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Inhibition of cell adhesion to the virus by synthetic peptides of fiber knob of human adenovirus serotypes 2 and 3 and virus neutralisation by anti-peptide antibodies

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Abstract

The fiber knob of adenovirus (Ad) causes the first step in the interaction of adenovirus with cell membrane receptors. To obtain information on the receptor binding site(s) several synthetic peptides derived from Ad2 and Ad3 fiber head sequences and their antisera were tested for interference with virus attachment to HeLa and FL cells and cell adhesion to viruses. The anti-peptide sera were also evaluated in ELISA and virus neutralisation test. Ad2 (of subgroup C) and Ad3 (of subgroup B) attachment was not significantly inhibited by peptides corresponding to the amino acid residues 535–554, 555–573, 562–582 of Ad2 fiber or 210–225, 267–283, 291–306 and 300–319 of Ad3 fiber. However, microplate pre-adsorbed Ad3 fiber residues 210–225 and 267–283 could bind FL and HeLa cells, and 1 mg/ml of Ad3 fiber residues 267–283 inhibited the cell adhesion to Ad3 virus to approximately 90%. This peptide may participate in the receptor binding site of Ad3 fiber. ELISA reactive anti-peptide antibodies against the homologous peptide and virus did not significantly reduce the cell adhesion to the immobilised virus or the virus attachment to cells, but in the neutralisation assay antibodies raised to Ad2 fiber residues 555–573 and 562–582 and Ad3 fiber residues 210–225 caused neutralisation of the homologous virus at serum dilutions of 1:500 and 1:32, respectively. The corresponding peptides and one further peptide of Ad2 fiber and two of Ad3 fiber seem to contain neutralisation epitopes.

Keywords: Adenovirus; Fiber knob peptides; Cell receptor binding site; Cell adhesion; Virus attachment; Virus neutralisation

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1. Introduction

The fibers of human adenoviruses serotype 2 (Ad2) of subgroup C and serotype 3 (Ad3) of subgroup B are already well characterised. The fiber 2 is a thin projection, 37 nm long (Ruigrok et al., 1990), terminating in a knob. The N-terminus of the polypeptide chains of the fibers, located in the tail region, interacts with the penton base, and the C-terminus is in the knob (Devaux et al., 1987; Caillet-Boudin et al., 1988; Weber et al., 1989). The length of the fiber of human Ad3 is only 16 nm (Ruigrok et al., 1990). The Ad2 fiber is glycosylated (Ishibashi and Maizel, 1974) but the Ad3 fiber apparently not (Caillet-Boudin et al., 1989). The fibers are trimers of identical subunits (Van Oostrum and Burnett, 1985; Albiges-Rizo et al., 1991).

The first step in the interaction of the adenovirus with a permissive cell is the attachment of the virus, via the head domain of the fiber, to a primary cell plasma membrane receptor (Bai et al., 1993; Mathias et al., 1994; Louis et al., 1994; Di Guilmi et al., 1995) and via the RGD sequence motif of the penton base to a second receptor. Despite the extensive study of the fiber there are some questions, so it is not known which sequences of the fiber knob participate in the virus attachment.

Synthetic peptides can be useful not only for the diagnosis of human adenovirus serotypes and for immunological questions (Modrow and Wolf, 1990), but also for the study of virus cell interaction. In this paper we evaluated the influence of some selected peptides of Ad3 and Ad2 fiber knob on the first step of interaction between adenoviruses and permissive cells. The requirement for an influence of peptides is the existence of continuous determinants in the binding site(s). The influence was also studied with labeled viruses and labeled cells. Especially, the adhesion of HeLa and FL cells to these peptides, to Ad2 and Ad3 virus and the attachment of Ad2 and Ad3 to the cells was examined. Additionally, the ability of prepared anti-peptide sera to bind and to neutralise the virus serotypes Ad2 and Ad3 was investigated.

2. Materials and methods

2.1. Peptides

The amino acid (aa) sequences of the fiber protein of Ad2 and Ad3 were published previously (Herisse and Galibert, 1981; Signäs et al., 1985) and compared with Ad5 fiber by Chroboczeck and Jacrot (1987). The sequence of the knob starts with the amino acid W of the conserved sequence T–L–W–T. Peptides of the fiber knob of Ad2 and Ad3 were selected partly on the basis of variability and antigenicity (Wolf et al., 1988). Peptides of Ad2 fiber and Ad3 fiber (Table 1, Fig. 1A,B) and Ad7 fiber residues 306–321 were synthesised by a solid phase synthesiser using F-moc protected amino acids. Cysteine (C) and lysine (K) were added for carrier conjugation. The peptides were purified by preparative RP-HPLC and characterised by analytical HPLC and amino acid analysis (Haist et al., 1992). Peptides of Ad3 fiber were purchased from Bio-TZ, Berlin-Buch, Germany.

