Immunological Relationship Among Human Adenoviruses of Subgenus D*

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Summary
Antigenic relationships between adenoviruses of subgenus D were determined by neutralization tests in HeLa cell cultures by CPE inhibition. For cross-testing, several antisera of the same species were tested against the prototype viruses. 39 wild strains belonging to 12 different virus species were also studied. Marked variation in the degree of cross-neutralization between individual sera of the same species was often observed. However, virus strains within a species mostly showed identical serological reactions. Hence, antigenic specificity appears to be a fairly constant property of any one species.

Strong cross-neutralizations between species are presumably due to a relationship of the ε (hexon) antigen, whereas weak cross-neutralizations found between viruses related by hemagglutination-inhibition are due to the γ (fiber) antigen.

Viruses related to adenovirus 15 (Mastadenovirus h 15) showed a variety of cross-reactions in neutralization tests. In view of the new species definitions of adenoviruses and to facilitate identification, changes in the classification of Ad 15, 25, 29, and 15/H9 are proposed. The prototypes of Ad 13, 15, 25, 29, and 30 have been cloned by terminal dilution.

Introduction
Recently a definition of adenovirus species, mainly based on neutralization (SN), has been acknowledged by the International Committee on Taxonomy of Viruses (10). The subgenus D (formerly subgroup D or subgroup II) of human adenoviruses comprises a large number of virus species, most, but not all of them (24) agglutinating rat blood cells. Apart from adenovirus 8 (Ad8; Mastadenovirus h 8), the main causative agent of epidemic keratoconjunctivitis, these viruses have rarely been isolated from man. Several of the formerly described

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“types” appear questionable as true separate species defined by the newly formulated criteria. On the other hand, few data are available on the immunological properties of nonprototype strains of viruses of subgenus D.

This study was made with the following aims:

a) to delineate the antigenic relationships among prototype adenoviruses of subgenus D more clearly, especially by the use of several antisera of a given species in SN,

b) to examine how homogeneous strains of any one species are in their antigenic properties.

Both parts of the study lead to a reassessment of the validity of the virus species (formerly types) in this subgenus.

Materials and Methods

Viruses

Besides the prototype adenovirus strains, we studied 39 wild strains from 12 different species (Table 1). No wild strains were available for Ad9, 15, 20, 22, 23, 24, 26, 32, and 36. The virus strains from Germany were isolated and identified in our laboratory. All Ad8 and Ad37 strains were isolated from ocular material, most other strains from fecal material. Earlier isolates were kindly provided by Drs. Edwin H. Lenette, Berkeley, Wade P. Parks, Houston, Leon Rosen, Honolulu, and Jan van der Veen, Nijmegen.

The viruses were passaged 2 to 5 times in HeLa cell cultures in our laboratory. The virus strains from Drs. Lenette, Rosen, and van der Veen had been passed beforehand twice in human diploid cells, 3 times in KB cells, or 4 times in HeLa cells respectively.

The term Ad15/H9 was devised to indicate the relationship to Ad15 by SN and to Ad9 by hemagglutination-inhibition (HI); “H” stands for hemagglutinin. This virus has been found by CRAMBLETT et al. (3) and was described in detail as a “serologically intermediate strain Ad9—15” (22).

Antisera

Adenovirus antisera — two or more against each virus species — were prepared by repeated intravenous inoculation of unpurified virus material, obtained by freezing and thawing of infected HeLa cell cultures, into rabbits. For the NS tests with prototype viruses, we used additional antisera raised in rabbits and kindly provided by Mr. Kirk M. Donovan, NIH, Bethesda, by Drs. M. S. Pereira, London, Leon Rosen, Honolulu, and Wallace P. Rowe, Bethesda, or in horses, prepared by Drs. Jan C. de Jong, Bilthoven, NL, and John C. Hierholzer, Atlanta.

Neutralization

SN tests were performed in HeLa tube cultures or micro plate cultures by CPE inhibition according to established procedures (18). Wild strains were screened by the micro method with one dilution (1:5) of two antisera of each of the subgroup D species; positive reactions were subsequently quantitated. Most SN tests with prototypes were done in tube cultures.

Tabulation of Results with Prototype Viruses

To save space and yet to provide a maximum of information, the observed cross-reactions are presented in a condensed format (Table 2). The numbers listed are log2 values of the ratio of homologous to heterologous SN titer reciprocals (HHTR). If for example (Table 2A) the Ad8 antiserum “Hi” neutralized Ad8 in 1:160 and Ad9 in 1:10 dilution, the HHTR is 160:10 = 16, and log2 16 = 4. The ratio for the homologous SN is always = 0 (log2 1 = 0). Some sera with a homologous SN titer of 1:40 allowed