Growth of fastidious adenovirus serotype 40 in HRT 18 cells: Interactions with E1A and E1B deletion mutants of subgenus C adenoviruses

S. A. Gomes1, 2, C. Niel2, *, and J. C. D’Halluin2

1 Departamento de Virologia, Instituto Oswaldo Cruz, Rio de Janeiro, Brasil
2 Laboratoire de Virologie Moléculaire, INSERM U.233, Lille, France

Accepted October 4, 1991

Summary. Growth of fastidious adenovirus serotype 40 (Ad 40) in several cell lines was investigated. Ad 40 was able to readily propagate in human intestinal cell line, HRT 18. Coinfection assays were made in non-permissive and permissive cells between Ad 40 and Ad 5dl 312 or dl 1520, mutants deleted in E1A and E1B regions, respectively, to test the ability of Ad 40 to complement these mutants and vice versa. Ad 40 could enhance Ad 5dl 312 DNA synthesis in HRT 18 and HeLa cells, although its own DNA disappeared in the presence of this mutant in HRT 18 cells. In coinfection with dl 1520, Ad 40 DNA synthesis was inhibited by dl 1520 in HRT 18 cells and dl 1520 DNA synthesis was inhibited by Ad 40 in 293 cells. This might reflect the presence of unusual products encoded by Ad 40 E1B region.

Introduction

Adenoviruses are important agents of infantile viral gastroenteritis and serotypes 40 and 41 (Ad 40 and Ad 41) are responsible for a large majority of cases [4]. In spite of this pathogenicity, Ad 40 and Ad 41 have been discovered later than most of the serotypes due to their difficulty to be propagated in vitro. Contrary to conventional human adenoviruses, the so-called fastidious adenoviruses could not be serially passaged in primary cells such as human embryonic kidney cells (HEK) or human diploid fibroblasts [1]. However, successful growth of Ad 41 in primary cells in defined conditions has been recently reported [24]. Ad 40 and Ad 41 can replicate with variable success in human cell lines such as HeLa, Hep-2, KB and A 534 cells [7, 23, 25, 37].

Replication of fastidious adenoviruses in human cell lines is thought to be

* Present address: CNRS URA 1160, Institut Pasteur, Lille, France.
blocked at an early stage in their growth cycle [29] and these viruses have been grown to some extent in the Ad 5-transformed human embryonic kidney cell line 293 [16, 28], which contains and expresses the Ad 5 early region E 1 [12]. This has led to the hypothesis that these viruses were defective in early regions E 1 A and E 1 B or in one of them and could be complemented by early gene functions of Ad 5 integrated in the genome of these cells [28, 19].

The Ad 2 E 1 A region gives rise to two major mRNAs (12 S and 13 S). The polypeptides encoded by these mRNAs are identical except for an internal domain of 46 amino acids within the largest product due to differential splicing of RNAs. Adenovirus mutants in E 1 A region that express only the 13 S gene product, replicate normally, are capable of trans-activating viral gene transcription and can immortalize primary rodent cells. Adenovirus mutants expressing only the 12 S gene product do not replicate in HeLa cells at low multiplicity of infection (for review see [3, 11]).

Ad 2 E 1 B region governs the synthesis at early times of two major polypeptides. A 22 S mRNA encodes a 19 k and a 55 k polypeptide. A 13 S mRNA encodes only the 19 k polypeptide. The 55 k polypeptide is essential for a complete lytic cycle in HeLa cells and is involved in accumulation of viral mRNA during productive infection. E 1 B mutants in 55 k polypeptide are altered in expression of early mRNAs and in DNA synthesis. Mutants in 19 k cause a rapid cytopathic effect and induce degradation of host and viral DNAs [9, 27, 36].

The nucleotide sequence of early regions E 1 A and E 1 B of fastidious adenoviruses has been determined [14, 34]. There is no major difference in the structure of E 1 A and E 1 B regions of nonfastidious and fastidious adenoviruses.

An increase of the replication of fastidious adenoviruses when cultivated in the presence of nonfastidious adenoviruses has been observed. Tiemessen and Kidd [30, 31] determined that Ad 2 could complement the growth of Ad 41 in Chang conjunctival and HEF cells by measuring the rate of late antigens with monoclonal antibodies specific to Ad 41.

For Ad 40, Mautner et al. [19] found that Ad 40 could be complemented in HeLa cells by E 1 B 55 K protein of nonfastidious adenoviruses.

