenesis of arenaviruses. *Bull. W.H.O.* **52**:409-419.
13. Nettleton, P. F., and M. M. Rweyemamu. 1980. The association of calf serum with the contamination of BHK-21 clone 13 suspension cells by a parovirus serologically related to the minute virus of mice (MVM). *Arch. Virol.* **64**:359-374.
14. Sabin, A. B. 1941. Toxoplasmic encephalitis in children. *J. Am. Med. Assoc.* **116**:801-807.
15. Scheid, W., K.-A. Jochheim, and W. Mohr. 1956. Laboratoriumsinfektionen mit dem Virus der lymphocytären Choriomeningitis. *Dtsch. Arch. Klin. Med.* **203**:88-109.
16. Sheinbergas, M. M. 1975. Antibody to lymphocytic choriomeningitis virus in children with congenital hydrocephalus. *Acta Virol.* **19**:165-166.
17. Traub, E. 1935. A filterable virus recovered from white mice. *Science* **81**:298-299.
18. van der Zeijst, B. A. M., B. E. Noyes, M.-E. Mirault, B. Parker, A. D. M. E. Osterhaus, E. A. Swyryd, N. Bleumink, M. C. Horzinek, and G. R. Stark. 1983. Persistent infection of some standard cell lines by lymphocytic choriomeningitis virus: transmission of infection by an intracellular agent. *J. Virol.* **48**:249-261.
19. Vermeil, C., and J. Maurin. 1953. Toxoplasmose expérimentale et chorio-meningite lymphocytaire chez la souris. *Ann. Parasitol. Hum. Comp.* **28**:5-13.
20. Weiner, L. P. 1973. Pathogenesis of demyelination induced by a mouse hepatitis virus. *Arch. Neurol.* **28**:298-303.
21. Wilsnack, R. 1966. Lymphocytic choriomeningitis. Viruses of laboratory rodents. *Natl. Cancer Inst. Monogr.* **20**:77-92.