Exp. Anim. 46(4), 311–313, 1997

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Replication of Murine Coronaviruses in Mouse Embryonic Stem Cell Lines in vitro

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Abstract: *Replication of murine coronaviruses in eight mouse embryonic stem (ES) cell lines of several genetic backgrounds was examined. Both mouse hepatitis virus (MHV) type 2 and MHV, strain A59 replicated well with no or minimal cytopathic effect in all the ES cell lines tested. The results suggest the possibility that MHV-infected ES cells may disseminate MHV in mouse colonies due to embryo manipulation.*

Key words: *embryonic stem cells, mouse hepatitis virus, viral replication*

Contamination of transplantable tumors and cell lines with murine viruses are sometimes found [1, 2, 14, 15, 22] and has been suspected of being a cause of infections in laboratory animals. A recent study demonstrated that such biological materials were still contaminated with rodent viruses. Of 297 tumors examined, 75 (25.3%) were contaminated, whereas the rate of contamination was lower than that done 20 years before [15]. Especially, transplantable tumors propagated *in vivo* were contaminated at a higher rate than those propagated *in vitro*, suggesting that they may be contaminated in infected animals and that they may disseminate infectious agents to naïve animals.

For genetic engineering of mouse, the use of mouse embryonic stem (ES) cells has been increasing in many biomedical science laboratories [17, 19]. Since chimeric embryos containing ES cells are transplanted into psuedopregnant females, the use of contaminated ES cells may disseminate infectious agents to naïve mouse colonies. Murine coronaviruses, namely, mouse hepatitis viruses (MHV), are important viral agents in mice, since MHV infection induces reproductive disorder and modifies the results of biomedical experiments [9, 18]. In a previous study, we found that MHV type 2 (MHV-2) replicates well in A3-1 cells, a 129/SvJ-derived ES cell line with little cytopathic effect and establishes a persistent infection *in vitro* [16]. In this note, I examined the replication of MHV-2 and MHV, strain A59 (MHV-A59) in eight ES cell lines used in many laboratories throughout the world.

AB1 ES cells [12] were obtained from Dr. A. Bradley, Baylor College of Medicine, Texas. BL/6-III [10], D3 [6] and J1 [11] ES cells were obtained from Dr. T. Saito, Chiba University, Dr. T. Muramatsu, Nagoya University and Dr. T. Noda, Cancer Research Institute, respectively. E14.1 [5] and R1 [13] ES cells were obtained from Dr. Y. Iwakura in our institute. TT2 ES cells [20] were purchased from GIBCO BRL, Tokyo.

(Received 26 February 1997 / Accepted 3 May 1997)

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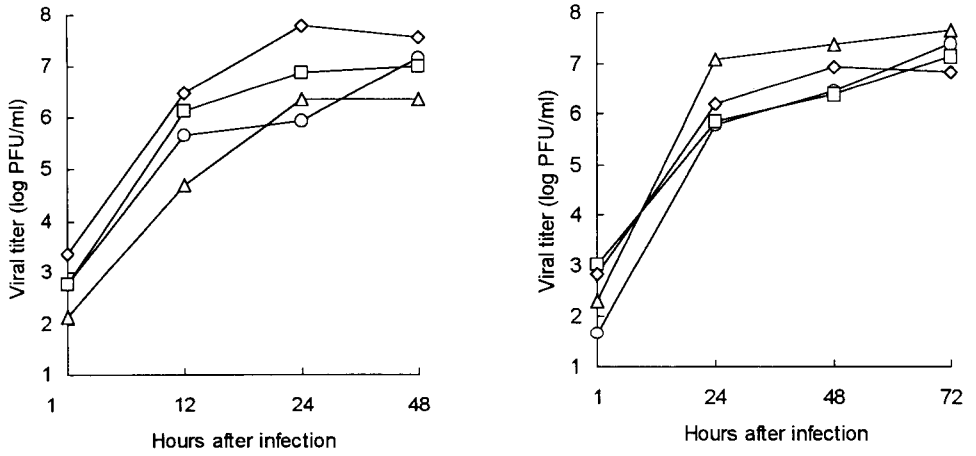

Fig. 1. Replication of MHV-2 in several ES cell lines. AB1 (◇), BL/6-III (○), D3 (△) and J1 (□) (left panel), and A3-1 (○), E14.1 (□), R1 (△) and TT2 (◇) (right panel) ES cells were infected with MHV-2 at an moi of 0.1. Supernatants were collected at the times indicated and the viral titers were determined by plaque assay.

BL/6-III and TT2 ES cell lines were derived from C57BL/6 and (C57BL/6 × CBA)F1 mouse, respectively. The ES cell lines except for BL/6-III and TT2 were derived from 129/Sv mouse or the substrains. They were cultured on gelatinized tissue culture dishes in a mixture of Dulbecco's modified eagle's medium and nutrient Ham's F-12 (DME/F-12) (Sigma) supplemented with 20% fetal calf serum (FCS), 10^{-4} M 2-mercaptoethanol and 1,000 unit/m*l* LIF (ESGRO™) (AMRAD, Victoria, Australia) at 37°C in a 5% CO_2/95% air atmosphere. FCS was selected by culturing the TT2 ES cell line as described elsewhere [17]. A non-fusogenic MHV-2 and fusogenic MHV-A59 were prepared as previously described and stocked at −80°C until use [16]. ES cells were inoculated with MHV at a multiplication of infection (moi) of 0.1. Viral titers in the supernatants were determined by conventional plaque assay by DBT cells [16].

Although eight ES cell lines of several genetic backgrounds were used, MHV-2 replicated well in all the ES cell lines tested, the maximum viral titers in the supernatants being over 10^6 PFU/m*l* (Fig. 1). ES cell lines also supported viral replication of MHV-A59 (Fig. 2). The results indicate that ES cell lines easily allow replication of MHV. In addition, MHV induced little cytopathic effect on ES cell lines and established persistent infection *in vitro* (data not shown), as reported previously [16].

Opportunities for contaminating ES cell lines with MHV may be infrequent, but ES cells are usually cocultured with feeder cells such as mouse embryonic fibroblasts, which can be contaminated with MHV. Since it is difficult to judge the contamination of ES cell lines with MHV by light microscopic observation because there is little cytopathic effect, it is better to regularly check the contamination of ES cell lines by virus isolation, the infant mouse bioassay, the mouse

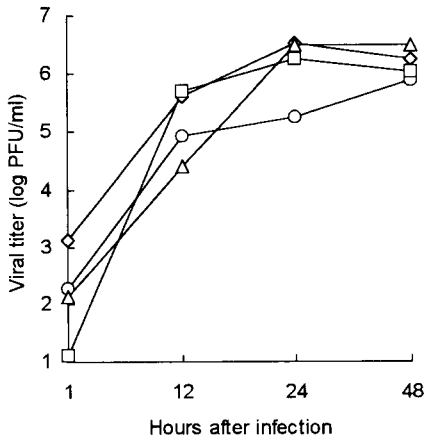

Fig. 2. Replication of MHV-A59 in several ES cell lines. AB1 (◇), BL/6-III (○), D3 (△) and J1 (□) ES cells were infected with MHV-A59 at an moi of 0.1. Supernatants were collected at the times indicated and the viral titers were determined by plaque assay.

antibody production test, monoclonal antibody solution hybridization assay, or polymerase chain reaction [2, 3, 7, 8, 15, 21].

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