In this study, we investigated the possible helper function of early regions E 1 A or E 1 B of nonfastidious adenoviruses in complementing DNA replication of Ad 40. We show that Ad 40 E 1 A region is functional. Coinfection assays of Ad 40 with a nonfastidious adenovirus deleted in E 1 B region (dl 1520) led to an inhibition of the synthesis of both DNAs.

**Materials and methods**

**Cells**

Human cell lines used were 293, HRT 18, an intestinal cell line [17] and HeLa. Monolayer cultures from these cell lines were grown in Dulbecco's medium containing 10% foetal bovine serum and divided twice a week at an appropriate subculture ratio.
**Viruses**

Ad 5 dl 312 is a deletion mutant lacking E1A activity [15]. dl 1520 is a hybrid Ad 2/Ad 5 E1B mutant which does not synthesize E1B 55K protein and shows a reduced rate of E1B 19K protein synthesis [2].

Stocks of dl 1520 and Ad 5 dl 312 were prepared in 293 cells, stock of Ad 2 WT (prototype strain provided by Dr. J. F. Williams, Pittsburg, Pa.) was made in HeLa cells. These viruses were purified as previously described [10]. Stock of Ad 40 (prototype strain Dugan) was obtained in HRT 18 cells after 10 passages of the virus in this cell line. Stock of Ad 40 was maintained as a crude extract after freezing and thawing of cell cultures three times.

Titers of Ad 40, dl 1520 and Ad 5 dl 312 stocks on 293, HRT 18 and HeLa cells, were determined at 48 h post-infection, by a fluorescent focus assay as previously described [10], using as first antibody a rabbit polyclonal anti-Ad 2 raised against purified virions and as a second antibody, fluorescein conjugated sheep anti-rabbit immunoglobulin. Titers were expressed as fluorescent focus units per ml (FFU/ml).

**Enzyme linked immunosorbent assay (ELISA)**

Ad 40 infected cultures were freeze and thawed three times then serially diluted and tested for adenovirus antigens by ELISA as previously described [22].

**Culture conditions for infection and coinfection assays**

Cell monolayers of 293, HRT 18 or HeLa grown in 25cm² flasks were single or double infected with virus stocks. The input virus concentration, expressed in FFU/cell, was variable. Virus inocula were adsorbed for 2h at 37°C. After this time, medium containing 2% foetal bovine serum was added. Each infected culture was incubated at 37°C until total cytopathic effect (CPE). In cases of absence of CPE, infected cells were recovered after 8 days.

**Extraction and digestion of viral DNAs**

Viral DNAs were extracted from 5 x 10⁶ infected cells using the Hirt [13] procedure modified by Wadell and de Jong [35]. Half of the extracted DNA was digested by Hind III restriction endonuclease and electrophoresed in a 0.8% agarose horizontal slab gel in Tris-Borate buffer at 50 V. Gels were stained in ethidium bromide and photographed under short UV light.

**Isolation of RNA**

Total RNA was prepared from mock-infected and from Ad 40 infected HRT 18 cells at 24 h post-infection by the acid guanidinium thiocyanate extraction method [6].

**cDNA synthesis and PCR amplification**

cDNA synthesis was performed using random primers (Gene Amp RNA PCR kit, Perkin Elmer Cetus). PCR amplification was carried out with oligodeoxynucleotides representing nucleotides 2485 to 2506 (5' end) and the complementary sequence from nucleotides 3018 to 3039 (3' end) of the Ad 40 sequence [34]. These primers were purchased from Eurogentec S. A. and purified by HPLC chromatography. The amplification reaction was performed with Taq DNA polymerase (Promega) in 40 cycles of DNA denaturation (94°C, 1 min), annealing (55°C, 1 min) and elongation (72°C, 3 min). Amplified DNAs were electrophoresed in a 2% agarose gel.
Results

Growth of Ad40, Ad5dl312, and dl1520 in 293, HRT 18, and HeLa cells

Human cell lines 293, HRT 18, and HeLa were tested for their capability to support growth of Ad40. HRT 18 is a cell line derived from human rectal adenocarcinoma [32] and was chosen because it is permissive to bovine enteric fastidious coronavirus [17]. To estimate virus growth in each cell line, the rate of Ad antigens in cultures was measured after three serial passages by ELISA. In 293 and HeLa cells, antigen levels were very low and the virus was not readily propagated. HRT 18 cell line gave a higher level of Ad40 antigens, although Ad 40 grew more slowly than nonfastidious adenoviruses. Total cytopathic effect was observed after 6–7 days post-infection. A reference stock of the virus was then prepared in this cell line after several additional passages.

