JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1999, p. 3940–3945 Vol. 37, No. 12
0095-1137/99/$04.00+0

Adenoviruses from Human Immunodeficiency Virus-Infected Individuals, Including Two Strains That Represent New Candidate Serotypes Ad50 and Ad51 of Species B1 and D, Respectively

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Received 3 May 1999/Returned for modification 9 July 1999/Accepted 13 September 1999

Adenovirus (Ad) isolates from a large number of human immunodeficiency virus (HIV)-infected individuals were compared serologically and genetically with Ad isolates from immunocompetent patients. Between 1982 and 1994, stool and urine samples from 137 subjects with AIDS hospitalized in The Netherlands yielded 143 Ad strains. Forty additional Ad strains were obtained from 35 HIV-positive patients in Manchester, United Kingdom, in 1992 and 1993. Of these 183 HIV-associated Ad strains, 84% belonged to species D and 3% belonged to species C. These strains were compared with 2,301 Ad strains collected during general diagnostic examinations in The Netherlands from 1973 to 1992. Of the latter strains, 5% belonged to species D and 49% belonged to species C. Two of the Ads isolated from fecal specimens of AIDS patients represent new serotypes: candidate Ad serotype 50 (prototype strain, Wan) of subspecies B1 and candidate Ad serotype 51 (prototype strain, Bom) of species D. The DNA restriction enzyme patterns of strains Wan and Bom differed from the patterns of all established prototypes.

Genera of the adenovirus (Ad) family have been subdivided into numerous serotypes. As reported by Francki et al. (8), "A serotype is defined on the basis of its immunological distinctiveness, as determined by quantitative *neutralization* with animal antisera (from other species). A serotype has either no cross-reaction with others or shows a homologous-to-heterologous titer ration of >16 in both directions. If neutralization shows a certain degree of cross-reaction between two viruses in either or both directions (homologous-to-heterologous titer ratio of eight or 16), distinctiveness of serotype is assumed if: (i) the hemagglutinins are unrelated, as shown by lack of cross-reaction on hemagglutination inhibition; or (ii) substantial biophysical/biochemical differences of DNAs exist."

To date, 49 serotypes of human Ads have been recognized (16) and grouped into six species (formerly called subgenera) (3) on the basis of their hemagglutinating properties and biophysical and biochemical criteria: species A, B (subdivided into subspecies B1 and B2), C, D, E, and F (19). The seven most recently described serotypes all belong to species D and were first isolated from human immunodeficiency virus (HIV)-infected patients (13, 16). For reasons still not understood, many patients with AIDS shed Ads that are rarely or never isolated from immunocompetent individuals (10, 13, 14). In the present paper, we describe Ad strains isolated from stool and urine specimens of patients with AIDS and compare them with Ad isolates obtained from immunocompetent patients during a similar time frame. We also describe two new serotypes isolated from the AIDS patients; we propose the names Ad serotype 50 (Ad50) (subspecies B1) and Ad51 (species D) for the two new serotypes.

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MATERIALS AND METHODS

Origins of virus strains. Prototype Ad strains were obtained from the American Type Culture Collection (Manassas, Va.). Wild-type Ad strains were recovered from several hospitals in both The Netherlands and Manchester, United Kingdom. From 1973 to 1992, 2,301 Ads were isolated at the general virus diagnostic laboratory of the Dutch National Institute of Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieuhygiëne [RIVM]) from clinical samples taken from immunocompetent patients living in The Netherlands, with each isolate originating from a different patient. Isolation and typing procedures remained essentially unchanged during the 20-year period. The number of isolates from clinical specimens, which were mainly sent by hospital-based physicians, varied between 67 and 200 per year. Nine Ad isolates could not be typed, mainly because they seemed to be mixtures of viruses belonging to different serotypes. Their separation proved difficult and their characterization would therefore be too time-consuming. It cannot be excluded that one of the strains represents a new serotype.

