“Reversion” of a Less Tumorigenic *cyt* Mutant of Adenovirus 12 in Induction of the Cell Surface Change

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Received for publication 27 March 1973

Five out of 120 clones of a *cyt* mutant of adenovirus 12 were not defective in induction of the cell surface change and in tumorigenicity.

Many of the *cyt* (cytotoxic) mutants of adenovirus 12 (Ad 12) are less tumorigenic in hamsters (2). The less tumorigenic (*lt*) mutants of Ad 12 isolated independently are similar or identical to the *cyt* mutants (5). Comparative studies of the *lt* mutants with the highly tumorigenic wild type (*w*) indicate that the mutants are defective in induction of the cell surface change, as revealed by lectin-agglutinability and by transplantation immunity (5). However, the *cyt* mutants are not always less tumorigenic. One of the mutants, *cyt* 143, which shows the

typical *cyt* character (large plaque and atypical cytopathic effect), is as highly tumorigenic as *w* (2) and is not defective in induction of the cell surface change (5). The *cyt* mutants obtained by mutagenization with ultraviolet irradiation are not leaky and their reversion rates to *cyt*⁺ (true reversion) are very low (3). However, the “reversion” of less tumorigenic to highly tumorigenic *cyt* mutants like *cyt* 143 has not yet been examined. The present communication describes this “reversion” (not true reversion), suggesting that the low tumorigenicity of *cyt*

TABLE 1. Tumor production in hamsters by clones of *lt* 5 mutant^a

Clone no.	Infectivity (lot PFU per 0.1 ml)	Agglutination by con A ^b (μg)	Tumors in hamsters	Probit	TPD ₅₀ (log PFU)
17	6.85	12	14/14 ^c (100%) ^d	7.00 ^e	5.1 ^f
32	6.80	12	7/8 (88)	6.17	5.8
38	6.85	12	8/10 (80)	5.84	5.7
82	6.35	50	4/7 (57)	5.17	6.1
114	6.90	12	8/9 (89)	6.22	6.0
26	6.80	> 200	5/8 (62)	5.31	6.7
71	6.80	> 200	4/8 (50)	5.00	6.8
74	6.85	> 200	6/12 (50)	5.00	6.9
92	6.75	> 200	5/11 (46)	4.88	7.0
<i>w</i> ^g		12			5.3
<i>lt</i> 5		> 200			6.8
<i>lt</i> 3		> 200			7.3
<i>cyt</i> 2		> 200			9.2
<i>cyt</i> 143		6			6.1

^a Newborn hamsters were injected subcutaneously with 0.1 ml of the virus preparation within 24 h after birth and observed for tumor development during 90 days thereafter.

^b An agglutination of HEK cells infected with each clone (20 PFU/cell) and incubated at 36 C for 30 h was carried out with twofold serial dilution of con A as described previously (5). A minimum amount of con A necessary for the positive agglutination is shown.

^c Number of hamsters developing tumors per number of hamsters observed.

^d Percent of tumor-positive hamsters.

^e Probits are calculated by the method of Finney (1).

^f Virus dose (log PFU) to produce tumors in 50% of hamsters was calculated by the probit method.

^g These figures of *w*, *lt* 5, *lt* 3, *cyt* 2, and *cyt* 143 are cited from previous reports (2, 5).

FIG. 1. Probit analysis of tumor production in hamsters. The probits are calculated from figures in Table 1 by the method of Finney (1) and plotted against virus doses (log PFU). Double circles show ag^+ clones and single circles ag^- clones. The regression line of w (●) and that of $lt\ 5$ (○) are cited from a previous report (5).

mutants is due to a small number of highly tumorigenic *cyt* "revertants."

The isolation of *lt 5* mutant and plaque assay in human embryonic kidney (HEK) cells were described previously (4, 5). Bottle cultures, in which 1 to 5 plaques of *lt 5* mutant were formed, were selected, and 120 clones each from a discrete plaque were propagated at 36°C in tube cultures of HEK cells in 1.0 ml of Eagle minimum essential medium. HEK cells in 2-oz. bottles (approximately 6 ml) infected with each of the undiluted clones were screened by the test for agglutination with concanavalin A (con A) as described previously (5). The amount of con A in the reaction mixture (0.1 ml) was 200 μ g. Five clones (numbers 17, 32, 38, 82, and 114) which brought about the cell surface change were selected and termed as ag^+ (agglutination-positive). Cells infected with each of the remaining 115 clones were agglutination-negative (ag^-). Virus stocks of the 5 ag^+ clones and 4 clones (numbers 26, 71, 74, and 92),

randomly selected from the remaining 115 ag^- clones, were prepared in HEK cells. These clones were further tested for infectivity (PFU), agglutination of infected cells with con A, and tumor production in hamsters (Table 1). The tumor-frequency (%) was applied to probit (1, 5) for calculation of mean tumor frequency (TPD_{50}). The average TPD_{50} of ag^+ clones was $10^{5.6 \pm 0.5}$, while that of ag^- clones $10^{6.8 \pm 0.2}$, equal to TPD_{50} of the parental *lt 5* mutant. The probits of ag^+ clones were distributed near the regression line of w , and those of ag^- clones near that of *lt 5* (Fig. 1).

This observation showed that the mutant stock contained about 4% (5/120) of highly tumorigenic *cyt* "revertants" like *cyt 143* in agreement with the low tumorigenicity ($3.1 \pm 0.3\%$) and suggested that the low tumorigenicity of the *lt 5* mutant was due to these "revertants" and not to an inherent property of the *cyt* mutants.

We are grateful to I. Tagaya for support of this work and to S. Uchida for review of the manuscript.

This investigation was supported in part by a grant from Ministry of Education of Japan.

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