Reference stocks of mutants Ad 5 dl 312 [15] and dl 1520 [2] showing large deletions in transcription units E 1 A and E 1 B, respectively, were prepared in 293 cells.

Each reference stock was titrated on 293, HRT 18, and HeLa cell lines. Results are shown in Table 1. The titer of Ad 5 dl 312 mutant of HeLa cells was about 10²-fold lower than on 293 cells. This was in agreement with the results published by Jones and Shenk [15]. The titers of Ad 5 dl 312 mutant in HRT 18 and HeLa cells were of the same order of magnitude. The titer of E 1 B mutant dl 1520 was 6-fold and 100 fold lower on HRT 18 and HeLa cells, respectively, than on 293 cells. For Ad 40, the difference between the titers obtained in the three cell lines during the first passage was less significant. Ad 40 behaved neither as an adenovirus mutated in E 1 A 13 S mRNA nor as an E 1 B mutant lacking 55 k protein.

Ad40 can complement Ad5 dl312 growth defect

To determine if a complementation could occur between Ad 40 and Ad 5 dl 312 or dl 1520, coinfection assays with Ad 40 and deletion mutants were carried out. The synthesis of viral DNAs in infection and coinfection assays was vis-

Table 1. Titers of Ad 40, Ad 5 dl 312 and dl 1520 reference stocks on 293, HRT 18 and HeLa cell lines

| Viruses  | Cells  | HRT 18  | HeLa    |
|----------|--------|---------|---------|
|          | 293    | 1.4 x 10⁶ | 2.4 x 10⁵ |
| Ad 40    | 7.0 x 10⁵ |         |         |
| Ad 5 dl 312 | 6.4 x 10¹¹ | 1.7 x 10⁷ | 7.0 x 10⁶ |
| dl 1520  | 3.5 x 10¹² | 2.1 x 10¹¹ | 3.5 x 10¹⁰ |

Viruses were titrated at 48 h post-infection by fluorescent focus assay [10]. Titers are expressed in focus forming units per ml (FFU/ml)
Ad 40 growth in HRT 18 cells

The cells were first single infected at a multiplicity of infection (m.o.i.) of 1 FFU/cell. This m.o.i. was calculated from reference stocks titrated in the most permissive cells, i.e., HRT 18 for Ad 40, 293 for the mutants. At this m.o.i., a clear restriction pattern of Hirt extracted DNAs was seen only with the most permissive cell line (Fig. 1).

Coinfection assays of Ad 40 and deletion mutants were performed in HRT 18, 293 and HeLa cells. Figure 2 shows DNAs extracted from cells coinfected with Ad 40 and Ad 5 dl 312. The m.o.i. of Ad 40 (titrated on HRT 18) was always 1 FFU/cell. The input concentration of Ad 5 dl 312, titrated on 293 cells, varies

Fig. 1. Hind III restriction pattern of Hirt extracted DNAs of Ad 5 dl 312, dl 1520 and Ad 40. Viruses were infected at a m.o.i. of 1 FFU/cell in permissive cell lines. 1 Ad 5 DNA (marker), 2 Ad 5 dl 312 (293 cells), 3 dl 1520 (293 cells), 4 Ad 40 (HRT 18 cells)

Fig. 2. Coinfection of deletion mutant Ad 5 dl 312 and Ad 40 analyzed by Hind III digestion of Hirt extracted DNAs. Coinfection was performed in HRT 18 (A, B) and HeLa (C) cells. The m.o.i. of Ad 40, titrated on HRT 18 cells, was 1 FFU/cell in all cases. The m.o.i. of Ad 5 dl 312, titrated on 293 cells, varies from 1 FFU/cell (A) to 10,000 FFU/cell (B and C). 1 Ad 5 dl 312, 2 Ad 40 + Ad 5 dl 312, 3 Ad 40
from 1 FFU/cell (Fig. 2 A) to 10,000 FFU/cell (Fig. 2 B and C). After infection of 1 FFU/cell of Ad 5 dl 312 in HRT 18 cells, the restriction pattern of this virus was not visible (Fig. 2 A, lane 1). Restriction patterns obtained in Ad 40 single infection (Fig. 2 A, lane 3) and in coinfection Ad 40 + Ad 5 dl 312 (Fig. 2 A, lane 2) were identical. At low m.o.i., no effect of one virus on DNA viral synthesis of the other virus was therefore observed.