2.2. Peptide-protein conjugation

Maleimide activated keyhole limpet hemocyanin (KLH) (Pierce) was the carrier for Ad2P1 and Ad2P3. (For example 3 mg of activated KLH (10 mg/ml) was added to 1.5 mg peptide (5 mg/ml) in phosphate buffered saline (PBS) and after 3 h incubation with gentle stirring the sample was dialysed against PBS). The three peptides of Ad3 fiber Ad3P1, Ad3P2 and Ad3P3 were conjugated with bovine thyroglobulin (TG) (Sigma, Aldrich) as carrier using glutaraldehyde, following a modified two-step method. The efficiency of peptide conjugation to the carrier was measured by gelfiltration or ultrafiltration with a 30 000 D cut-off and UV-photometry.

2.3. Adjuvants and immunisation

For the preparation of antisera adjuvants are essential in the induction of antibodies. Hence different adjuvant formulations, incomplete Freund's adjuvant together with Quil A (Liebermann et al., 1990) or immune stimulated complex (IS-

Table 1

Titers of antisera against fiber knob peptides of Ad2 and Ad3 in ELISA and neutralisation test (NT)

Peptide		Titer			
		ELISA		Neutralisation	
		a	b	c	d
Ad2P1	Ad2 fiber residues 538–554-C, GTSESTETSEVSTYSMSC	n.d.	$1.9 \times 10^{3\,e}$	128	<2
Ad2P2	Ad2 fiber residues 555–573-C, FTWSWESGKYTTETFATNSC	n.d.	$7.7 \times 10^{3\,e,g}$	500	2
Ad2P3	Ad2 fiber residues C-562–582, CGKYTTETFATNSYTFSYIAQE	4.9×10^5	$1.0 \times 10^{5\,e}$	560	100
Ad3P1	Ad3 fiber residues 210–225 LKTDLELKYKQTADFS	2.3×10^5	$4.1 \times 10^{4\,f}$	32	20
Ad3P2	Ad3 fiber residues 267–283, LEVTVMLNKRLPDSRTS	2.1×10^5	$2.0 \times 10^{4\,f}$	16	4
Ad3P3	Ad3 fiber residues K-291–306, KSLNAGLAPETTQATLI	$2.0 \times 10^{3\,h}$	$4.1 \times 10^{4\,f}$	16	4
Ad3P4	Ad3 fiber residues 300–319, TTQATLITSPFTFSYIREDD	n.d.	$3.1 \times 10^{4\,f,i}$	2	<2

n.d., not determined.

[a] Against the homologous peptide (0.05 μg per well).

[b] Against the homologous virus or fiber.

[c] NT (Titer, 50% neutralisation of the homologous virus at reciprocal serum dilution).

[d] NT with the heterologous virus.

[e] Against Ad2 fiber (0.25 μg per well).

[f] Against Ad3 virus (crude virus suspension, diluted 1:10).

[g] Not carrier conjugated.

[h] Low adsorption of this peptide under the chosen conditions (in sodium bicarbonate buffer pH 9.6).

[i] ISCOM.

COM) (Morein et al., 1990) were used. The preparation of ISCOM was analysed by sucrose density gradient centrifugation combined with UV-photometry and also by electron microscopy. Peptide carrier conjugate, unconjugated peptide and/or ISCOM were given to rabbits (two to five animals per peptide and 100 μg of peptide per animal and per injection) with 1–3 booster immunisations.

Monospecific antisera against the purified adenoviruses and the purified native fiber of Ad2 (Micromun, Greifswald, Germany) were obtained by injections into rabbits of the antigens mixed with complete and incomplete Freund's adjuvant.

The antisera were analysed using the indirect ELISA, the immunoblotting and a modified micro-neutralisation test (NT).

2.4. ELISA procedure

Anti-peptide antibodies from rabbits were tested by indirect ELISA against homologous peptides, crude virus suspension or purified virus. Wells of polystyrene microplates with high adsorption (Greiner or Nunc, Germany) were coated with 0.1 ml of peptide or virus (concentrations, see legends of Fig. 1) in bicarbonate buffer

