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Bovine adenovirus type 3 internalization is independent of primary receptors of human adenovirus type 5 and porcine adenovirus type 3

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Abstract

Usefulness of adenoviral vectors derived from human adenovirus (HAd) type 5 (HAd5) is mainly limited by wide prevalence of preexisting anti-HAd5 immunity as well as nonspecific tissue tropism of these vectors. As an alternative, nonhuman adenoviral vectors including bovine adenovirus type 3 (BAd3), are currently being investigated. Non-prevalence of BAd3 in humans and its ability to evade preexisting HAd immunity are some of the features that make BAd3 a promising vector for human gene delivery. BAd3 appears to have a tissue tropism distinct from that of HAd5 and also the repertoire of cells efficiently transduced by BAd3 is different. We performed antibody-mediated receptor blocking experiments to show that BAd3 internalization was independent of coxsackievirus adenovirus receptor (CAR), the primary determinant of HAd5 tropism, or integrin $\alpha_v\beta_3$, a secondary molecule involved in HAd5 entry. Using homologous and heterologous knob-mediated competition assays with recombinant knobs of HAd5, porcine adenovirus type 3 (PAd3), or BAd3, we observed that BAd3 internalization was independent of the primary receptors of HAd5 and PAd3. These results provide support for further exploration of BAd3 vectors for designing targeted vectors for human gene therapy.

INTRODUCTION

Nonhuman adenoviruses have been proposed as alternative gene delivery vectors in order to extend the range of adenoviral vectors and overcome the limitations associated with human adenoviral (HAd) vectors based on HAd type 5 (HAd5) [1-6]. In general, nonhuman adenoviruses are species specific, can enter human cells, and are naturally not prevalent in the human population [4;7]. Bovine adenovirus type 3 (BAd3) is a nonhuman adenovirus belonging to the genus *Mastadenovirus* and was originally isolated from a normal healthy cow [8] and is not known to cause any serious disease in its natural host. BAd3 genome organization and transcription map are similar to those of subtype C HAd [9]. BAd3 effectively evades HAd5-specific immune response [10] and is not cross-neutralized by HAd-specific preexisting neutralizing antibodies in humans [4]. Based on these observations, BAd3 vectors have been proposed as a promising gene delivery vehicle for human and veterinary use [11;12].

Therapeutic application of any gene delivery vector is dependent on its ability to specifically transduce the target cell types. Adenoviral transduction is mediated by high affinity interaction of the fiber knob domain with the cell surface receptor(s) followed by secondary interactions of the penton base with the cell surface integrins [13]. HAd5 entry involves coxsackievirus-adenovirus receptor (CAR) as a primary receptor [14], and integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ as secondary

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receptors [13]. Although BAd3 possesses a unique fiber protein consisting of an N-terminal tail, a long shaft region containing 46.5 hydrophobic repeats, and a globular carboxy-terminal knob [15], the nature of primary receptor(s) for BAd3 is unknown. Identification of the cellular receptors involved in BAd3 entry will be important in determining the potential cell or tissue types that are susceptible or refractory to BAd3 vectors.

Transduction efficiency of BAd3 vectors has been tested in various cell lines [11;16;17]. In a recent study, we observed significant differences in transduction efficiencies of BAd3, HAd5 and porcine adenovirus type 3 (PAd3) vectors in a panel of human, murine, bovine and porcine cell lines [11], suggesting that these vectors may use distinct primary receptors for entry into the susceptible cells. Furthermore, the exchange of the fiber knob domain of BAd3 with that of HAd5 resulted in a significant alteration in cell tropism of the chimeric BAd3 vector [18]. Using homologous and heterologous knob-mediated competition assays, here we provide the evidence that the primary receptors of BAd3 is/are distinct from that of HAd5 or PAd3. Further experiments are needed to define the exact nature of the BAd3 primary receptor/s, and to design BAd3-based targeted vectors for human gene delivery.

MATERIALS AND METHODS

Adenoviral genome sequence alignment

The complete genomic sequences of HAd5 (accession #NC_001405), PAd3 (accession #AF083132) and BAd3 (NC_001876) were obtained from GenBank to compare amino acid sequences of their receptor-binding fiber knob domains using CLUSALW alignment software [19].