With 10,000 FFU/cell of Ad 5 dl 312 in HRT 18, the restriction pattern of Ad 5 dl 312 was visible in single infection (Fig. 2 B, lane 1). Although the cells were washed before the viral DNA extraction, it could not be ruled out that the inoculum could be responsible for visible restriction pattern. As a control, a similar experiment was performed in which viral DNA extraction was made 1 h post infection. In this case, no visible band was seen after digestion with restriction enzyme. In co-infection assays, the restriction pattern of Ad 5 dl 312 was enhanced but Ad 40 DNA was absent (Fig. 2 B, lane 2). With 10,000 FFU/cell of Ad 5 dl 312 in HeLa cells, the same phenomenon of enhancement of Ad 5 dl 312 DNA was observed by co-infection assays (Fig. 2 C, lane 2) although the restriction pattern of Ad 40 was not visible in single infection (Fig. 2 C, lane 3).

These experiments indicated that Ad 40 could complement E1A functions from deletion mutant Ad 5 dl 312 in HRT 18 and HeLa cells, and that this complementation was dependent on the input concentration of Ad 5 dl 312. At high m.o.i. of Ad 5 dl 312, Ad 40 was able to stimulate the synthesis of Ad 5 dl 312 DNA although its own DNA disappeared in co-infection assays. In HeLa cells the restriction pattern of Ad 40 could not be visualized, but a similar enhancement of the synthesis of Ad 5 dl 312 DNA was observed when the two viruses were present.

**Interactions between Ad 40 and dl 1520**

To determine if Ad 40 could also stimulate the growth of a mutant deleted in the E1B region, co-infection assays of Ad 40 with dl 1520 were performed. The m.o.i. of Ad 40 and dl 1520 was 1 FFU/cell. A clear restriction pattern of dl 1520 was visible when the virus was infected in 293 cells (Fig. 3 A, lane 1). The bands visible on Fig. 3 B, lane 1 (dl 1520 grown in HRT 18 cells) result from leakage of this mutant (see Table 1). For Ad 40, a clear restriction pattern was observed in HRT 18 (Fig. 3 B, lane 3). In co-infection assays with dl 1520 and Ad 40 both DNAs disappeared in 293 and HRT 18 cells (Fig. 3 A and B, lanes 2).

In a similar manner, co-infection assays with Ad 2 and dl 1520 were performed in HRT 18 cells to determine if dl 1520 was able to inhibit DNA synthesis of nonfastidious adenovirus or if this inhibition was specific of fastidious Ad 40. Results of this assay are shown in Fig. 4, lane 2. No inhibition of Ad 2 DNA by dl 1520 was observed. A faint band of DNA visible under Hind III-B band of Ad 2 revealed the presence of dl 1520 DNA. As expected, this virus was therefore complemented by Ad 2.
Fig. 3. Coinfection of deletion mutant dl1520 and Ad 40 analyzed by Hind III digestion of Hirt extracted DNAs. Coinfections were performed in 293 (A) and HRT 18 (B) cells. The m.o.i. of dl1520 and Ad 40, titrated on 293 and HRT 18, respectively, was 1 FFU/cell. 1 dl1520, 2 Ad 40 + dl1520, 3 Ad 40

Fig. 4. Coinfection of deletion mutant dl 1520 and Ad 2 in HRT 18 cells analyzed by Hind III digestion of Hirt extracted DNAs. The m.o.i. of both viruses was 1 FFU/cell. The arrow indicates a specific dl1520 restriction fragment in double infection. 1 dl1520, 2 dl 1520 + Ad 2, 3 Ad 2