A

[---A---]   [----------B----------------]
400   410   420
TLWTTPDPSPN CRIHSDNDCK FTLVLTKCG

[----C----------------]   [-- ----D*--
430   440   450
SQV LATVAALA VSGD LSSMTGTV ASVSIFL

----]   [---E-----] [-F--]
460   470   480
RFDQNGVLMBN SSLKKHYWNFRNGNSTNA N

                                   [-----G*---
490   500   510
PYTNAVGPMPN LLAYPKTQSQTAKN NIVSQ

----]   [------H*----------]
520   530   540
VYLHQ DKTKPMILTI TLNQTIEATEFGSVS

[-----------I*----------]   [--------J-----]
550   560   570
TYSMB:IT WSWESGKYTTET FATNSYIFSYI

580
AQE

B

[---A---]   [----------B--
                140   150
NTLWTGPKPEANCIIEYGKQNPDSKLTLI

-----------]   [---------------C-------------]
160   170   180
LVKNGGIVNGYVTLMGASDYVNTLFKNKNV

[---------D*---------]   [-----E-----]   [-F---]
190   200   210
SINVELYFDATGHILPDSSSLKTDLELKYK

                                   [-----G*
220   230   240
QTADFSARGFMPSTTAYPFVLPNAGTHNEN

--------]   [-------H*---------]
250   260   270
YIFGQCYYKASDGALFPLEVTVMLNKRLPD

[---------I*----------]
280   290   300
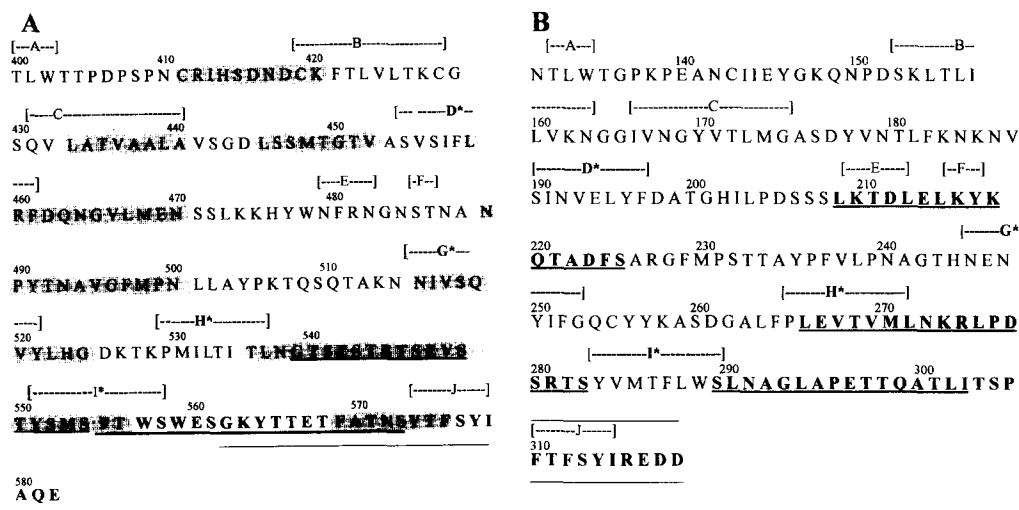SRTSYVMTFLWSLNAGLAPETTQATLITSP

[-------J-----]
310
FTFSYIREDD

Fig. 1. Primary sequences of the knob domain of Ad2 fiber (A) and of Ad3 fiber (B) with the evaluated peptides. The peptides are written in fat letters and are underlined (Ad2P3 and Ad3P4 are fine underlined). The shaded sequence motifs (Fig. 1A) show the antigenic regions/epitopes determined by Fender et al. (1995) and the ranges of D, G, H and I indicate the β-strands being the 'Receptor-sheet' (Xia et al., 1994). The ranges of A, B, C, and J form the other β-sheet, which is partially buried in the trimeric structure and named the 'V-sheet'. The small β-strands are parts of the DG loop (Xia et al., 1994, 1995). (The positions of the ranges of H, J and especially I are not quite the same in the publications of Xia et al. (1994, 1995). We used the first).

pH 9.6 or PBS pH 7.4 over night at 5°C. In later experiments the peptides were suspended in water, added to the microplates and incubated at 37°C over night to dryness. Duplicate samples of anti-peptide sera (0.1 ml) were tested using horseradish-peroxidase-labeled anti-rabbit IgG and o-phenylenediamine as a substrate. The last serum dilution was considered positive if its absorbance value was greater than twice the average value of negative control serum. Anti-virus antibodies and pre-immune sera were included as controls.

2.5. Cells and virus

For virus propagation FL or HeLa cells were grown in monolayer cultures in Eagle's minimal essential medium (MEM) containing 7–10% heat inactivated fetal calf serum (FCS) and subcultivated every 5–6 days. Adenovirus was propagated in medium containing 2% heat inactivated FCS. Cultures with complete cytopathic effect were frozen and thawed three times. After titra-

tion aliquots of viral stocks were stored at − 70°C until use.

2.6. Focus reduction neutralisation test

The test was performed as described recently (Mentel et al., 1996). In brief, 100 μl of serum dilution in medium were pipetted into a chamber slide for tissue culture (Lab Tek, Nunc, USA). Fifty microliters of virus suspension (30 focus forming units) were added and the mixture incubated at room temperature for 2 h. After this, 50 μl of FL cell suspension (400 000 cells/ml) were added and incubated at 37°C for 48 h in a humidified 5% CO_2 atmosphere. The supernatant was removed. The cells were air dried and fixed by acetone for 20 min at − 20°C. The cell sheet was stained with 100 μl of fluorescein isothiocyanate labeled antibodies against adenovirus at 4°C for 30 min. The fluorescent cells of the stained areals were counted microscopically. The protective effect of the antiserum was determined in relation to viral control.

