Human adenovirus serotypes 4 and 11 show higher binding affinity and infectivity for endothelial and carcinoma cell lines than serotype 5

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Adenoviruses are promising vectors for human cancer gene therapy. However, the extensively used adenoviruses serotypes 2 and 5 (Ad2 and Ad5) from species C have a major disadvantage in being highly prevalent; thus, most adults have an immunity against the two viruses. Furthermore, the expression of coxsackievirus and adenovirus receptors for Ad2 and Ad5 varies in different cells. This study aims to identify adenovirus serotypes with specific tropism for endothelial cells and epithelial tumour cells. Comparison of the binding affinities of Ad31, Ad11, Ad5, Ad37, Ad4 and Ad41, belonging to species A–F, respectively, to established cell lines of hepatoma (HepG2), breast cancer (CAMA and MG7), prostatic cancer (DU145 and LNCaP) and laryngeal cancer (Hep2), as well as to endothelial cells (HMEC), was carried out by flow cytometric analysis. Ad11 from species B showed markedly higher binding affinity than Ad5 for the endothelial cell line and all carcinoma cell lines studied. Ad4 showed a specific binding affinity for hepatoma cells and laryngeal carcinoma cells. The ability of Ad11, Ad4 and Ad5 to be expressed in hepatoma, breast cancer and endothelial cell lines was studied by immunostaining and 35S-labelling of viral proteins in infected cells. Ad11 and Ad4 manifested a higher proportion of infected cells and a higher degree of hexon expression than Ad5.

INTRODUCTION

Adenoviruses are attractive gene delivery vectors for cancer gene therapy, since they can be made safe, are capable of infecting both dividing and non-dividing target cells and can be produced in high titres. The adenovirus vectors used extensively are based on serotypes 2 and 5 (Ad2 and Ad5) from species C (Russell, 2000). However, their non-specific targeting of tumour cells may be suboptimal and limit the usefulness of adenovirus vectors for cancer gene therapy (Kim et al., 2002). The high prevalence of immunity against Ad2 and Ad5 in the general population results in only short-term expression of a transgene and this has also limited their application in vivo (D’Ambrosio et al., 1982). There is an obvious need to develop a more efficient adenovirus vector.

The coxsackievirus and adenovirus receptor (CAR) is the primary attachment protein for serotypes from all human adenovirus species, with the exception of species B (Bergelson et al., 1997; Roelvink et al., 1998). CAR is a component of tight junctions and is thus not readily accessible on epithelial cell surfaces (Cohen et al., 2001). Additional cellular receptors for adenovirus have been identified. One is the major histocompatibility complex class I (MHC-I) z2, which was suggested to be the receptor for Ad5 on CAR-deficient cells (Hong et al., 1997). Another is sialic acid (z2,3) (Arnegberg et al., 2000), the receptor for Ad37 (species D). However, the uptake of recombinant Ad5 in some human tumour cell lines has been shown to be independent of MHC-I. This would argue against MHC-I being a primary receptor for Ad5 on the cells studied (Davison et al., 1999; McDonald et al., 1999). Heparan sulfate glycosaminoglycans expressed on the cell surface were reported to be co-receptors involved in the binding of Ad5 and Ad2 to host cells (Dechecchi et al., 2000) and the attachment of adenovirus fibre protein to the primary cellular receptor represents the critical initial determinant of virus tropism. However, different strategies for internalization of adenovirus virions into host cells may affect tissue tropism. Multiple adenoviruses, e.g. Ad2, Ad3, Ad4 and Ad12, use binding to cell surface z, integrins via the Arg–Gly–Asp (RGD) sequence exposed in the variable loop of the penton base for their internalization (Mathias et al., 1994; Wickham et al., 1993). However, the number of adenovirus primary receptors and integrins expressed on the surface of tumour cells is highly variable and may influence gene transfer and expression of Ad5- or Ad2-based vectors (Li et al., 1999a, b; Pearson et al., 1999).