Amplification of cDNA from E1 B region of Ad 40

In order to determine of Ad 40 E1 B region could transcribe a mRNA coding for 55 k protein, cDNA PCR amplification was performed from RNA extracted from Ad 40 infected HRT 18 cells. Primers were chosen based on sequence data of van Loon et al. [34] who have found an open reading frame for 55 k protein in nucleotide positions 1719 to 3147. Primers corresponding to C-terminal domain of this protein (nt 2485 to 2506 and nt 3018 to 3039) were used. Figure 5 shows that a fragment of predicted size (522 bp) was present in Hirt extracted Ad 40 DNA (lane 2). A fragment of identical size was observed from amplified cDNA from total RNA extracted from Ad 40 HRT 18 infected cells (Fig. 5,
Fig. 5. PCR amplification of a fragment of Ad 40 E1B region. 1 Molecular weight standard, the sizes of fragments are indicated in bp on the left, 2 Hirt extracted Ad40 DNA from HRT18 cells, 3 cDNA amplification of Ad40 infected HRT18 cells, 4 PCR amplification from RNA of Ad40 infected HRT18 cells (without previous cDNA synthesis), 5 cDNA amplification of mock-infected HRT18 cells

Discussion

In the present study, we were able to cultivate Ad40 in a human intestinal cell line, HRT18, which has not been transformed in vitro by adenovirus genes. From an Ad40 stock obtained in HRT18 cells, there was a modest difference for antigen production during the first passage between HRT18, HeLa and 293 cell lines (Table 1). However, the yield of Ad40 during subsequent passages was constant for HRT18 cells whereas it decreases for HeLa and 293 cells. The ability of Ad40 to grow in HRT18 cells showed that the presence of adenovirus genes integrated in the DNA of the host cell is not an absolute requirement to
allow growth of this serotype. The capability of cultivating Ad40 in an intestinal cell line is in agreement with results from Kidd and Madeley [16] who cultivated Ad40 in foetal intestinal organ cultures. Ad40 grows very well in its target tissue, the intestinal epithelium and it has been detected in large amounts in stool of children with gastroenteritis [4, 5, 21, 33]. It is likely that some cellular factors from intestinal cells can promote the lytic cycle of Ad40 as suggested by Tiemessen and Kidd [30]. However, even in HRT18 cells, Ad40 grows more slowly than nonfastidious adenoviruses and total cytopathic effect was observed only after 6–7 days post-infection. Attempts to propagate Ad40 in HRT18 using a high m.o.i. were unsuccessful (results not shown). This was consistent with results previously reported showing a similar Ad40 growth inhibition at high m.o.i. in WK, A549 and 293 cells [37].

To test the hypothesis that E1A or E1B regions of Ad40 were defective, complementation assays were made between Ad40 and E1A deletion mutant Ad5dl312, and between Ad40 and E1B deletion mutant dl1520. The DNAs of both coinfecting viruses were visualized after Hirt extraction and restriction enzyme digestion.

In HRT18 cells, Ad40 could not complement Ad5dl312 infected at low m.o.i. At high m.o.i. of Ad5dl312, the amount of DNA of Ad5dl312 in double infection was enhanced but Ad40 DNA synthesis was inhibited (Fig. 2B, lane 2). This inhibition might be the result of a phenomenon of dominance of one serotype by another as studied by Delsert and D'Halluin [8]. However, results depending on m.o.i. values should be interpreted with caution. For example, 10,000 FFU/cell of Ad5dl312 titrated on 293, corresponds to 0.25 infectious units on HRT18 cells and to 0.1 on HeLa cells (Table 1). HeLa cells infected singly with high m.o.i. of Ad5dl312 supported moderate DNA synthesis (Fig. 2C, lane 1) and residual virus growth (Table 1). This is in agreement with previous results showing some leakage for this mutant [20]. In coinfection with Ad40, the level of Ad5dl312 DNA was augmented, although Ad40 DNA was not detected (Fig. 2C, lane 2). E1A functions of Ad40 are therefore capable of complementing Ad5dl312 in cells permissive (HRT18) and non-permissive (HeLa) to Ad40.

Mautner et al. [19] have detected by slot blot hybridization a complementation of Ad40 growth by Ad5dl312 and vice versa in HeLa cells. Due to relatively low yields of Ad40, these authors were not able to obtain visible restriction patterns of Hirt extracted DNA. We also used slot blot hybridization (results not shown) and found an amount of Ad40 DNA lower in cells coinfected by Ad5dl312 and Ad40 than in cells infected with Ad40 only. A possible explanation for this discrepancy is that the Ad40 reference stocks have not been obtained in the same cell line.