Between 1982 and 1994 in The Netherlands, 143 Ad infections were established by virus isolation from 115 AIDS patients hospitalized at the Academic Medical Center and the Municipal Health Laboratory, both in Amsterdam, and from 22 AIDS patients in other hospitals in The Netherlands. Four patients were infected with two Ad serotypes, and one patient was infected with three Ad serotypes. At North Manchester General Hospital, Manchester, United Kingdom, 40 Ad infections were established by virus isolation from stool and urine samples from 35 HIV-positive patients in 1992 and 1993; two different serotypes were isolated from five samples (14). Together, these 183 HIV-associated Ad strains form the subject of the present study.

Origins of antisera. Antisera were raised in rabbits by conventional procedures (7). Essentially, HEp-2 cell-grown virus preparations made in serum-free Eagle minimal essential medium were purified by extraction with Arcton 113 fluorocarbon, mixed with Freund's incomplete adjuvant, and injected intramuscularly into rabbits four times at weekly intervals. The animals were boosted 1 month later with Arcton 113 fluorocarbon-purified virus and were bled 10 days

TABLE 1. Ad isolates by site and species from non-HIV-infected and HIV-infected individuals from The Netherlands and the United Kingdom

HIV infection	Specimen source	No. (%) of the following species[a]:							
		A	B1	B2	C	D	E	F	Total
No	All sites[b]	83 (4)	593 (26)	14 (1)	1,119 (49)	110 (5)	54 (2)	328 (14)	2301 (100)
Yes	All sites[c]	4 (2)	2 (1)	14 (8%)	6 (3)	153 (84)	3 (2)		182 (100)
Yes	Enteric[c]	3	2	1	3	145	1		155 (85)
Yes	Urine[c]	1		14	1	2			18 (10)
Yes	Respiratory[c]		1		1	8	2		12 (7)
Yes	Other[c]				1	2			3 (2)

[a] Blank entries, no Ad isolates.
[b] Ads isolated at RIVM from Dutch patients during the period from 1973 to 1992.
[c] Ads isolated in The Netherlands from AIDS patients during the period from 1982 to 1994 and Ads isolated in Manchester from AIDS patients for a previous study (14).

after that. Reference horse antisera were prepared at the Centers for Disease Control and Prevention (CDC) as described previously (12).

Serological analysis. All strains were serotyped at RIVM by virus neutralization (VN) assays; many strains were further analyzed by hemagglutination inhibition (HI) tests. VN and HI assays were performed as described previously (5–7, 18). VN tests were conducted with human diploid embryonic lung fibroblasts (strain GaBi) growing in 96-well microplates unless indicated otherwise. In accordance with the usual practice for Ads (17), in VN assays the cell cultures were inoculated with a virus dose that caused 100% of the cells to show cytopathic effects after 7 days of incubation at 37°C. HI assays were performed in microplates with either rat or rhesus monkey erythrocytes where appropriate.

Genetic purification and DREA. Virus strains were genetically purified by two consecutive passages at terminal dilution in human diploid fibroblasts (strain GaBi). Tenfold dilution series were inoculated onto tube cultures of GaBi cells with 10 tube cultures per dilution. After inoculation, the cells were incubated for 4 to 6 weeks in roller drums at 37°C. Positive tubes with the highest dilution yielding any growth were then subcultivated. If two tubes were positive at the same dilution, the tube with the latest-appearing cytopathic effect was selected. If more than two tubes were positive at the same dilution, the passage was repeated. A number of strains were characterized by DNA restriction enzyme analysis (DREA) as described previously (18).

RESULTS

Comparison of Ad serotypes circulating among immunocompetent and HIV-infected individuals. The Ad species and serotype distributions among the subjects varied according to HIV infection, which in most patients had advanced to AIDS (Tables 1 and 2). Ads from HIV-infected patients generally belonged to species D; certain serotypes were particularly related to the condition of AIDS, namely, serotypes 9, 17, 20, 22, 23, 26, 27, 42 to 49, and 51 (Table 2). Only rarely were Ads from immunocompetent patients among these serotypes.

