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Lifelong persistent infection of hamster brain by human adenovirus type 6.

Yoshiro Yabe* Kyoko Matsumoto†

Hajime Ogura‡

*Okayama University,
†Okayama University,
‡Okayama University,

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Lifelong persistent infection of hamster brain by human adenovirus type 6.*

Yoshiro Yabe, Kyoko Matsumoto, and Hajime Ogura

Abstract

To establish an experimental persistent infection of the brain with human adenoviruses, adenovirus type 6 (ad 6) was inoculated intracerebrally into young adult hamsters. Hamsters appeared languid for a few days after inoculation, but recovered rapidly. By cocultivation of tissue fragments with HeLa cells, ad 6 was always recovered from the brains of hamsters throughout their lives, as long as 29 months, indicating the establishment of a lifelong persistent infection. Except for the first few days after inoculation, however, attempts to recover virus by inoculation of tissue extracts onto HeLa cells or by cultivation of tissue fragments alone were unsuccessful.

KEYWORDS: adenovirus type 6, persistent infection, hamster brain

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—Brief Note—

Lifelong Persistent Infection of Hamster Brain by Human Adenovirus Type 6

Yoshiro Yabe*, Kyoko Matsumoto and Hajime Ogura

Department of Virology, Cancer Institute, Okayama University Medical School, Okayama 700, Japan

To establish an experimental persistent infection of the brain with human adenoviruses, adenovirus type 6 (ad 6) was inoculated intracerebrally into young adult hamsters. Hamsters appeared languid for a few days after inoculation, but recovered rapidly. By cocultivation of tissue fragments with HeLa cells, ad 6 was always recovered from the brains of hamsters throughout their lives, as long as 29 months, indicating the establishment of a lifelong persistent infection. Except for the first few days after inoculation, however, attempts to recover virus by inoculation of tissue extracts onto HeLa cells or by cultivation of tissue fragments alone were unsuccessful.

Key words : **adenovirus type 6, persistent infection, hamster brain**

Adenoviruses were first isolated from tissue cultures of human adenoids (1). Since then, the persistent infection of the lymphoid tissues of rabbits and guinea pigs with human adenoviruses has been established (2-4). The development of subacute adenovirus encephalitis has also been reported in a patient with malignant lymphoma (5, 6). To our knowledge, the persistent infection of the brain of laboratory animals with human adenoviruses has not been reported. We inoculated human adenovirus type 6 (ad 6) intracerebrally into young adult hamsters and found that it persisted in the brain throughout the lives of the hamsters.

Ad 6 (strain: Tonsil 99) was cultured in HeLa cells maintained in Eagle's minimum essential medium (MEM) containing 2% heat-inactivated calf serum at 37℃. The 50% tissue culture infective dose (TCD_{50}/0.1 ml) was determined by observing the development of cytopathic effects in virus-inoculated HeLa cells for 5 days.

Syrian golden hamsters of 4-5 weeks of age were inoculated with 0.02 ml ($2\times10^{3.5}$ TCD_{50}) of ad 6 into the right temporal region of the brain under ether anesthesia. Hamsters appeared languid for 4-6 days after inoculation, but all recovered rapidly. At various intervals after inoculation, hamsters were bled under ether anesthesia. The virus-inoculated region of the brain was cut out, minced with sharp scissors and washed thoroughly in phosphate-buffered saline (PBS). A portion of the minced tissue fragments was further ground in a

* To whom correspondence should be addressed.

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chilled mortar, suspended in PBS to 10% and centrifuged at 2,000 rpm for 10 min at 4℃. The supernatant thus obtained was used as the tissue extract.

Virus isolation was performed by three methods: inoculation of tissue extracts (0.2 ml per tube) onto HeLa cells; cultivation of tissue fragments alone (approximately 10 fragments of about 1 mm^3 per tube) in Maitland type cultures (7); and cocultivation of tissue fragments with HeLa cells, in which approximately 10 fragments per tube were planted onto HeLa cells and maintained together. These cultures were maintained in MEM containing 2% calf serum (2 ml per tube) at 37℃ for 4 weeks (2 weeks, in Maitland type cultures), with medium changes every 4 days. When cytopathic effects were not observed during this period, one whole culture including fluid, cells and/or tissue fragments was frozen and thawed once, and inoculated into new tubes of HeLa cells (0.2 ml/tube). The second passage was observed for 2 weeks. The neutralization test of isolated viruses was done as described previously (8).

Two days after inoculation, ad 6 was recovered from the brain by both inoculation of tissue extracts onto HeLa cells and cocultivation of tissue fragments with HeLa cells (Table 1). Thereafter, attempts to isolate virus by inoculation of tissue extracts onto HeLa cells or by cultivation of tissue fragments alone were always unsuccessful. By cocultivation of tissue fragments with HeLa cells, however, virus was isolated from the brains of all hamsters sacrificed at various intervals from 8 days to 29 months after inoculation, and adenovirus-specific cytopathic effects developed in HeLa cells always in 8-17 days, mostly in 10-13 days. The isolated viruses were identified as ad 6 by the neutralization

Table 1 Virus recovery from the brains of hamsters intracerebrally inoculated with adenovirus type 6

Intervals after inoculation	No. of hamsters examined	No. positive by		
		Inoculation of tissue extracts onto HeLa cells[a]	Cultivation of tissue fragments[b]	Cocultivation of tissue fragments with HeLa cells[c]
2 days	1	1 (7)[d]	ND[e]	1 (3)
8 〃	1	0	ND	1 (8)
1 month	1	0	0	1 (11)
2 months	1	0	0	1 (13)
3 〃	2	0	ND	2 (11, 13)
6 〃	1	ND	ND	1 (9)
8 〃	1	ND	ND	1 (11)
12 〃	1	ND	ND	1 (13)
18 〃	1	ND	ND	1 (10)
24 〃	2	ND	ND	2 (12, 16)
25 〃	1	ND	ND	1 (10)
27 〃	1	0	0	1 (17)
29 〃	1	0	0	1 (13)

a: Tissue extracts were inoculated onto HeLa cells (0.2 ml per tube), and maintained.
b: Tissue fragments were planted into a tube (about 10 fragments per tube), and maintained.
c: Tissue fragments were planted onto HeLa cells (about 10 fragments per tube), and maintained together.
d: Days from inoculation of tissue extracts or tissue fragments onto HeLa cells to development of cytopathic effects are given in parentheses.
e: ND, Not done.

test with ad 6-specific antiserum.

The results of the present study indicate that ad 6 inoculated intracerebrally into young adult hamsters persists in the brain throughout the lives of the animals. The successful isolation of virus from tissue extracts 2 days after inoculation, suggests that ad 6 replicates to a limited extent for the first few days. Braithwaite has also reported the semipermissive replication of ad 5 in the rat brain in the first 2 days after inoculation (9). After the first few days, however, ad 6 probably persists in an inactive state, though the continuation of very weak viral replication cannot be completely denied.

In the present study, ad 6 was recovered from the brains of hamsters throughout their lives, as long as 29 months. To our knowledge, this is the longest period reported so far for the persistence of human adenoviruses in laboratory animals. It is not known at present, however, what cells in the brain harbor ad 6 and what mechanism makes its stable persistence possible. The elucidation of these problems must await further studies. Similar attempts with ad 12 have so far been unsuccessful.

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