2.7. Radioactive labeling of adenovirus particles

For [35S]methionine and cysteine labeling of Ad2 or Ad3 25 cm³-flasks with a confluent FL or HeLa cell culture were infected with 500 μl virus (about 10^6 CID_{50}/ml) each. After adsorption for 30 min at 37°C 10 ml MEM with 2% newborn calf serum and 1/100 volume 350 mg L-arginine and 10 g glucose per 100 ml were added and incubated for 24 h. After washing 2 ml of methionine- and cysteine-free medium and approximately 15 MBq [35S]methionine and cysteine (Amersham) were added. A second 1 ml of the activity was added 24 h later. The virus was harvested at a full CPE about 1 day later for purification.

2.8. Virus purification

After three freeze-thaw cycles [35S]methionine and cysteine labeled adenovirus was generally purified by freon-treatment and one or two CsCl density gradient centrifugations at 36 000 rpm for 2 h in a 6 × 11 ml swing-out rotor (Sorvall TH641). CsCl was removed by dialysis, Sephadex G25-gel filtration or by ultrafiltration in a 'Microcon' filtration unit with 30 kDa cut-off (Amicon). Glycerol (10%) or 1% of bovine serum albumin (BSA) respectively, was added before ultrafiltration or dialysis. Unlabeled virus was concentrated by PEG 6000 precipitation or by ultrafiltration as described previously (Liebermann, 1982) prior to gradient centrifugation. The concentration of the purified virus was determined optically, using $E_{260nm} = 3.3$ cm³/mg. The virus mass in the crude virus suspension was determined by sucrose density gradient centrifugation, following measurement in a UV flow photometer (Uvicord SD, LKB-Pharmacia) as previously described (Liebermann and Mentel, 1994).

2.9. Cell adhesion assay

For cell adhesion assays, monolayer FL or HeLa cell culture was labeled with [35S]methionine and cysteine (approximately 2 MBq per 2 ml). About 24 h later the cells were removed using 0.2% trypsin and 0.2% EDTA and resuspended in adhesion buffer (Wickham et al., 1994, Dulbecco's minimum essential medium (DMEM) supplemented with 2 mM $MgCl_2$, 1% BSA and 20 mM HEPES). Approximately 5×10^3 labeled cells per well were added to individual wells of 96-well plates, coated overnight with virus, fiber protein or peptides (four wells for each antigen and concentration) and blocked with 5% BSA in PBS pH 7.4 for 1 h at room temperature (RT). For the inhibition of the cell adhesion the cells were also incubated with peptides in adhesion buffer for 1 h at RT and then added to the virus coated wells. The adhesion time was 60 min at 37°C. (In some cases a pH correction of the mixture of peptide and cells was necessary). The radioactivity of the supernatant and of the adsorbed cells after lysis by RIPA buffer (0.15 M NaCl, 10 mM Tris–HCl pH 7.4, 1% sodium deoxycholic acid, 1% Nonidet P40, 0.1% SDS) from each well was determined in a counter.

2.10. Virus binding assay

Binding of [35S]methionine and cysteine labeled Ad2 and Ad3 to FL and HeLa cells respectively was performed similarly to previous descriptions (Persson et al., 1985; Defer et al., 1990). Cell monolayers in 96 or 24-well plates were washed with cold PBS and incubated with or without competition antigen, the peptides or fiber, and then with labeled virus in PBS or TN buffer with 1–2.5% BSA for 45 min at 20°C or more than 2 h at 4°C. The specific radioactivity ranged from 1.8×10^{-5}–1.5×10^{-4} cpm per virion. The first supernatant and the second after rinsing with PBS were pooled. The activity of this unadsorbed virus was measured after drying on filterpaper in toluene scintillator in a liquid scintillation counter. The cell-adsorbed virus was determined by lysis of the cells by RIPA buffer over night at RT and by counting. Non-specific binding of the virus was determined by measuring the total and bound activity in the presence of a 100-fold excess of unlabeled virus.

2.11. SDS–PAGE and immunoblotting

For immunoblot (Dernick and Heukeshoven, 1989) virus and fiber samples were denatured under reducing conditions and separated by SDS–PAGE with a 10% polyacrylamide gel (Mini protean II, Bio-Rad) and transferred to a 0.2 or 0.45 μm nitro-cellulose membrane semi dry (by Sartoblot II, Sartorius). Non-specific binding of proteins was blocked by 10% non-fat dry milk or 5% BSA. After some washings the filter strips were incubated with different dilutions of rabbit anti-peptide, anti-virus and/or anti-fiber sera, respectively. The reaction was detected by anti-rabbit alkaline phosphatase or peroxidase conjugate and chemiluminescence kits (Serva or Amersham, respectively).