To know if Ad40 could complement a nonfastidious adenovirus mutated in E1B transcription unit, coinfection of Ad40 and dl1520 was performed. Surprisingly, this coinfec tion led to an inhibition of DNA synthesis of both viruses. As a control, cells were coinfected with dl1520 mutant and Ad2 wild
type. No inhibition of Ad 2 DNA synthesis was observed. Furthermore, dl 1520 was complemented by Ad 2 (Fig. 4, lane 2). We do not presently know the reasons of the mutual inhibition between Ad 40 and dl 1520. Interactions between two adenoviruses lacking E 1 B products should not lead to an inhibition of the DNA synthesis of both viruses. Several findings have suggested that Ad 40 E 1 B region is transcribed in an unusual manner [18, 26] although it is structurally similar to E 1 B region of nonfastidious adenoviruses [34]. The presence of unusual E 1 B proteins of Ad 40 associated with viral or cellular factors might explain the inhibition of dl 1520 DNA synthesis in coinfection experiments. Our PCR results show that at least one mRNA is synthesized in a region corresponding to the 55 k messenger of nonfastidious adenoviruses. To know why dl 1520 DNA synthesis is inhibited by Ad 40, further experiments are necessary to detect and characterize the Ad 40 E 1 B mRNAs and proteins.

Acknowledgements

We are grateful to Dr. H. G. Pereira and to C. Cousin for critical reading of the manuscript, to Drs. T. Shenk and A. Berk for providing us Ad 5 dl 312 and dl 1520, respectively, to Dr. T. H. Flewett for the gift of Ad 40, to N. Helbecque for oligonucleotide purification, and to D. Petite for cell culture assistance. S. A. Gomes is a recipient of a fellowship from CNPq (Brazil).

References

1. Albert MJ (1986) Enteric adenoviruses. Brief review. Arch Virol 88: 1–17
2. Barker DD, Berk AJ (1987) Adenovirus proteins from both E 1 B reading frames are required for transformation of rodent cells by viral infection and DNA transfection. Virology 156: 107–121
3. Berk A (1986) Adenovirus promoters and E 1 A transactivation. Annu Rev Genet 20: 45–79
4. Brandt CD, Kim HW, Rodriguez WJ, Arrobio JO, Jeffries BC, Stallings EP, Lewis C, Miles AJ, Gardner MK, Parrott RH (1985) Adenoviruses and pediatric gastroenteritis. J Infect Dis 151: 437–443
5. Brandt CD, Kim HW, Yolken RH, Kapikian AZ, Arrobio JO, Rodriguez WJ, Wyatt RG, Chanock RM, Parott RH (1979) Comparative epidemiology of two rotavirus serotypes and other viral agents associated with pediatric gastroenteritis. Am J Epidemiol 110: 243–254
6. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156–159
7. de Jong JC, Wigand R, Kidd AH, Wadell G, Kapsenberg JG, Muzerie CJ, Wermenbol AG, Firtzlaff RG (1983) Candidate adenoviruses 40 and 41: fastidious adenoviruses from human infant stool. J Med Virol 11: 215–131
8. Delsert C, D’Halluin JC (1984) Genetic expression of human adenoviruses in simian cells. Evidence for interserotypic inhibition of viral DNA synthesis. Virus Res 1: 365–380
9. D’Halluin JC, Cousin C, Niel C, Boulanger P (1984). Characterization of an early temperature-sensitive and cytocidal double mutant of adenovirus type 2. J Gen Virol 65: 1305–1317
10. D’Halluin JC, Martin GR, Torpier G, Boulanger P (1978) Adenovirus type 2 assembly analyzed by reversible cross-linking of labile intermediates. J Virol 26: 357–363
Ad 40 growth in HRT 18 cells