Cells and adenoviral vectors

Madin Darby bovine kidney (MDBK) cells, human embryonic kidney cells expressing adenoviral E1 (293), fetal bovine retina cells expressing adenoviral E1 (FBRT HE1)(van Olphen et al., 2002), and fetal porcine retina cells expressing adenoviral E1 (FPRT HE1-5) [4] were propagated as described previously [4]. The generation and propagation of replication-defective HAd5 (HAd-GFP), PAd3 (PAd-GFP) and BAd3 (BAd-GFP) vectors carrying the green fluorescent protein (GFP) gene driven by the cytomegalovirus promoter is described elsewhere [11]. BAd-GFP, HAd-GFP, and PAdGFP were grown and titrated in FBRT HE1, 293, or FPRT HE1-5, respectively. These vectors replicated similar to their respective wild-type counterparts in these E1-complementing cell lines. The physical particle count of purified vector stocks was estimated by spectrophotometry [20]. Briefly, each vector stock was diluted to 10- and 100-fold in 10 mM Tris (pH 8.0), and the absorbance was measured at 260 nm using a spectrophotometer. The optical density value of 1 at 260nm was considered equivalent to 4×10^{11} particles. The ratio of particle number to the plaque forming unit (p.f.u.) for purified preparations of BAd-GFP, HAd-GFP, and PAd-GFP vectors were 120, 80 and 138, respectively.

Antibodies

The mouse hybridoma RmcB that expresses anti-hCAR antibody [21] was purchased from American Type Culture Collection (ATCC), Rockville, MD; and purified as previously described [22]. The mouse anti-human $\alpha_v\beta_3$ integrin (clone LM609) monoclonal antibody was obtained from Chemicon International, Inc., Temecula, CA.

Expression, purification and characterization of recombinant BAd3, HAd5 and PAd3 fiber knobs

BAd3 fiber sequences encoding the knob domain and the last shaft motif present between nucleotides 30278 and 30898 (corresponding to the BAd3 genome sequence in GenBank accession #NC_001876) were amplified by PCR using the forward 5'-GCAGACGGATCCCAGGTTAGGGTTA-3' and the reverse 5'-GCTAAGCTTCCAACCTTATTTAAGTCCAG-3' primers, and the genomic BAd3 DNA as a template. The PCR product was ligated into the *Bam*HI-*Hind*III site of pQE30 vector (Qiagen, Inc., Valencia, CA) to obtain pQE30.B3knob, and expressed in *E. coli* M5 (pREP4) (Qiagen) following the supplier's recommendations. Briefly, the bacterial culture was grown to an OD₆₀₀ of 0.5-0.6 followed by induction of protein expression by 1 mM isopropyl β -D-thiogalactopyranoside (IPTG) and the incubation was continued for 3-4 h at 37°C. Due to the presence of the 6xHis-tag in-frame with the knob, the recombinant protein was purified from the soluble fraction by nickel-nitrilotriacetic acid (Ni-NTA) metal chelation chromatography using His.Bind protein purification kit (Novagen, Madison, WI) as per the supplier's instructions. Purified knobs were dialyzed against PBS (137 mM NaCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, and 2.7 mM KCl, pH 7.4) and protein concentrations were determined by Coomassie Protein Assay Reagent (Pierce Biotechnology, Inc., Rockford, IL) using bovine serum albumin (BSA) as a standard. Purification of HAd5 and PAd3 trimeric knob domains was carried out as described previously [22]. The ability of the recombinant adenoviral knobs to form trimers was verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using both boiled and unboiled samples followed by Coomassie staining or Western blot using a rabbit anti-BAd3, anti-HAd5, or anti-PAd3 hyperimmune serum (data not shown).

Transduction assay in MDBK cells

Approximately 1×10^5 MDBK cells were seeded in 12-well tissue culture plates. Twenty-four h later, cells were infected with BAd-GFP, HAd-GFP, or PAd-GFP at a multiplicity of infection (m.o.i.) of 4, 20, or 100 p.f.u. per cell. At 24 h post-infection, cells were visualized under Olympus IX 70 fluorescence microscope (Olympus America Inc., Melville, NY) and photographed using Olympus DP70 CCD digital camera (Olympus). Subsequently, cells were harvested and analyzed by flow cytometry as described below.