3. Results

3.1. Antibodies against fiber peptides

For antisera preparation the three peptides of Ad3 fiber knob Ad3P1, Ad3P2 and Ad3P3 were conjugated to bovine TG as carrier, because in comparison with other carriers TG delivered the highest neutralisation titers in previous immunisation experiments with VP1 peptides of foot-and-mouth disease virus (FMDV) and various carriers (Liebermann et al., 1990). These peptides of Ad3 fiber induced antibodies with high titers in the ELISA against the homologous peptide (Table 1). The adsorption of Ad3P3 to the microplate under the chosen conditions using sodium bicarbonate pH 9.6 was inefficient, this was the reason for the low values in ELISA against this peptide.

The peptides Ad3P4 and Ad7 fiber residues 306–321 were incorporated into ISCOMs for comparison. ISCOM was selected because it is known to be a very good adjuvant without significant side effects (Morein et al., 1990). The resulting titer of the antiserum in ELISA with Ad3 as antigen was similar to the other carrier conjugated peptides (Table 1). The highest titers against the homologous peptide, Ad2 fiber protein, Ad2 and Ad3 were yielded after immunisation of two rabbits with Ad2P3-KLH conjugate (Table 1). The

ELISA results with the whole virus indicate that all tested peptides contain antigenic determinants (Fig. 1A,B).

The results with the anti-Ad2 fiber peptide sera correspond with the peptide-based epitope mapping by Fender et al. (1995). The peptide Ad2P1 is completely in the antigenic region residues 535–556, determined by Fender et al. (1995). Ad2P2 contains the motif FATNS of the epitope, residues 569–576 and Ad2P3 contains this epitope completely. In relation to the X-ray crystallographic model of Ad5 fiber head by Xia et al. (1994) with an eight stranded antiparallel β-sandwich of the monomer the peptide Ad2P1 contains the YSMS motif of the β-strand I. The β-strands I, D, G and H form the β-sheet, which is exposed to solvent and presumably faces the cellular receptor; hence it is called the R-sheet (Xia et al., 1994). Ad2P2 contains the FTWS motif of the β-strand I. Comparing the Ad2P3 has the β-strand J as a part of the other β-sheet, the V-sheet, composed of β-strands J, C, B and A and partially buried in the trimeric structure (Fig. 1A). Ad3P1 contains the β-strands E and F as parts of the DG-loop and Ad3P2 has seven residues of the β-strand H. Ad3P3 is located between the β-strands I and J in the IJ loop and Ad3P4 contains the β-strand J (Fig. 1B).

For the immunoblot we used crude virus, partially and highly purified virus and fiber protein in the SDS–PAGE. The fibers of Ad2 and Ad3 were detected by some antisera obtained from Ad2 or Ad3 fiber peptides, respectively. Anti-Ad2P2 and anti-Ad2P3 antibodies reacted well with the fiber polypeptide of the purified fiber and of the crude virus samples in the immunoblot (not demonstrated).

3.2. Cell adhesion

The influence of the peptides on the first stage of virus cell interaction was tested by cell adhesion to selected fiber peptides. Subsequently the blocking of the cell adhesion to the virus by these peptides was tested.

The peptides Ad3P1 and Ad3P2, pre-adsorbed to microplate, were able to bind FL cells. We did not find this cell binding capacity for the peptides
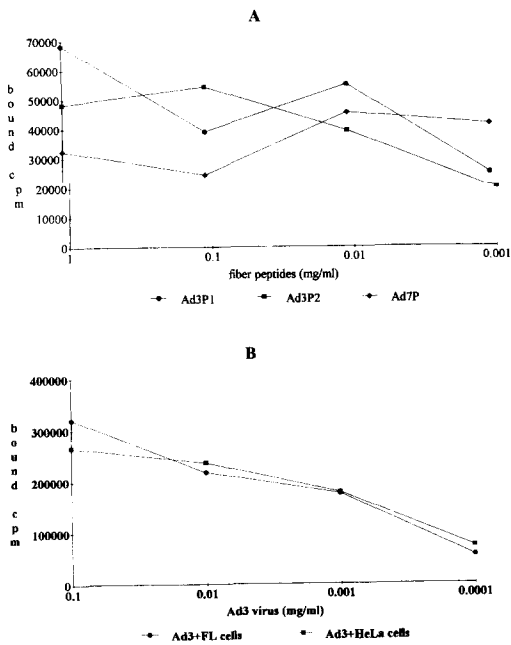
Fig. 2. Cell adhesion to Ad3 fiber peptides and Ad3. (A) Adhesion of ^{35}S-labeled FL cells to Ad3P1, Ad3P2 and Ad7P (Ad7 fiber residue 306–321, TSATTLVTSPFTFYYI); the pre-adsorption of the peptides to microplates was about 20 h at 37°C. (B) Adhesion of ^{35}S-labeled FL and HeLa cells to the adenovirus (mean values of two measurements each). The activity of the added cells (about 7400 cells/100 μl/well) was 410 000 cpm/well. Unspecific adsorption, 20 000 cpm.

cially to immobilised peptides, in the next experiments the cells were pre-incubated with peptides. Ad3P2 inhibited the adhesion of cells to Ad3 virus (Fig. 4). Approximately 1 mg/ml of this peptide inhibited the HeLa cell adhesion to Ad3 virus to about 90%. The result suggests, that this peptide inhibited sterically the binding of cell-receptor to virus and could be a part of the receptor binding site of Ad3 fiber.