11. Flint J, Shenk T (1989) Adenovirus E1A protein paradigm viral transactivator. Annu Rev Genet 23: 141–161
12. Graham FL, Smiley J, Russell WC, Nairn R (1977) Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J Gen Virol 36: 59–72
13. Hirt B (1967) Selective extraction of polyoma DNA from infected mouse cell cultures. J Mol Biol 26: 365–369
14. Ishino M, Ohashi Y, Emoto T, Sawada Y, Fujinaga K (1988) Characterization of adenovirus type 40 E1 region. Virology 165: 95–102
15. Jones N, Shenk T (1979) Isolation of adenovirus type 5 host range deletion mutants defective for transformation of rat embryo cells. Cell 17: 683–689
16. Kidd AH, Madeley CR (1981) In vitro growth of some fastidious adenovirus from stool specimens. J Clin Pathol 34: 213–216
17. Laporte J, Bobulesco P, Rossi F (1980) Une lignée cellulaire particulièrement sensible à la réplication du coronavirus entéritique bovin. C R Acad Sci D: 625–623
18. Mautner V, Mackay N, Morris K (1990) Enteric adenovirus type 40: expression of E1B mRNA and proteins in permissive and nonpermissive cells. Virology 179: 129–138
19. Mautner V, Mackay N, Steinthorsdottir V (1989) Complementation of enteric adenovirus type 40 for lytic growth in tissue culture by E1B 55K function of adenovirus types 5 and 12. Virology 171: 619–622
20. Nevins J (1981) Mechanism of activation of early viral transcription by the adenovirus E1A gene product. Cell 26: 213–220
21. Niel C, Gomes SA, Leite JPG, Pereira HG (1986) Direct detection and differentiation of fastidious and nonfastidious adenoviruses in stools by using a specific nonradioactive probe. J Clin Microbiol 24: 785–789
22. Pereira HG, Azeredo RS, Leite JPG, Andrade ZP, De Castro L (1985) A combined enzyme immunoassay for rotavirus and adenovirus (EIARA). J Virol Methods 10: 21–28
23. Perron-Henry DM, Herrmann JE, Blacklow NR (1988) Isolation and propagation of enteric adenoviruses in HEP-2 cells. J Clin Microbiol 26: 1445–1447
24. Pieniazek D, Pieniazek NJ, Macejak D, Luftig RB (1990) Enteric adenovirus 41 requires low serum for growth in primary cells. Virology 178: 72–80
25. Pieniazek D, Pieniazek NJ, Macejak D, Coward J, Rayfield M, Luftig RB (1990) Differential growth of human enteric adenovirus 41 (TAK) in continuous cell lines. Virology 174: 239–249
26. Steinthorsdottir V, Mautner V (1991) Enteric adenovirus type 40: E1B transcription map and identification of novel E1A-E1B cotranscripts in lytically infected cells. Virology 181: 139–149
27. Stillman B (1986) Functions of adenovirus E1B tumour antigens. Cancer Surv 5: 389–403
28. Takiff HE, Straus SE, Garon CF (1981) Propagation and in vitro studies of previously non-cultivable enteral adenoviruses in 293 cells. Lancet ii: 832–834
29. Takiff HE, Straus SE (1982) Early replicative block prevents the efficient growth of fastidious diarrhea-associated adenoviruses in cell culture. J Med Virol 9: 93–100
30. Tiemessen CT, Kidd AH (1988) Helper function of adenovirus 2 for adenovirus 41 antigen synthesis in semi-permissive and non-permissive cells. Arch Virol 103: 207–218
31. Tiemessen CT, Kidd AH (1990) Adenovirus growth in semi-permissive cells shows multiple-hit kinetics. Arch Virol 110: 239–245
32. Tompkins WAF, Watrach AM, Schmale JD, Schultz RM, Harris JA (1974) Culture and antigenic properties of newly established cell strains derived from adenocarcinomas of human colon and rectum. J Nat Cancer Inst 52: 1101–1110
33. Unhoo I, Wadell G, Svensson L, Johansson ME (1984) Importance of enteric adenovirus 40 and 41 in acute gastroenteritis in infants and young children. J Clin Microbiol 20: 365-372
34. van Loon AE, Ligtenberg M, Reemst AMCB, Sussenbach JS, Rozijn TH (1987) Structure and organization of the left-terminal DNA regions of fastidious adenovirus types 40 and 41. Gene 58: 109-126
35. Wadell G, de Jong JC (1980) Restriction endonucleases in identification of a genome type adenovirus 19 associated with keratoconjunctivitis. Infect Immun 27: 292-296
36. White E, Grodzicker T, Stillman BW (1984) Mutation in the gene encoding the adenovirus E1 B 19K tumour antigen causes the degradation of chromosomal DNA. J Virol 52: 410-419
37. Witt DJ, Bousquet EB (1988) Comparison of enteric adenovirus infection in various human cell lines. J Virol Methods 20: 295-308

Authors' address: Dr. J. C. D'Halluin, Laboratoire de Virologie Moléculaire, INSERM U.233, 2 place de Verdun, F-59045 Lille Cedex, France.

Received April 30, 1991