Deviant Ads from HIV-infected patients. During the typing procedures, a number of unusual isolates that presented typing problems were detected. They may have represented as yet unknown Ad serotypes and are described below.

(i) Subspecies B1 candidate Ad50: strain Wan. Ad strain Wan was isolated from a fecal sample taken in January 1988 from a 34-year-old man with diarrhea who was hospitalized with AIDS at a hospital in Amsterdam. Strain Wan grew exceptionally well in GaBi cells, yielding crude cell lysates with infectious virus titers up to 10^9 50% tissue culture infective doses per ml. The virus was genetically purified by passages at terminal dilution. In the VN tests, none of the 49 rabbit antisera to the established Ad prototype strains and the rabbit antiserum to strain Bom (see below) reacted with strain Wan to titers higher than 0.01 the homologous titers (data not shown). Weak cross-reactivities were observed with Ad21 (subspecies B1) and Ad14, Ad34, and Ad35 (subspecies B2), and all were below the threshold of 1/16 the homologous VN titers (Table 3). Conversely, rabbit anti-Wan antiserum did not react with any of the 49 prototype strains or with strain Bom with a VN titer higher than 0.01 the homologous titer (data not shown). A low VN titer was found only with Ad21 (Table 3).

Strain Wan agglutinated erythrocytes from rhesus monkeys. According to differential hemagglutination (HA) subgrouping, this strain could be classified as HA subgroup 1B (9, 11). In the HI tests, the strain could not be clearly distinguished from Ad21 (subspecies B1) or from Ad34 and Ad35 (subspecies B2) when antiserum to the Ad21 prototype strain was used (Table 4). The phenomenon that a strain belonging to serotype *x* is reacting in HI assays like prototype *y* was reported before (13, 20) and was observed extensively in the present study (data not shown). In view of the serotype definition for Ad and the behavior in the VN assays, this observation is not taxonomically significant. Only a low HI titer to strain Wan was observed with antiserum to Ad34, and no reactivity was detected with antisera to the other members of species B (see Table 6). Conversely, rabbit antiserum to strain Wan inhibited the HA of Ad21, Ad34, and Ad35 to titers that differed only fourfold or less from the homologous titer.

By DREA with restriction enzyme *Sma*I, strain Wan produced eight bands (Fig. 1), confirming its allocation to species B (19). By using five restriction enzymes, DREA patterns were determined for strain Wan and the Ad14 and Ad21 prototype strains (Fig. 1). The patterns proved to be different from those of the Ad1 to Ad41 prototype strains (1), the Ad42 prototype strain (21), the Ad43 to Ad47 prototype strains (13), and to Ad47 and Ad48 prototype strains (16).

Earlier, strain Wan was examined by a novel PCR method for species identification of human Ads and was found to belong to subspecies B1 (15) (Table 2). Recently, the two extreme ends of the hexon gene, which together comprise 872 nucleotides, were sequenced and were compared with the same regions of all eight established species B prototype strains. In this analysis, the regions of strain Wan proved to be identical to those of the Ad21 prototype strain (subspecies B1) and different at a total of 84 to 95 nucleotide positions from the corresponding regions of the other seven prototype strains of species B (4). This is in line with the identical reactivities of strain Wan and Ad21 prototype strain in HI tests and the classification of strain Wan as subspecies B1 in the species-specific Ad PCR.