To date, 51 human adenovirus serotypes belonging to six species, A–F, have been recognized. They show a wide range of tissue tropism and are associated with several clinical syndromes, such as respiratory, cardiac, gastrointestinal, ocular and urinary tract diseases. It would be useful to
explore the natural tropism of different adenovirus serotypes in order to identify alternative adenovirus vector candidates with high affinity for epithelial tumour cells. Our aim has been to identify adenovirus serotypes with specific tropism for endothelial cells and epithelial tumour cells. In this study, we screened the binding affinity of representatives from every species of human adenovirus for established cell lines of hepatoma, breast cancer, prostate cancer and laryngeal cancer and also a cell line of endothelial origin by flow cytometric analysis. A comparison of the ability of these adenoviruses to be expressed in hepatoma, breast cancer and endothelial cell lines was performed by immunostaining of viral structural proteins, 35S-labelling of proteins in infected cells and titration of the infectivity of the virus particles produced.

**METHODS**

**Viruses.** Ad31 (prototype strain 1315/63), Ad11 (prototype strain Slobitski), Ad5 (vector strain pFG140), Ad37 (prototype GW), Ad4 (prototype Ri-67) and Ad41 (prototype Tak) were used in this study as representatives of adenovirus species A–F, respectively. All were grown in A549 cells and virions were purified by equilibrium centrifugation in CsCl (Mei et al., 1998). Their identity was confirmed by restriction pattern analysis (Adrian et al., 1986).

**Cell lines and culture conditions.** Eight human cell lines were used in this study: A549, from human oat cell carcinoma of the lung; HepG2, established from hepatoblastoma; Hep2, from laryngeal carcinoma; LNCaP and DU145, both derived from metastatic prostate carcinoma; and MG7 and CAMA, both from breast carcinoma. HMEC was an immortalized human microvascular endothelial cell line (Ades et al., 1992a). A549, HepG2 and Hep2 cells were grown at 37 °C in DMEM containing 5 or 10% foetal calf serum, 0.1 mM non-essential amino acids, 2 mM glutamine and 2 mM methionine. Cells were subcultured every 3 days. LNCaP, DU145, CAMA and MG7 cells were all grown at 37 °C in RPMI 1640 containing 10% FCS, 20 mM HEPES, 0.75 g NaCO3 l−1, 100 IU penicillin G ml−1 and 100 μg streptomycin sulfate ml−1. These three cell lines were subcultured every 3 days. LNCaP, DU145, CAMA and MG7 cells were all grown at 37 °C in RPMI 1640 containing 10% FCS, 20 mM HEPES, 0.75 g NaCO3 l−1, antibiotics (as above), and 10−3 M methylthiuronilone (NEN Life Science) for LNCaP cells, 1 mM pyruvate and 2 mM glutamine for DU145 cells or 0.2 IE insulin ml−1 (Pharmacia Upjohn) for MG7 cells. HMEC cells were grown in endothelial basal medium MCDB131 containing 10% dialysed FCS, antibiotics (as above), 2 μM hydrocortisone, 5 ng human epidermal growth factor ml−1 (Roche) and 2 mM glutamine. These five cell lines were subcultured every 5–7 days. For all cell lines, the concentration of FCS was decreased to 2% after virus infection.

**Virus labelling.** Virions were desalted on a NAP-10 column (Pharmacia) in labelling buffer (50 mM NaHCO3, 2 mM MgCl2 and 135 mM NaCl, pH 8–8). Then, 100 μl N-hydroxysuccinimidobiotin (Sigma) in 1 mg DMSO ml−1 was added to 1 ml of the virions (1–5 mg ml−1). Virions were then mixed with biotin overnight by shaking at 4 °C in the dark. This solution was passed through the NAP-10 column equilibrated with PBS and free biotin was removed. The concentration of biotinylated virions was determined by spectroscopy. Glycerol was added to a concentration of 10% of the total volume and the virions were then aliquoted in small volumes and kept at −70 °C until use.

The extent of biotinylation of virions from the different serotypes was assayed by SDS-PAGE with silver staining and Western blot. The hexons of adenoviruses demonstrated a similar concentration both in silver-stained gels and in Western blot, where biotinylated hexons for each adenovirus species were detected by streptavidin–HRP and then ECL. Results indicated that measurements of viral protein and biotin bound were comparable and reliable for quantification of virus binding.

