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Letter to the Editor

Implications, Monitoring, and Control of Accidental Transmission of Lymphocytic Choriomeningitis Virus within Hamster Tumor Cell Lines

SUMMARY

Contamination of tumor cell lines of hamster origin with lymphocytic choriomeningitis virus may be assumed to be widespread. These contaminated lines can cause the infection of hamster and other rodent populations used for tumor research. Such infections may spread the virus to humans in contact with the animals and may invalidate the experimental results. Human infections range from a subclinical course to one with signs of severe encephalomeningitis; the etiology may be missed if the physician is not aware of the patient's contact with possibly infected animals. Serological assay of adequate numbers of the laboratory rodent population for lymphocytic choriomeningitis virus is essential to proper surveillance. Elimination of the rodent infection requires primarily the destruction of contaminated and exposed animals, fumigation of the work area, and monitoring of new cell lines to prevent reinfection.

During the last decade, several reports in the medical literature described a high rate of LCM[1] in persons who had contact with hamsters bearing tumor cell lines (1, 3, 7, 14). These tumor cell lines were proven to be contaminated with LCM virus and were strongly suspected of being the cause of LCM infection in the hamster population. A recent outbreak in university personnel[2] demonstrated that many persons working with tumor-bearing hamsters are still unaware of these risks and consequently of the means to minimize them. Besides the hazards to human health, the validity of research results may be affected. The following is a brief compilation of relevant data which may help workers in the field assess the risk in their own institutions and to manage and prevent LCM virus contamination.

Introduction and Spread of LCM Virus in Laboratory Animal Populations. LCM virus is endemic in wild rodents, particularly mice (Ref. 13, p. 116). It may spread to laboratory animals if wild mice infest the animal and food storage areas of breeders, distributors, and research institutions. In hamsters, the illness is frequently asymptomatic, and infection of the laboratory animal population is therefore usually not noticed. Animals excrete the virus in urine, feces, and saliva; such excretion from asymptomatic hamsters may last from several weeks in mature animals (21) to several months in young animals (26). Once introduced, the infection may be perpetuated in several ways. Airborne transmission, facilitated by the use of open-wire cages in many laboratories, is probably the most common.[2] Transplacental spread has been demonstrated in mice (8) and may also contribute to the survival of LCM virus in laboratory animal populations.

A different mode of transmission is possible when rodents harboring LCM virus are used in research as donors or sources of tissue samples which are injected into other susceptible animals. These samples may serve as vehicles of the virus. This type of transmission is facilitated if the material contains living cells for transplantation experiments, such as are common in tumor research. LCM virus may multiply within the tumor cells, although there are no data to suggest that it does so preferentially (26). Technical procedures that are applied to keep the cells or tissues viable during handling or storage are also apt to preserve the otherwise rather labile virus. Intralaboratory transfer of tumor cell lines may then introduce the virus into new settings. A number of reports describe contamination of leukemic and other tumor cells with LCM virus and other viral agents, some of them resembling LCM virus (6, 10–12, 15, 16, 23–25, 27). It is reasonable to assume that many hamster cells lines are now contaminated.

This assumption is supported by the results of an ongoing study at the Center for Disease Control, Atlanta, Ga., in which so far 11 lines of tumor cells of hamster origin were found to be contaminated with LCM virus (P. D. Walter, personal communication). These lines included duodenal, prostatic, pituitary, and melanotic carcinomas; adenocarcinomas of the endometrium, pancreas, renal cortex, small bowel, and breast; plasmacytoma; and cystoadenocarcinoma of the liver. LCM virus was also isolated from various rat and mouse tumor lines (P. D. Walter, personal communication). Recognizing this problem, the major supplier of such tumor cell lines stopped distribution.

Human LCM Virus Infection. Factors influencing the transmission of LCM infection to humans are not fully understood, although airborne spread has been implicated as 1 route (4, 7, 19). The risk of illness increases with frequency of exposure to the infected animals, but illness had also occurred after very minimal exposure, and direct contact with the animals is not necessary.[2] Attack rates vary. In a university laboratory outbreak, one-quarter of the exposed personnel and students became ill.[2] Person-to-person transmission is not known to occur (19).

The human LCM infection may vary from a subclinical

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[1] The abbreviation used is: LCM, lymphocytic choriomeningitis.

[2] R. J. Biggar, T. A. Schmidt, and J. P. Woodall. Lymphocytic Choriomeningitis Infection Associated with Hamsters in a University Laboratory, submitted for publication.

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course to one with signs of severe encephalomeningitis. The incubation period appears to be from 6 days to several weeks (Ref. 4; Ref. 13, p. 90). Deaths are rarely reported (20). Among 57 patients who acquired LCM from contaminated pet hamsters (4), most had a "flu-like" disease. The most common signs of illness included fever, headache, myalgia, nausea, and vomiting; sore throat and photophobia were also observed. Unusually severe pain during eye movements was occasionally mentioned; less common signs were rash, diarrhea, cough, swollen glands, and orchitis. In one-quarter of the cases, the illness was biphasic. Approximately 20% of the patients were hospitalized with a clinical diagnosis of encephalitis or meningitis. Delirium, amnesia, or transient paralysis of the bladder was reported for some of these individuals.

Abnormalities of the cerebrospinal fluid include lymphocytic pleocytosis with up to several thousand cells, a moderately low glucose level, occasionally as low as 22 mg/dl,[2] and elevated protein levels. Peripheral white blood counts may be low or normal, and atypical lymphocytes suggestive of mononucleosis are sometimes observed, although the differential count is usually normal in distribution.

Physicians who were not alerted by the circumstances surrounding the illness or the patient's history frequently diagnosed LCM infections as influenza, mononucleosis, herpes encephalitis, or tuberculous meningitis, until laboratory studies revealed the LCM virus etiology. This is not surprising, since none of the manifestations is unique to LCM (Ref. 13, pp. 92, 96) and since other agents causing similar symptoms may be widespread in the community (5).