(ii) Species D candidate Ad51: strain Bom. Ad strain Bom was isolated at a hospital in Amsterdam from a stool sample taken in June 1989 from a 51-year-old male AIDS patient with fever, *Pneumocystis carinii* pneumonia, and diarrhea. The patient died in February 1991. This strain grew readily in GaBi cells and epithelial cell lines such as HEp-2 cells. It was genetically purified by passages at terminal dilution. In the VN assays, all of the 49 rabbit antisera to the Ad prototypes and

TABLE 2. Ad infections by species and serotype of non-HIV-infected patients from The Netherlands and HIV-infected patients from The Netherlands and Manchester, United Kingdom[a]

Species	Type	No. of infections		
		NL		UK, HIV
		Non	HIV	
A	12	23		
	18	2		
	31	58	2	1
B1	3	230		1
	7	345		
	16	2		
	21	16	1	
	50		1	
B2	11	13	11	
	14	1		
	34		1	
	35		1	1
C	1	383	2	
	2	521	1	3
	5	204		
	6	11		
D	8	57		1
	9	2	6	2
	10	1	2	
	13	1	1	
	15	6	1	
	17	4	2	6
	19	7	1	6
	20		8	1
	22		9	
	23		5	2
	24			
	25	3	2	
	26	2	6	2
	27	2	5	1
	28	2	1	
	29		2	
	30			
	32	1	2	
	33		1	
	36			
	37	9	3	
	38		1	
	39	1	1	1
	42		2	
	43		7	1
	44		10	1
	45		6	
	46	1	3	1
	47		3	3
	48		14	3
	49	2	10	1
	51		3	
	NT	7	4	1
E	4	54	2	1
F	40	57		
	41	271		
?	NT	2		
Total		2,301	143	40

[a] Each double and triple infection is entered separately. Abbreviations: NL, The Netherlands; UK, United Kingdom; Non, not HIV infected; HIV, infected with HIV; NT, nontypeable; blank entries, no Ad infections.

the rabbit antiserum to strain Wan reacted with strain Bom to titers lower than 0.01 the homologous titers (data not shown). The same was true for rabbit antiserum to strain Bom when titrated against the 49 prototype strains and strain Wan. The CDC reference horse antisera to 29 prototype strains of species D, i.e., all species D prototype strains except the Ad48 and Ad49 prototype strains, were also titrated against strain Bom. The most reactive of these antisera were those against Ad33, Ad43, and Ad44, which showed 32- to 64-fold lower titers to strain Bom than to the corresponding homologous viruses (Table 5). The recently proposed serotypes Ad48 and Ad49 did not show any cross-reactivity with strain Bom. Together, these results demonstrate the uniqueness of strain Bom in the VN tests and its position as a candidate new prototype of species D.

Strain Bom agglutinated erythrocytes from rats. According to the differential HA subgrouping scheme of Hierholzer (9, 11), this strain could be classified as HA subgroup 2E. In the HI tests, strain Bom did not react with antisera to the Ad serotypes of species D except with antisera to the Ad43 prototype strain and weakly with antisera to the Ad27, Ad44, Ad48, and Ad49 prototype strains (Table 6). Horse anti-Ad43 serum reacted with strain Bom to roughly the same HI titer as to Ad43 strain 1373; the Ad43 prototype strain (strain 1309) did not hemagglutinate satisfactorily in our hands and thus could not be used in the HI tests. Conversely, antiserum to strain Bom reacted in HI assays only with Ad43 strain 1373 and Ad49 strain Sak (but not with the Ad49 prototype strain itself) and weakly with the Ad27, Ad44, and Ad48 prototype strains. Apparently, strain Bom shares its fiber antigen with Ad43 strain 1373 and Ad49 strain Sak.

By DREA with restriction enzyme *Sma*I, strain Bom produced 15 bands (Fig. 2), confirming its allocation to species D (19). With five restriction enzymes, DREA patterns were determined for strains Bom, Ben (Ad48), and Kam (Ad49), the Ad48 prototype strain, and the Ad49 prototype strain (Fig. 2). The DREA patterns of strain Bom proved different from those of the Ad1 to Ad41 prototype strains (1), the Ad42 prototype strain (21), the Ad43 to Ad47 prototype strains (13), and the Ad47 and Ad48 prototype strains (16).

