Antigenic Variation Among Adenovirus Type 12 Strains

ROBERT R. RAFAJKO AND JANICE C. YOUNG

Department of Virus Research, Microbiological Associates, Inc., Bethesda, Maryland

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Recent reports relative to the oncogenicity of adenovirus types 12 and 18 (Trentin, Yabe, and Taylor, *Science* **137**:835, 1962; Huebner, Rowe, and Lane, *Proc. Natl. Acad. Sci. U.S.* **48**:2051, 1962) have caused a renewed surge of interest in the adenovirus group. In addition to the prototype strain Huie, Huebner and co-workers investigated eight adenovirus type 12 strains, recovered from anal swabs of children, for their ability to produce tumors in hamsters. Strains were put through a limited number of KB passages after isolation in KB or HEP-2 cells. Four strains were also reisolated in primary human embryonic kidney cells and carried through one additional passage in the same cells. All were inoculated into newborn hamsters. Three of the eight field isolates produced tumors in hamsters identical to those produced by the Huie strain. The proven oncogenicity of at least four different strains of type 12 precludes the possibility that the ability to cause tumors in newborn hamsters is a property unique to the prototype. Although additional factors were involved, the apparent inability of five of eight field isolates to produce tumors questions the homogeneity of adenovirus type 12 strains. For this reason, eight laboratory strains of adenovirus type 12 were examined in cross-neutralization tests with "early" antisera to see whether any intratypic differences could be detected.

The prototype Huie strain used in this investigation was obtained through the courtesy of Robert J. Huebner, Laboratory of Infectious Disease, National Institutes of Health, Bethesda, Md. All other strains studied were obtained from Klaus Schell, Microbiological Associates, Inc., Bethesda, Md. In our laboratory, viruses were put through a limited number of passages in HeLa cells free from pleuropneumonia-like organisms, and a working stock pool produced in the same cells. Virus pools were identified as adenovirus type 12 by use of the following criteria: (i) presence of a soluble adenovirus group CF antigen when tested against specific convalescent human serum; (ii) inability to agglutinate rat, Rhesus monkey, or African Green monkey erythrocytes; (iii) inhibition of cyto-

pathic effects (CPE) in HeLa cells by type 12 (Huie) hyperimmune serum but not by type 18.

Antisera were prepared by a method previously described (Rafajko, *Proc. Soc. Exptl. Biol. Med.* **113**:238, 1963) in which rabbits received a single intravenous inoculation of 1.5 ml of undiluted virus. Animals were exsanguinated 1 week later by cardiac puncture.

Neutralization tests were carried out in 24-hr-old HeLa tube cultures. Cells were planted in minimal essential medium with 10% agamma calf serum (Hyland Laboratories, Los Angeles,

TABLE 1. *Cross-neutralization results between adenovirus type 12 strains homologous with Huie virus*

Virus	Sera				
	Huie	10096	10904	10531	97838
Huie	80*	80	320	80	<5
10096	20	80	20	20	5
10904	5	5	20	—	<5
10531	20-80	80-320	20-80	20-80	5
97838	<5	<5	<5	5	80

* Neutralizing antibody titer as reciprocal of highest serum dilution causing complete inhibition of virus CPE; 32 to 40 TCID₅₀ were in the test at time of reading.

Calif.) and maintained on the same medium containing 5% serum. Serial fourfold dilutions of heat-inactivated sera were prepared in Hanks basal salt solution, and each dilution was mixed with equal volumes of virus diluted to contain 100 TCID₅₀ per 0.1 ml. Virus-serum mixtures were allowed to incubate 30 min at room temperature before inoculating triplicate HeLa cell cultures with 0.2 ml. Challenge virus was always titrated in the actual test. Cell cultures were read 7 days later.

When studied by reciprocal cross-neutralization tests, it was found that all strains were not identical. Results of cross-neutralization tests between Huie and 97838 viruses indicated a lack of relationship, and, therefore, these two strains

TABLE 2. *Cross-neutralization results between adenovirus type 12 strains homologous with 97838 virus*

Virus	Sera				
	97838	97844	13316	10516	Huie
97838	80*	20	80	<5	<5
97844	5-20	20	5	5	<5
13316	20	20	80	20	5
10516	20	20	20	5	<5
Huie	<5	<5	<5	<5	80

* Neutralizing antibody titer as reciprocal of highest serum dilution causing complete inhibition of virus CPE; 32 to 40 TCID₅₀ were in test at time of reading.

were chosen as prototypes to represent two serologically different intratypic groups.

Three strains appeared to be homologous with Huie. Results of cross-neutralization tests with prototypes Huie and 97838 are found in Table 1. As can be seen, there is almost a complete lack of relationship between 10096, 10904, and 10531 and the prototype 97838, whereas a very close relationship exists between these viruses and Huie. Likewise, immune serum to each of these strains contains a level of antibody to Huie equivalent to that of the isologous virus although containing little or no antibody to 97838.

Strains 97844, 13316, and 10516 are heterologous with respect to Huie virus and homologous

with 97838 (Table 2). Sera to all three strains without exception lack neutralizing antibody to Huie, although they contain antibody to 97838 equal to that of the isologous virus. The singular exception is 10516 antiserum, which does not contain antibody to Huie or 97838. However, 97838 antiserum contains neutralizing antibody to 10516 equivalent to that against 13316 and 97844. Huie antiserum contains little or no neutralizing antibody to 97844, 13316, and 10516. Immune serum to each of the three strains homologous with 97838 contains neutralizing antibody to the other homologous strains comparable with that of the immunizing virus.

It is apparent from the aforementioned results that the use of "early" antiserum in cross-neutralization tests allows the differentiation of adenovirus type 12 strains into two serological intratypic groups dependent upon their cross-reaction with 97838 and Huie viruses. The lack of relationship between strains in a different intratypic group is complete in most instances. Resolution among poliovirus strains is best carried out with "early" antisera (McBride, Virology 7:45, 1959), and it appears that adenovirus strains can likewise be differentiated. What correlation, if any, these serological groupings have on the possible oncogenicity of a virus remains to be resolved; however, it is tempting to speculate that cross-neutralization tests such as those described could possibly be a simple way to demonstrate potential tumorigenic activity of an adenovirus type 12 strain.