**Binding experiments by FACScan flow cytometry.** For each binding experiment, 1.25 × 106 or 2.5 × 106 cells were used. The cells were incubated with three different concentrations, 1, 3 and 6 μg per cell, of biotinylated Ad11, Ad5, Ad4, Ad31, Ad37 and Ad41 virions in PBS containing 2% FCS and 0.01% NaN3 (PBS/FCS/NaN3) buffer in a total volume of 100 μl at 4 °C and shaken for 30 min. The cells were washed once with 120 μl PBS/FCS/NaN3 buffer, followed by the addition of a 1:100 dilution of streptavidin–FITC (Dakopatts) in PBS/FCS/NaN3 buffer and shaken for another 30 min at 4 °C. Then the cells were washed once with the buffer described above and finally resuspended in 300 μl PBS/FCS/NaN3 buffer containing 1 μg propidium iodine ml−1 to exclude dead cells from FACS analysis. Cell samples were measured with a FACScan (Becton Dickinson) flow cytometer and then analysed using the LYSYSII software program (Becton Dickinson).

**Immunostaining procedures.** Cell lines HepG2, HMEC and MG7 (2 × 105 cells) were cultured in a 24-well plate (2 cm2 per well). Per cell, 1 × 104 physical particles of Ad11, Ad5 and Ad4 in 1 ml medium were adsorbed to the cells for 1 h at 37 °C on a shaking table, followed by rinsing twice with PBS. Then medium containing 2% FCS was added to the wells and the cells were further cultured. After 20, 40 and 72 h post-infection (p.i.), the medium was discarded and the cells were washed once with PBS and allowed to dry. The cells were fixed in 100% ice-cold methanol at 4 °C for 10 min, washed three times with PBS for 2 min, blocked with 0.2% BSA in PBS for 15 min at room temperature and incubated with 1:200 dilutions of hyperimmune virion-specific rabbit antiserum for 1 h at 37 °C. Type-specific hyperimmune anti-virion sera were titrated by ELISA. Titres ranged from 10−3 (Ad5) to 10−2 (Ad4) using 50 μg ml−1 of the monotypic virions as antigens in the ELISA. Hence, an excess of virion-specific antibodies was used for all virus types. The cells were then washed three times with PBS for 2 min each over a 15 min period and incubated for 30 min at 37 °C with the secondary antibody, an FITC-conjugated swine anti-rabbit IgG (Dakopatts) diluted 1:40 in PBS. The cells were washed again as described previously and covered with anti-fading solution (90% glycerol and 1% O-phenylenediamine in PBS) prior to storage. Stained cells were examined and the percentage of FITC-positive cells per well (2 × 105 cells) was calculated using a fluorescence microscope (ZEISS, Axiovert 25). Micrographs were taken at a magnification of × 200.

**35S-labelling of infected cell proteins.** HMEC, MG7, HepG2 and A549 cells (1·5 × 105) were infected with 2 pg per cell (corresponding to 7·2 × 104 virus particles per cell) of Ad5, Ad11 and Ad4 virions. Virions were absorbed by shaking for 90 min in 1 ml DMEM without FCS for A549 and HepG2 cells and RPMI 1640 for MG7 and HMEC cells and then unbound virions were discarded. The infected cells were washed once with methionine- and cysteine-free RPMI 1640 medium 22 h p.i. and incubated for 2 h in 2·5 ml methionine- and cysteine-free DMEM (ICN) or RPMI 1640 (ICN) containing 5% FCS, 20 mM HEPES and antibiotics, as before, in order to deplete endogenous methionine and cysteine. At 24 h p.i., the cells were labelled with 0·45 μCi per bottle of 35S-labelled methionine and cysteine (1175 Ci mmol−1, 10·5 μCi ml−1; ICN). At 1 and 4·5 h after labelling, 50 μl cold cysteine (100 mM) and 25 μl cold methionine (100 mM) were added, respectively. After labelling for 24 h, unlabelled methionine and cysteine were added again. Infected cells were harvested 72 h p.i. and washed twice in a wash buffer containing 0·1 M Tris/HCl (pH 8·0), 5 mM EDTA and 1 mM PMSF and resuspended in 90 μl of the same buffer to a final volume of 100 μl. Then the samples were analysed by SDS-PAGE and autoradiography, as described below.
SDS-PAGE and autoradiography. Of each labelled sample, 10 μl was taken for electrophoresis in SDS-polyacrylamide gels containing 12 % acrylamide: bisacrylamide at a ratio of 29:1. Protein samples were mixed with an equal volume of 2× loading buffer and heated at 95 °C for 8 min before loading. Electrophoresis was performed at 200 V for 3–5 h until the bromophenol blue dye reached the bottom of the gel. Then, the gels were stained in Coomassie brilliant blue for 3–5 h and destained in 40 % methanol and 10 % acetic acid for 12–16 h. Gels were dried using a gel drier prior to autoradiography. Photographic film (Fuji-RX) was exposed for 1–3 days. The density of the hexon bands was analysed using the Gel-Pro ANALYSER program.