(iii) Subspecies B1: Ad21 variant strain Wek. Ad strain Wek could not be typed in the VN assays with rabbit antisera to all 49 serotypes or horse antisera to the subspecies B1 prototype strains. However, rabbit antisera to strain Wek did neutralize the Ad21 prototype strain to a titer that was 40 times higher than the VN titer to strain Wek itself (Table 3). Apparently, strain Wek is an Ad21 strain with a low avidity for VN antibodies, but with a VN antibody-inducing capacity similar to that of the Ad21 prototype strain. Strain Wek did not react with horse antiserum to the Ad21 prototype strain. This was not unexpected, however, because the strength of this antiserum did not allow the detection of titers more than 32-fold lower than the homologous titer. In the HI tests, strain Wek could not be distinguished from the Ad21 prototype strain (Table 4).

(iv) Species D: Ben-like strains. In the VN tests, a group of 17 closely related strains was identified as Ad48, although strain Ben, which is representative of these viruses, differed significantly from the Ad48 prototype strain in these assays. Antiserum to strain Ben, however, showed a fivefold higher VN titer to the Ad48 prototype strain than to the homologous virus (Table 5). Apparently, strain Ben is a less "avid" variant of Ad48. In the HI tests, strain Ben reacted like the Ad27 prototype strain (Table 6).

(v) Species D: Kam-like strains. Another group of deviant strains comprised 22 viruses. We identified the strains of this group as Ad49. The representative strain, Kam, was isolated

TABLE 3. VN assays with subspecies B1 candidate Ad50 strain Wan and other species B virus strains

Virus strain	VN titers of antisera to the following Ad types (source)[a]:										
	Ad50 P (R, RIVM)	Ad3 P (G, RIVM)	Ad7 P (G, RIVM)	Ad11 P (R, CDC)	Ad14 P (R, RIVM)	Ad16 P (R, RIVM)	Ad21			Ad34 P (R, RIVM)	Ad35 P (R, RIVM)
							P (R, RIVM)	P (H, CDC)	Wek (R, RIVM)		
Ad50 P (Wan)	20,480	<	<	<	128	≤16	32	<		16	16
Ad3 P	<	20,480	<	<	<	≤16	<			<	<
Ad7 P	<	32	**2,048**	<	<	≤16	<			<	<
Ad11 P	<	<	<	**20,480**	16	≤16	<			<	128
Ad14 P	<	<	<	<	**20,480**	≤16	<			<	<
Ad16 P	<	32	<	<	<	**40,960**	<			<	<
Ad21 P	32	<	<	64	<	≤16	**20,480**	**512**	20,480	<	<
Ad21 (Wek)	<	<	<	<	<	≤16	32	<	**512**	<	<
Ad34 P	<	<	<	<	<	≤16	<			**20,480**	32
Ad35 P	<	<	<	<	<	≤16	<			<32	**4,096**

[a] R, rabbit antiserum; G, goat antiserum; H, horse antiserum; P, prototype strain; <, <16; ≤16, antiserum was cytotoxic at dilutions up to 1:16; blank space, not done. Homologous titers are in boldface type.

from a fecal specimen of a 1-year-old boy with bronchopneumonia in Amsterdam in 1975. This strain belongs to Ad49 but deviated in the VN tests from the Ad49 prototype strain in one direction, showing a homologous titer-to-heterologous titer ratio of 20 when the Ad49 prototype strain was tested against Kam antiserum (Table 5). Since strain Kam did not hemagglutinate in our hands, strain Sak, which was closely related to strain Kam in the VN assays, was used in the HI assays. It reacted like the Ad43 prototype strain (Table 6).