We tested also the influence of anti-peptide antibodies on the cell adhesion to the virus. The immobilised virus was pre-incubated with anti-serum dilutions and after washing HeLa cells were
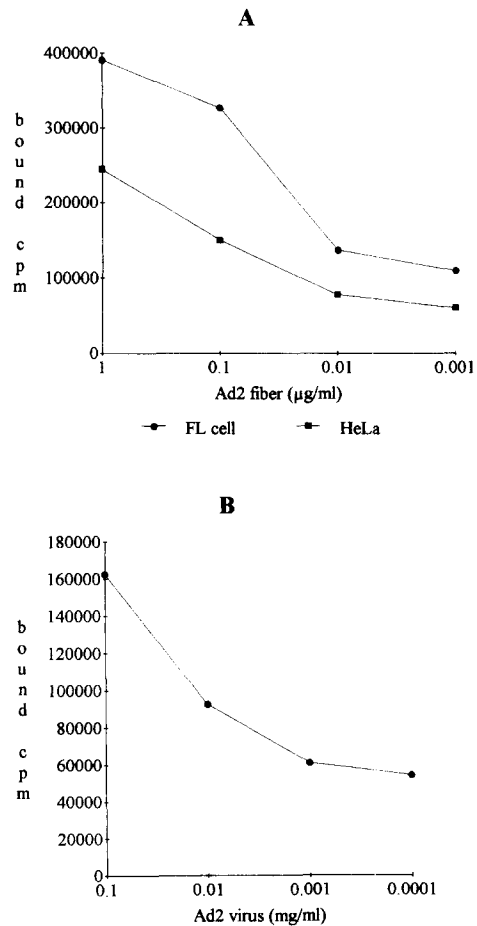

Fig. 3. Adhesion of ^{35}S-labeled FL and HeLa cells to Ad2 fiber protein (A) and of FL cells to Ad2 (B). Conditions, see legend of Fig. 2. The pre-adsorption of the antigens to microplates was over night at 4°C. Unspecific adsorption, approximately 20 000 cpm. (Average of two measurements each).

Ad7P (Ad7 fiber residues 306–321) (Fig. 2A) or Ad2P3 and other nonviral peptides (not shown). In the control experiment Ad3 virus was pre-adsorbed and either FL or HeLa cells were added (Fig. 2B). Approximately 100 μg/ml of peptides adsorbed approximately the same amount of FL cells as 0.1 μg/ml of virus. The reason for the low cell-binding capacity of the peptides may be that the peptide contains only a part of the receptor binding site and steric conditions. Fig. 3 demonstrates the results of binding FL and HeLa cells to Ad2 fiber and the binding of FL cells to the complete Ad2. Comparing these results, it was expected that 100 μg/ml of Ad2 and 1 μg/ml of Ad2 fiber would bind the same amount of FL cells, because the mass portion of fiber is approximately 1% of the virion. The cell-binding capacity of the virus was however lower. Because of the difficulties mentioned for the binding of cells espe-
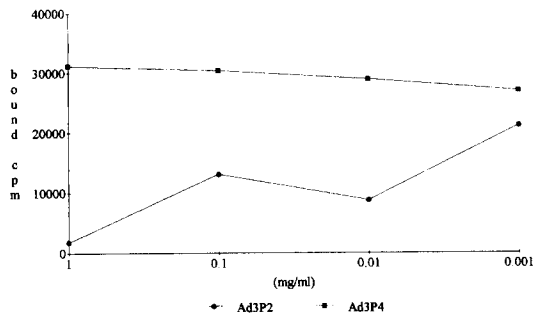
Fig. 4. Inhibition of HeLa cell adhesion to Ad3 by pre-incubation of radiolabeled cells with Ad3P2 in comparison with Ad3P4. The concentration of microplate pre-adsorbed purified virus was 0.02 mg/ml. 43 000 cpm were added per well and 33 000 cpm were bound without peptide.

added (1 h incubation at 37°C) and then the bound activity counted. These antibodies, diluted 1:10–1:10 000, did not significantly reduce the cell adhesion to the immobilised virus (not shown).