Titration of virus infectivity. Of each of the cell lines HepG2, MG7, HMEC and A549, 1 × 10⁵ cells were incubated with 2 pg per cell of Ad4 in duplicate tubes for 1, 12, 24, 48 and 96 h. After 1 h of adsorption at 37 °C in 200 μl DMEM or RPMI 1640 containing 2 % FCS with agitation, all cells were washed twice in PBS and then maintained in 1 ml fresh medium. Two tubes of infected cells were pooled together at the end of the incubation and freeze–thawed three times. Lysates were diluted in tenfold steps and each dilution was used to inoculate five tubes of A549 cells in parallel. Development of cytopathic effect (CPE) was monitored every day for 12 days.

RESULTS

Ad11 showed high binding efficiency for the endothelial cell line and all carcinoma cell lines studied

The binding affinity of human adenoviruses from different species for different carcinoma cell lines established from

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**Fig. 1.** Analysis of the binding capacity of Ad11, Ad5, Ad4, Ad31, Ad37 and Ad41 to endothelial (HMEC) and tumour A549 (lung cancer) cell lines, and to HepG2 (hepatoma), LNCaP and DU145 (prostatic carcinoma), CAMA and MG7 (breast carcinoma) and Hep2 (laryngeal carcinoma) cells. Three different concentrations of biotin–streptavidin, FITC-labelled adenoviruses (1, 3 and 6 pg per cell) were incubated with cells of different origin and the percentage of virus-labelled cells was evaluated by flow cytometry. Values represent the means of three independent experiments.
Ad4 at a high m.o.i. (10^3 physical particles per cell) and (HMEC). Cell cultures were infected with Ad5, Ad11 and (HepG2), breast cancer (MG7) and endothelial cells in order to evaluate whether they were infectious to hepatoma cells to be studied further in an immunostaining procedure in

Ad4 and Ad11 exhibit higher expression of viral proteins than Ad5 in HepG2, MG7 and HMEC cell lines

Ad4, Ad11 and the commonly used vector Ad5 were selected to be studied further in an immunostaining procedure in order to evaluate whether they were infectious to hepatoma (HepG2), breast cancer (MG7) and endothelial cells (HMEC). Cell cultures were infected with Ad5, Ad11 and Ad4 at a high m.o.i. (10^3 physical particles per cell) and harvested 20, 40 and 72 h p.i. Anti-virion sera were used in an immunostaining procedure to reveal expression of viral structural proteins.

Infected cells showed a pronounced CPE and bright green fluorescence (Fig. 2). The intensity of fluorescence was weaker in Ad5-infected cells and more pronounced in cells expressing Ad11 and Ad4 viral proteins. Already at 20 h p.i., the number of infected cells differed between these three serotypes (Table 1). Ad11 and Ad4 were more infectious than Ad5 in hepatoma, breast cancer and endothelial cells. No sign of Ad5 infection was noted in breast cancer (MG7) cells.

Ad4 and Ad11 viral structural and non-structural proteins are expressed efficiently in HepG2 and MG7 cell lines

To confirm the immunostaining results and to detect which viral proteins were induced in these tumour and endothelial cells, newly synthesized proteins were labelled with [35S]methionine. HepG2, MG7, HMEC and A549 cells were infected with 2 pg per cell of Ad11, Ad4 and Ad5 and labelled with [35S]methionine and [35S]cysteine 24 h p.i.; A549 cells were used as a positive reference in this procedure. For each cell line, a mock-infected sample was included as a negative control. The density of the hexon band seen after SDS-PAGE of A549 cells infected with Ad5 was set to 100. The densities of the hexon bands obtained in the other experiments were then normalized to this value.