(vi) Species D: Aus-like strains. Another potentially new serotype was represented by strain Aus. It was isolated from a stool sample of an HIV-infected Australian patient in 1991. Rabbit antiserum to the Ad44 prototype strain demonstrated an almost 100-fold lower VN titer to strain Aus than to the homologous strain, and rabbit antiserum to strain Aus differentiated the two viruses even by a ratio of 640. Horse antiserum from the CDC to the Ad44 prototype strain, however, showed only a fourfold lower VN titer to strain Aus than to the homologous strain (Table 5). Strain Aus was therefore classified as Ad44. It did not display HA activity in our tests.

DISCUSSION

This study confirms the results presented in earlier papers (10, 13, 14) reporting that most of the Ads infecting the gastrointestinal tracts of HIV-infected patients represent serotypes or intermediate serotypes of species D that are rarely isolated from clinical specimens of immunocompetent patients or of patients suffering from other kinds of immunodeficiencies. Typical AIDS-associated Ad serotypes include types 9, 17, 20, 22, 23, 26, 27, and 42 to 51 (Table 2). The cause of this predilection is not known. It has been suggested that the long-term infection characteristic for AIDS patients may provide the opportunity for mutations to occur within a strain or for recombinational events between coinfecting serotypes to take place and could explain the unusual frequency and variety of deviating Ad strains in the gastrointestinal tracts of such individuals (10). Alternatively, these patients may be more likely to develop symptomatic Ad infection and may thus be more likely to be examined by virological analysis, although the pathogenic significance of Ad infections in AIDS patients is unclear and asymptomatic infection is not uncommon (10, 14).

Another explanation for the emergence of novel Ad serotypes from AIDS patients may be a more quantitative one. As is apparent from Table 2, even the "typical" AIDS-associated Ads are not strictly confined to this group of patients, nor has it been established that they circulate more frequently among such patients. In this regard, we note that data on the Ad serotype distributions in the guts of non-HIV-infected adults are scanty. At the general diagnostic department of RIVM, for instance, a total of 1,478 Ad strains were typed during the period from 1981 to 1992. Only seven of these strains were isolated from fecal specimens of non-HIV-infected patients ages 25 to 59 years. Perhaps, therefore, the "new" Ads have just come to light since stool samples from that particular age group are now examined more frequently because AIDS patients frequently suffer from intestinal disorders of various etiologies. Possibly, nucleotide sequence analysis can produce evidence for and against these various hypotheses (2).

Some typing problems were encountered during the present

TABLE 4. HI assays with subspecies B1 candidate Ad50 strain Wan and other species B virus strains

Virus strain	HI titers of antisera to the following Ad types (source)[a]:								
	Ad50 P (R, RIVM)	Ad3 P (R, RIVM)	Ad7a P (R, RIVM)	Ad11 P (R, CDC)	Ad14 P (R, RIVM)	Ad16 P (R, RIVM)	Ad21 P (R, RIVM)	Ad34 P (R, RIVM)	Ad35 (R, RIVM)
Ad50 P (Wan)	**320**	<	<	<	<	<	1,280	40	<
Ad3 P	<	**1,280**	<	<	<	<	<	<	<
Ad7a P	<	20	**1,280**	160	80	<	20	<	<
Ad11 P	<	<	80	**2,560**	320	<	20	<	<
Ad14 P	20	<	640	160	**10,240**	<	20	20	80
Ad16 P	<	<	<	<	<	**640**	20	<	<
Ad21 P	80	<	<	<	<	<	**640**	<	<
Ad21 (Wek)	320		20	<	<	<	640	<80	<40
Ad34 P	160	<	<	<	<	<	320	**640**	160
Ad35 P	160	<	<	<	<	<	640	640	**320**

[a] R, rabbit antiserum; P, prototype strain; <, <20; blank space, not done. Homologous titers are in boldface type.