Effects of LCM Virus Contamination on Tumor Cell Lines. In addition to the hazards to the laboratory worker in tumor research, the validity of results of experiments using LCM virus-contaminated cell lines is questionable for several reasons. Although hamsters have not been studied, experimental evidence in mice and guinea pigs indicates that a concurrent or preceding LCM virus infection may alter the course of illness due to leukemias and virus-induced tumors. A longer than average survival time has been reported in L_2B (16) and L_2C (11) leukemia-transplanted guinea pigs that were infected with LCM near the time of cell inoculation. A slower growth of local tumors and a lesser degree of splenomegaly were noted (16). Similarly, experimental LCM virus infection of weanling mice induced significant protection against subsequent inoculation with Rauscher virus, as evidenced by lower incidence of leukemia, prolonged incubation period, and delayed death. In newborn mice inoculated with LCM virus, the protection was even more marked (27).

Some evidence suggests that leukemia failed to develop because the concurrent LCM virus interfered with reproduction of Rauscher virus by the target cell (27). An agent, designated as M-P virus and antigenically related if not identical to LCM virus, was reported to suppress transplanted mammary carcinoma and spontaneous leukemia in mice (15). A protective effect of LCM infection against polyoma virus-induced tumors in mice has also been noted; the incidence of tumor formation was lower when LCM infection was introduced into newborn mice within a day of polyoma virus inoculation (8).

Finally, the permissiveness of cells in certain tumors to invasion of LCM virus will result in altered genetic and antigenic characteristics and may influence their rates of mitosis and survival. A valid interpretation of experimental results obtained with LCM-contaminated cell lines may not be possible.

Surveillance of Laboratory Animals. Since hamsters used in tumor research have been the main species involved thus far in the transmission of LCM virus to laboratory personnel, recommendations for surveillance deal primarily with them. However, similar hazards may be encountered with other rodent species used in tumor research. Skinner and Knight (18) have recently summarized the problems associated with mouse colonies contaminated with LCM virus.

All hamsters used in tumor cell research, regardless of the source of tumor cells, should be serologically assayed for LCM antibodies. It is advisable to sample first the animals most likely to have acquired the virus, *i.e.*, the older animals that have been held for a considerable time within the institution and the animals that have had tumor cell implants. An adequate number should be tested, since the probability of detecting antibody carriers depends on the number of animals surveyed, as well as the incidence of antibody-positive animals in the population (*e.g.*, to detect by random sampling of a population of infinite size and incidence at a 10% level with 95% confidence, 28 animals should be tested). The method of choice for assay of LCM antibody in hamsters is the complement-fixation test because of its sensitivity, low costs, and general availability.

Detection of LCM antibody in healthy animals indicates a prior infection at an undetermined time. A high prevalence of antibody in the animal population suggests a current contamination of the colony, which may be confirmed by isolation. For example, virus isolation was easily achieved from hamsters from a small laboratory in which 83% of selected hamsters without anticomplementary activity were seropositive.[2] Virus isolation may also be attempted from stored tumor cell lines. However, isolation work requires special precautions, in particular, adequate ventilation of the animal facilities and safe handling of exhaust air from these areas in order to prevent spread of virus to personnel or to other areas of the animal facilities.

Elimination of LCM Virus from the Animal Population and Cell Lines. If LCM virus is found in the animal population, all animals that are susceptible to this virus and that have been exposed must be killed and incinerated. The rooms in which contaminated animals have been kept should be fumigated, after which the cages and equipment may be removed and autoclaved, and the premises thoroughly cleaned.

LCM virus is inactivated by formalin (2). Fumigation may therefore be carried out by using formalin (40% formaldehyde in water) at the rate of 1 ml/cu ft, as a spray. Alternatively, 0.3 g paraformaldehyde per cu ft may be vaporized in a high-temperature silicone fluid at 205°. It is important to keep the relative humidity over 70% during fumigation. Precautions should be taken to avoid the spread of vapor to adjoining areas through ventilation systems or cracks in doors and windows. After the fumigated area has been kept

closed for 8 hr, it should be thoroughly ventilated. Further details of these fumigation techniques are given by Songer *et al.* (22). Since LCM virus is thermolabile, a further safeguard would be to leave the area empty for 7 to 10 days.

Elimination of LCM and other unidentified viral agents from contaminated tumor cell lines has been attempted by repeated passages of these cells through animals immune to the contaminating agent (6, 12, 17, 24). Further investigation of the effectiveness of this method is necessary. Such an investigation should not rely solely on conventional isolation attempts but should include passages of the treated cell lines in nonimmune animals, followed by a search for antibody conversion in these animals.

Homograft rejection of infected cells has been postulated as a pathogenic mechanism leading to elimination of LCM virus (9). The usefulness of decontamination procedures involving serial passage in immune animals may therefore be limited if a subpopulation of the contaminated tumor cell line produces complete or incomplete LCM virus, since successful elimination of virus may be associated with removal of virus-susceptible cells. Such alternation of the cell population could change other characteristics of the cell line as well, affecting subsequent research results.

Prevention of Reinfection by LCM. Monitoring by several methods is necessary to check the adequacy of control measures. New hamster cell lines should be screened for virus to exclude contamination before they are introduced into the laboratory. Serological testing of animals 3 to 4 weeks after implantation of any new tumor cell material is an important safeguard even if no virus could be isolated from the material initially. Measures to exclude contact with wild rodents should be rigorously enforced. Semiannual surveillance of an appropriate sample of the animal population (see "Surveillance of Laboratory Animals") will detect contamination from indigenous wild rodents infected with LCM.

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