Viral structural proteins of Ad11, Ad4 and Ad5 were produced to a varied extent in the A549, HepG2, MG7 and HMEC cell lines, with the exception that no Ad5 structural proteins were detected in MG7 cells (Fig. 3). Twofold more Ad11 and Ad4 hexon protein than Ad5 hexon protein was produced in A549 cells (Fig. 4). This was in agreement with the differences in binding affinity seen (Fig. 1). In MG7 cells, viral proteins were detected in the case of Ad11 and Ad4 but not in the case of Ad5 (Fig. 3a) and this was also corroborated by the immunostaining experiment, where virtually no Ad5-infected cells were seen (Fig. 2). A five- and sixfold higher expression of Ad11 and Ad4 hexon, respectively, than Ad5 hexon was seen in HepG2 cells (Fig. 4). The efficient shut-off of the expression of cellular proteins as a consequence of efficient infection was seen in Ad11- and Ad4- but not in Ad5-infected A549 and MG7 cells. A unique cellular 50 kDa protein was strongly expressed in MG7 and HepG2 cells. The expression of this 50 kDa protein was effectively turned off by Ad11 but was not affected by Ad4 and Ad5 infection (Fig. 3). The lowest relative expression of viral proteins was seen in HMEC cells and cellular protein shut-off was not efficient for all three serotypes (Figs 3A and 4). It was noteworthy that Ad4 proteins were expressed more efficiently than the Ad11 proteins in HepG2 and MG7 cells, even although Ad11 showed a higher binding efficiency to these cells.

Ad4 infectious particles are produced efficiently in HepG2, MG7 and HMEC cells

The extent of permissiveness for Ad4 was studied by performing growth analysis in A549, HepG2, MG7 and HMEC cells. The yield of infectious Ad4 virus particles was assayed in A549 cells. Cells were incubated with Ad4 virions at an m.o.i. of 2 pg per cell for 1, 12, 24, 48 and 96 h. The lowest infectivity at 1 h p.i. was a log TCID50 of 4.6–5.2 for the A549, HepG2, MG7 and HMEC cells. Afterwards, infectivity increased to 9.5 log TCID50 at 24 h p.i. for A549 cells and to 8.8, 9.5 and 8.7 log TCID50 at 48 h p.i. for HepG2, MG7 and HMEC cells (Fig. 5). The results described above showed that although HepG2, MG7 and HMEC cells are all permissive to adenovirus infection, the
production of infectious particles in these cell lines was somewhat delayed compared to A549 cells.

**DISCUSSION**

The commonly used adenovirus vectors, which are based on Ad5 and Ad2 from species C, have been demonstrated to be expressed efficiently in some tumour cell lines but less efficiently in others (Haisma et al., 1999; Kim et al., 2002; Zabner et al., 1994). These observations have been confirmed in our study of Ad5. Even with a high concentration of virions (6 pg per cell), the proportion of labelled cells did not exceed 15% for any of the cell lines studied. CAR is the primary adenovirus receptor for Ad2 and Ad5 (Bergelson et al., 1997). Little is known about the extent of the expression of CAR on different tumour cells. However, CAR appears to be expressed on many different types of tumour cells with variable density, e.g. the expression of CAR was variable in head and neck squamous cell carcinoma (Legrand et al., 1999) and bladder cancer cell lines (Li et al., 1999b), even in cell lines displaying the same histology, and certain tumour cells could lose CAR.

*Fig. 2.* The infectivity of Ad5, Ad11 and Ad4 in HMEC, HepG2 and MG7 cell lines. Immunofluorescence staining was performed 20, 40 and 72 h p.i. with Ad5, Ad11 and Ad4 virions (10^3 physical particles per cell) using anti-virion sera. Uninfected cells were included as a negative control (NC). Magnification, × 200.
expression (Li et al., 1999a). Thus, this variability in CAR expression could restrict the Ad5-mediated gene transfer to tumour cells of different origin (Pearson et al., 1999).