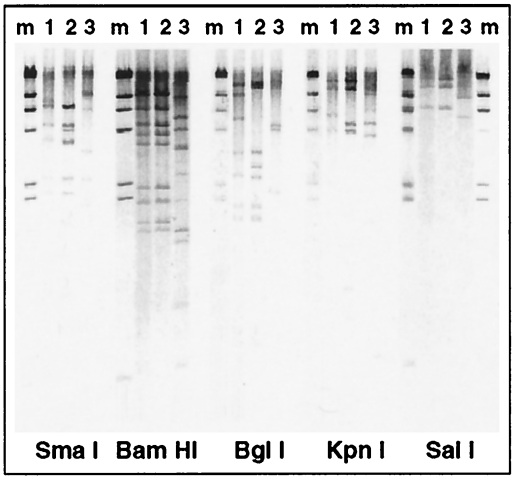

FIG. 1. DREA of strain Wan (Ad50) and some related strains of species B. Lanes of electrophoresis gels: m, molecular weight markers; 1, Ad50 prototype strain Wan; 2, Ad21 prototype strain; 3, Ad14 prototype strain.

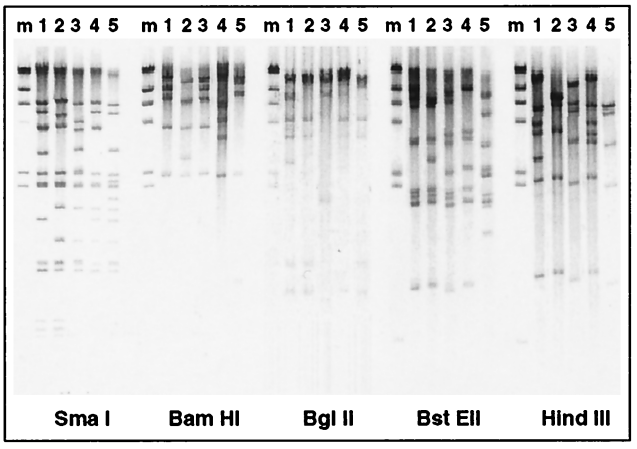

FIG. 2. DREA of strain Bom (Ad51) and some related strains of species D. Lanes of electrophoresis gels: m, molecular weight markers; 1, Ad51 prototype strain Bom; 2, Ad48 prototype; 3, strain Ben (Ad48); 4, Ad49 prototype; 5, strain Kam (Ad49).

study. A few arose from the phenomenon that in VN assays horse anti-Ad antisera are generally less discriminatory than rabbit antisera. For example, strain Aus of species D was a novel serotype in the VN tests with rabbit antisera, while it belonged to Ad44 in the VN assays with reference horse antisera from CDC (Table 5). The definition of Ad serotype does not specify the animal species in which the antisera to be used in the VN assays should be prepared (see the introduction). There are arguments, therefore, to call strain Aus a new serotype. To avoid confusion, however, we think that it is sensible to create a new serotype only when it proves to be novel in VN assays with both kinds of antisera. The practical problem is that antisera for routine Ad typing are usually prepared in rabbits, whereas the widely used CDC reference antisera, instrumental for the recognition of new serotypes, were prepared in horses. In routine typing work, therefore, Ad strains like Aus will remain untypeable.

Another kind of typing problem was presented by Ad21 strain Wek. For unknown reasons, strain Wek escaped significant neutralization by anti-Ad21 antiserum. It was recognized as belonging to serotype 21 only with the aid of rabbit antiserum to strain Wek (Table 3), which is not available in routine typing laboratories.

On the basis of their uniqueness in the VN assays, we propose that strains Wan and Bom be considered prototypes of Ad50 and Ad51, respectively. Their weak cross-reactivities in the VN and HI assays with members of species B and D, respectively, their capacity to agglutinate rat and monkey erythrocytes, and the number of bands produced with the restriction enzyme *Sma*I confirm that they belong to species B and D, respectively. Molecular evidence showed that strain Wan belongs to subspecies B1 and is closely related to the Ad21 prototype strain of this subspecies.