3.3. Inhibition of the virus attachment to cells

Anti-Ad2P1, anti-Ad2P2 and anti-Ad2P3 antibodies (dilution from 1:1 to 1:10 000) did not significantly influence the adsorption of Ad2 to HeLa or FL cells, respectively. Only the anti-Ad2 fiber protein serum reduced moderately the virus attachment (Fig. 5). Under the chosen conditions, the fiber peptides of Ad2 listed did not block the virus adsorption to these cells either. For comparison, the native Ad2 fiber protein was used to inhibit Ad2 attachment to cells (Fig. 6A). A concentration of 1 μg/ml of Ad2 fiber was necessary for a 94% reduction of the attachment taking account of unspecific adsorption. About 1 μg/ml of fiber protein has the same blocking effect as 100 μg/ml of unlabeled Ad2 (Fig. 6B). This corresponds to the fiber mass of the virus particle. In comparison, Ad3 did not prevent the Ad2 adsorption to FL cells (Fig. 6B). Only at an Ad3 concentration of 1.4 mg/ml the reduction of attachment was about 30% (taking account of unspecific adsorption), an indication of the different primary receptors of these serotypes.

The blocking of the virus adsorption to cells by anti-Ad3 fiber peptide antibodies was weak and not significant (not shown).

3.4. Virus neutralisation

We were interested in the question whether the anti-fiber peptide antibodies are able to prevent virus infection. Therefore anti-fiber knob peptide antibodies were examined in the neutralisation assay against the homologous and heterologous virus (Table 1). For all three anti-Ad2 peptide antibodies neutralisation activity was detected. Especially anti-Ad2P2 and Ad2P3 sera showed high neutralisation titers against Ad2. Evidently the corresponding peptides contain neutralisation epitopes.

In the cross reaction the titer of anti-Ad2P3 serum was much higher (value 100) than that of anti-Ad2 fiber protein (value 6) and anti-Ad2 serum (value 2).

The neutralisation titer of the anti-Ad3 peptide sera are generally lower than the titers of the anti-Ad2 peptide sera. The anti-Ad3P1, Ad3P2 and Ad3P3 sera were significantly neutralising for the Ad3 (Table 1). The corresponding peptides should have weak neutralisation epitopes.

4. Discussion

To obtain information about region of the knob which participate in the first steps of interaction between adenovirus and susceptible cells, and especially on the binding of the primary receptor
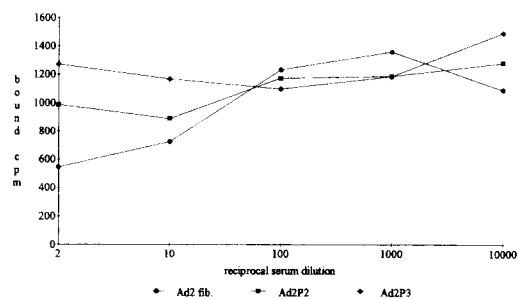

Fig. 5. Influence of anti-Ad2 fiber, anti-Ad2 fiber peptide Ad2P2 and Ad2P3 sera on the attachment of [^{35}S]methionine and cysteine labeled Ad2 to HeLa cells (monolayer in microplates). Labeled virus was pre-incubated with antiserum dilutions 2 h at RT. After washing HeLa cells were incubated with the pre-incubated virus (15 200 cpm per well) 2 h at 4°C.
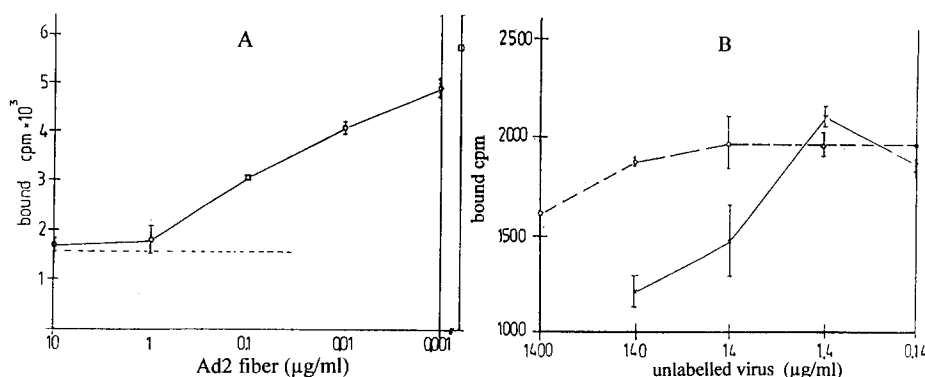
Fig. 6. Inhibition of Ad2 attachment to cells. (A) Blocking of ^{35}S-labeled virus adsorption to FL cells by Ad2 fiber protein. Pre-incubation of cells together with Ad2 fiber for 45 min at RT. □, control value without fiber; – – –, unspecific adsorption. (B) Blocking of labeled Ad2 adsorption by unlabeled Ad2 and Ad3. o– – –, after pre-incubation of cells with Ad3 for 1 h at 20°C; average from two independent experiments. x——, with Ad2. Specific activity, 1.46×10^{-4} cpm per virus particle. Adsorption time

of permissive cells, we used chemically synthesized peptides of the fiber knob of Ad2 and Ad3 and the corresponding antisera.