Table 1. Percentage of fluorescent cells infected by adenoviruses

Immunostaining was performed on cultures of HMEC, HepG2 and MG7 cells at 20, 40 and 72 h after infection by Ad5, Ad11 and Ad4 virions (10^5 physical particles per cell) using homotypic anti-virion sera. The input of physical particles equals the total number of infectious and non-infectious virus particles. The concentration of the purified virion was determined by subtracting A_{260} from A_{330}. Hence, 10^{12} virion particles or 280 µg virus proteins A_{260–330} = 1. The table presents the proportion of positive (fluorescent) cells per well (corresponding to a total of 2 × 10^5 cells). The designation ‘few’ represents less than 100 positive cells per well.

<table>
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<th>Time (h p.i.)</th>
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<th>40</th>
<th>72</th>
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<td>Ad11</td>
<td>Ad4</td>
<td>Ad5</td>
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<td>Few</td>
<td>10</td>
<td>30</td>
<td>5</td>
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<tr>
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<td>0</td>
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<tr>
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<td>Few</td>
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Fig. 3. (a) Expression of viral structural and non-structural proteins in HMEC, MG7 and A549 cell lines. (b) Expression of viral structural and non-structural proteins in the hepatoma (HepG2) cell line. Of the different cell lines, 1·5 × 10^6 cells were infected with Ad11, Ad5 and Ad4 (7·2 × 10^3 physical virus particles per cell), labelled with [35S]methionine and [35S]cysteine at 24 h p.i. and harvested at 72 h p.i. The same quantities of cell lysates from different cell lines were separated by SDS-PAGE on a 12 % gel and autoradiographed for 1–3 days. Arrows show the main structural polypeptides of Ad11. Lysates of Ad11, Ad5 and Ad4 virions were also separated and used to identify viral proteins. Mock-infected samples (negative control, NC) were included for each cell line and used to differentiate cellular and viral proteins. *, hexon band was 120 kDa for Ad11 and 110 kDa for Ad5; **, represents unknown 50 kDa protein.

Fig. 4. Evaluation of the expressed hexon bands from Ad11, Ad5 and Ad4 in HMEC, HepG2, MG7 and A549 cells. The density of the Ad5 hexon band expressed in A549 cell is given as a standard control. The densities of all other hexon bands derived from expression in the different cell lines were compared to the density of Ad5 hexon expressed in A549 cells.
In our study, we found that Ad11 from species B2 showed an impressively high binding efficiency for all cell lines tested, including the endothelial cell line and hepatoma, breast cancer, prostatic cancer and laryngeal cancer cell lines. A high binding affinity of Ad11 for several haematopoietic cell lines has also been observed (Segerman et al., 2000). Adenoviruses of species B use a different primary receptor (Defer et al., 1990; Stevenson et al., 1995), which has not yet been characterized. Remarkably, Ad4 showed a high binding affinity only to the cell lines of hepatoma and laryngeal cancer origin (HepG2 and Hep2) with a unique binding pattern. It seems that only HepG2 and Hep2 can express sufficient amounts of specific receptor for Ad4 but this is not true of all tumour cells and endothelial cells. Ad4 is the only member of species E adenovirus and can cause both conjunctivitis and respiratory disease. The head domain of fibre gene sequences contains the CAR-binding motifs and displays similarity to species C members Ad5 and Ad2 (Chroboczek et al., 1995; Roelvink et al., 1998). However, our binding results suggest that Ad4 may use different receptors as well. A CAR-receptor-binding site on the fibre protein was determined recently (Roelvink et al., 1999; Santis et al., 1999). Ad5 fibre amino acid residues S1408, K417, K430, and Y477, which are involved in the binding of CAR, are also conserved in Ad4 fibre. However, since the Ad4 knob sequence shares only 52% identity with the fibre knob sequence of Ad5 (Chroboczek et al., 1995), the overall structure of the receptor-binding site may be sufficiently different to explain the diverse binding characteristics of Ad4 and Ad5 (Skog et al., 2002). The infectivity of Ad4 on these cell lines was confirmed by titration of infectious virus particles. Infectious virions were produced efficiently on HepG2, MG7 and HMEC (listed in the order of decreasing log TCID_{50} cell lines, indicating that these three cell lines are permissive to Ad4 infection.