It is striking that the last nine new Ad serotypes were identified in HIV-infected subjects. One might speculate that still more new Ad types will be detected among these immunocompromised individuals in the future. PCR-based methods may be helpful for tracking down such viruses, especially when they would not be readily growing, like the fastidious serotypes Ad40 and Ad41 (6), or even not cultivable at all. In the latter case, VN assays are not possible and an alternative taxonomic concept based on sequence data must be developed to express the uniqueness of such viruses. For cultivable Ads, the current type definition seems appropriate from a medical point of view. Immunity to Ads appears to be closely linked to the presence of neutralizing serum antibodies, and the application

TABLE 5. VN assays with species D candidate Ad51 strain Bom and other species D virus strains

Virus strain	VN titers of antisera to the following Ad types (source)[a]:											
	Ad51 P Bom (R, RIVM)	Ad33 P		Ad43 P		Ad44			Ad48		Ad49	
		R, RIVM	H, CDC	R, RIVM	H, CDC	P (R, RIVM)	P (H, CDC)	Aus (R, RIVM)	P (R, RIVM)	Ben (R, RIVM)	P (R, RIVM)	Kam (R, RIVM)
Ad51 P (Bom)	**20,480**	<	32	<	32	<	128		<	<	<	<
Ad33 P	<	**2,048**	**1,024**	<	≤16	<		<	<	<	<	<
Ad43 P	<	<	16	**8,192**	**1,024**	<		<	<	<	<	<
Ad44 P	<	16	<	<	≤16	**20,480**	**8,192**	32	96	<	<	<
Ad44 (Aus)			<		32	256	2,048	**20,480**				
Ad48 P	<	<	<	<	≤16	1,024	256	<	**4,096**	20,480	<	<
Ad48 (Ben)	<	<	<	<	32	64	≤16		8,192	**4,096**	<	<
Ad49 P	<	<	128	<	≤16	<		<	<	<	**10,240**	2,048
Ad49 (Kam)	<	<	64	<	32	<	256		<	<	10,240	**40,960**

[a] R, rabbit antiserum; H, horse antiserum; P, prototype strain; <, <16; ≤16, antiserum was cytotoxic at dilutions up to 1:16; blank space, not done. Homologous titers are in boldface type.

TABLE 6. HI assays with species D candidate Ad51 strain Bom and other species D virus strains

Virus strain	HI titers of antisera to the following Ad types (source)[a]:								
	Ad51 P Bom (R, RIVM)	Ad27 P		Ad43 P (H, CDC)	Ad44 P (R, RIVM)	Ad48 P (R, RIVM)	Ad48 Ben (R, RIVM)	Ad49 P (R, RIVM)	Ad49 Kam (R, RIVM)
		R, RIVM	H, CDC						
Ad51 P (Bom)	**20,480**	40	80	640	80	20	20	80	<
Ad27 P	20	**10,240**	**10,240**	40	80	160	20,480	<	80
Ad43 (1373)[b]	20,480	80	160	1,280	160	160		640	
Ad44 P	40	40			**5,120**	2,560	20	80	<
Ad48 P	20	80			2,560	**1,280**	40	80	<
Ad48 (Ben)	20	20,480	160	320	80	<	**20,480**	<	40
Ad49 P	<	20			<	<	<	**320**	<
Ad49 (Sak)[b]	10,240	20	80	640	80	40	40	80	20

[a] R, rabbit antiserum; H, horse antiserum; P, prototype strain; <, <20; blank space, not done. Homologous titers are in boldface type.
[b] Strains Ad43 P and Ad49 (Kam) did not hemagglutinate and were replaced by strains 1373 and Sak, respectively, in the HI tests.

of gene therapy with Ad vectors may be seriously hindered by such antibodies.

ACKNOWLEDGMENTS

We thank M. J. Cardosa and D. Perera for allowing us to use their nucleotide sequence data for Ad species B, A. S. Bailey for providing the AIDS-associated Ad strains from Manchester, and D. Sanders for retrieving data for Ad isolates obtained at RIVM from the data files of the Institute.

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