The fiber carries antigenic determinants that are important in the serological differentiation of adenoviruses (Wadell and Norby, 1969). Some antisera we produced against fiber peptides are also suitable after preadsorption to differentiate serotypes in the ELISA (not published).

The results in ELISA and neutralisation test with three anti-Ad2 fiber knob peptide sera are conform with findings of Fender et al. (1995) using peptide scanning with anti-Ad2 fiber and fiber knob antibodies. In comparison we also used anti-Ad2 fiber peptide and anti-Ad3 fiber peptide sera. Additionally virus-cell attachment and cell adhesion experiments were performed. The results with the Ad3 fiber peptides and their antisera can be described in relation to the model of Ad5 fiber head of Xia et al. (1994, 1995). According to this the virus neutralisation by anti-Ad3P1, anti-Ad3P2 and anti-Ad3P3 seems probable, because of the involved parts of the R-sheet and the IJ loop, respectively. It has been noted in the literature that the host receptor binding site of adenovirus is probably a conformation determinant of the trimeric knob (Henry et al., 1994; Di Guilmi et al., 1995; Fender et al., 1995). In this case, in relation to the dimensions of the receptor complex sequence motifs, especially of the β-strands G and

H may participate in the receptor binding and Ad3P2 with the β-strand H as a part of the R-sheet (Xia et al., 1994) could contain a part of the receptor binding site.

Our results about the influence of anti-Ad2 fiber antibody on the attachment of Ad2 to FL or HeLa cells do not quite agree with the data of Wohlfart et al. (1985). These authors showed that anti-fiber neutralised virions attached to cells to a three to five times greater extent than the untreated control virus. We did not find such a strong effect. Other antigen–antibody proportions may be the reason (Dimmock, 1995).

It was not expected that Ad3 fiber peptides would inhibit the attachment of Ad2, because the Ad3 virus could only inhibit the Ad2 virus adsorption to FL cells at a high concentration (Fig. 6B). The absence of inter-serotype competition between adenoviruses of subgroups B and C for cell membrane receptors was already shown by Defer et al. (1990).

Using a modified micro-neutralisation test it was also possible to quantify moderate neutralisation effects. The manner of virus neutralisation has not been evaluated up to now by us. The sucrose gradient analyses of the neutralising antibodies to demonstrate any aggregation due to immune complex formation are outstanding in the reaction with radiolabeled virus particles. The high titer of anti-Ad2P3 antibody in ELISA and

especially in NT was not quite expected, because the virus neutralisation titer of the corresponding antiserum of Ad3P4 was low. A reason for the low neutralisation titer of Ad3P4 antibody may be using ISCOM instead of KLH. Another reason may be the biological variability of the rabbits, which was used for antisera preparation. It is known that the carrier can drastically influence peptide immunogenicity (Liebermann et al., 1990; Daniel et al., 1994). It is also possible that differences in the sequences induce another secondary structure. But in the plot structure Ad3P4 contains more antigenic indices over 1.2 than Ad2P3 (not shown).

Certainly the conformation of a fiber peptide is not quite the same as in the whole fiber protein. The common sequence stretch of five amino acids of the peptide Ad2P3 and of the corresponding Ad3 fiber peptide is perhaps more accessible than in the whole fiber. This can be the reason for the high cross neutralisation titer of its antiserum against Ad3.

The receptor binding site of other viruses can be a part of the neutralisation immunogen, for example the RGD sequence of VP1 residues 140–160 of the FMDV (Pfaff et al., 1982; Surovoy et al., 1988; Fox et al., 1989; Liebermann et al., 1991). Antibodies against the amino acid sequence of VP2 residue 156–170 of human rhinovirus type 2 recognised and neutralised native virus, apparently by preventing attachment to cells (Barnett et al., 1993). It was interesting, whether this is similar for the adenovirus. Anti-Ad2P1, anti-Ad2P2 and anti-Ad2P3 sera were neutralising, but a reducing effect on the cell adhesion to virus could not be found under the chosen conditions. The possible virus aggregation by these and the other neutralizing antisera as described for anti-fiber serum by Wohlfart et al. (1985) could be the reason for this and also for the non-significant inhibition of virus attachment to cells. Logically also their peptides must not block of necessity the virus attachment to cells.

Now we also evaluate the blocking capacity of peptides of the fiber knob of Ad2 and Ad3 against adenovirus infection in vitro. Using combinations of some peptides and of their antisera, synergistic effects on the attachment and the virus neutralisation can also be expected.

Acknowledgements

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