The fibre plays a role not only in the binding of virus to target cells but also on the initiation of an infection (Legrand et al., 1999). Consequently, Ad5 with its lower binding affinity for all cell lines studied showed, in a similar manner, a lower ability to infect cells in immunostaining experiments, as seen by the weaker intensity of fluorescence and lower proportion of cells infected. Also, ^{35}S-labelling revealed little or no expression of Ad5 hexons in HepG2, HMEC and MG7 cells. Contrary to Ad5, Ad11 caused higher levels of fluorescence on the cell surface and a higher proportion of infected fluorescent cells, and also high expression of hexons. In accordance with our observation, Ad3 (species B) and Ad17 (species D) displayed more efficient infectivity than Ad5 (Krasnykh et al., 1996; Zabner et al., 1999). An unknown 50 kDa protein was observed only in the breast cancer (MG7) and hepatoma (HepG2) cell lines. The 50 kDa protein was turned off quite effectively as a consequence of Ad11 virus replication but not during infection with Ad4 or Ad5. Ad4 showed a higher capacity of expression than Ad11, even though the binding capacity of Ad4 was lower. This was indicated by more pronounced CPE and higher expression of hexon in the ^{35}S-labelling experiment. Such a result may be explained in part by a higher efficiency of internalization determined by interaction of integrins on the host cell surface with the RGD motif on the penton base of adenovirus. Adenoviruses of species A, B, C and E of adenovirus use \( \alpha_\beta_1/\beta_5 \) integrins for internalization (Mathias et al., 1994). The requirement for integrins in adenovirus internalization is supported by the case of Ad41, which is devoid of the RGD \( \alpha_\text{v} \), integrinbinding motif and has shown insufficient uptake into A549 cells (Albinsson & Kidd, 1999). Perhaps additional cellular molecules also intervene in internalization and infection, as adenovirus can internalize in breast cancer cells by employing an integrin-independent pathway (Kim et al., 1999). As an example, hepatocytes are almost \( \alpha_\text{v} \) integrin-deficient cells but are still permissive to Ad11 and Ad4 (Hautala et al., 1998). Furthermore, the fibre could modulate the route of virus trafficking in experiments using adenoviruses from different species (Miyazawa et al., 1999, 2001).

Endothelial cells are essential target cells for gene therapy because they are involved in disease processes associated with inflammation and angiogenesis and are readily accessible to gene therapy vectors via the circulation. However, the mechanism of transduction of endothelial cells by adenovirus vectors is poorly understood. Endothelial cell lines can be permissive to adenovirus (Ades et al., 1992b; Friedman et al., 1981). In our study, the endothelial cell line was shown to be less permissive for Ad5 but more permissive for Ad4 and Ad11. Cellular protein synthesis is usually downregulated by adenovirus replication through impaired transport of cellular mRNA transport to the cytoplasm (Babich et al., 1983; Yoder & Berget, 1985). Adenovirus may also act in a different way to regulate cellular protein synthesis by activating the expression of cellular endogenous genes (Ramalingam et al., 1999).
In summary, Ad11 virions are most effectively bound to all cell lines in this study originating from endothelia and tumours of the lung, breast, liver and prostate and Ad11 virions were binding most efficiently to glioblastoma, medulloblastoma and neuroblastoma cell lines (Skog et al., 2002), whereas both Ad11 and Ad35 virions bound efficiently to established cell lines of haematopoietic origin (Segerman et al., 2000). Our observations of this binding capacity of Ad11 and Ad35 have been confirmed by Shayakhmetov et al. (2000) for haematopoietic cell lines and also by Havenga et al. (2002) who demonstrated that chimeric Ad5 with fibres from different members of species B adenoviruses bind efficiently to tumour cell lines of haematopoietic, pancreas, breast and cholangiocarcinoma origin. Ad11 is a likely vector candidate for gene transfer in ex vivo cancer gene therapy and for gene therapy of vascular diseases. Since it is capable of targeting HepG2 cells, Ad4 appears to be a promising serotype for use in in vivo applications involving gene therapy of hepatoma and liver diseases.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Cancer Foundation, the Swedish Science Council and the local Umeå Cancer Foundation. We thank J. C. Hierholzer at the Biological Products Branch, Centers for Disease Control, Atlanta, Georgia, USA, for kindly providing us with the endothelial cell line (HMEC); we thank Magnus Evander at the Department of Virology, Umeå, who contributed the breast cancer cell lines (CAMA and MG7).

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