Antibody-mediated blocking experiments

Anti-CAR antibody or anti- $\alpha_v\beta_3$ antibody-mediated blocking assays for BAd-GFP, HAd-GFP, or PAd-GFP in MDBK cells were conducted essentially as previously described [22].

Fiber knob-mediated virus inhibition assays

Experiments involving BAd3 (B3), HAd5 (H5), or PAd3 (P3) knob-mediated inhibition in transduction efficiencies of homologous and heterologous adenoviral vectors in MDBK cells were performed as described previously [22].

Flow cytometric analysis of GFP-expressing cells

Flow cytometry analyses were carried out as described [4]. Briefly, vector-infected MDBK cells were harvested by trypsinization, washed once with PBS and resuspended at a density of approximately 10^6 cell per ml in PBS containing 2% formaldehyde and immediately used for flow cytometry at Purdue University Flow Cytometry Laboratories using Cytomics™ FC-500 Flow Cytometer (Beckman Coulter, Inc., Fullerton, CA) with Ar 488 nm excitation filter and FL-1 525 nm BP emission filter. At least 5000 cells were enumerated for each sample.

Statistical analysis

Data were compared by Student's 't' test for statistical significance. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Comparison of BAd3, HAd5 and PAd3 fiber knob amino acid sequences

The initial attachment of adenovirus to the cell surface receptor/s is mediated by the adenoviral fiber knob, hence, it was important to compare the amino acid sequences of BAd3, HAd5 and PAd3 fiber knobs. For a number of HAd types including HAd5, CAR serves as the primary receptor [14]. Mutagenesis studies of fiber knob domains of a number of CAR-binding HAd types have led to identification of amino acid residues that are critical for or peripherally effect CAR binding [23;24]. Comparison of BAd3, HAd5 and PAd3 fiber knob amino acid sequences showed that the residues critical in CAR-binding (Ser₄₀₈, Pro₄₀₉, Tyr₄₇₇ and Leu₄₈₅) or those that peripherally effect CAR-binding (Ala₄₀₆, Arg₄₁₂ and Arg₄₈₁) were not conserved in the BAd3 or PAd3 fiber knob (Fig.1). The consensus Thr-Leu-Trp-Thr (TLWT) motif that marks the beginning of the knob domain in *Mastadenovirus* species [25] was also conserved in the BAd3 and PAd3 fiber knobs. The similarity between BAd3 and HAd5, PAd3 and HAd5, or BAd3 and PAd3 fibers was 29.3, 29.1, and 26.2%, respectively. When compared at the fiber knob domains, these values were 25.3%, 27.3, and 33.6%, respectively. In addition, the BAd3 fiber is very long (976 residues with 46.5 repeats in the shaft region) [15], the PAd3 fiber is short (448 residues with 15 repeats in the shaft region) [9;26], compared to the HAd5 fiber (581 residues with 22 repeats in the shaft region) [25]. Furthermore, the integrin-binding motif such as the Arg-Gly-Asp (RGD) or Leu-Asp-Val (LDV) is absent in the BAd3 or PAd3 penton base [9]. On the basis of above-mentioned dissimilarities in fiber knob domains of BAd3, HAd5 and PAd3, it was anticipated that BAd3 internalization into susceptible cells might be CAR- as well as integrin-independent. Recently we have demonstrated that PAd3 internalization was independent of CAR and $\alpha_v\beta_3$ or $\alpha_v\beta_5$ integrin [22].

Transduction by BAd-GFP, HAd-GFP, or PAd-GFP in MDBK cells

We used bovine MDBK cell line for performing all competition experiments in this study. None of these vectors replicate in MDBK cells due to a lethal deletion in the E1 region of all three vectors. We have previously shown that transduction efficiencies of BAd-GFP, HAd-GFP, and PAd-GFP in MDBK cells at an m.o.i. of 25 p.f.u. per cell were 85, 58 and 48%, respectively [11]. Similar differences were also observed in the transduction experiments conducted in this study. As indicated by the number of green fluorescent cells, BAd-GFP showed significant transduction at an m.o.i. of 4 p.f.u. per cells, whereas both HAd-GFP- and PAd-GFP-mediated transduction was clearly visible at an m.o.i. of 20 p.f.u. per cell (Fig. 2). These results clearly demonstrated a dose-dependent increase in transduction with each vector. The number of green fluorescent cells was determined by flow cytometry at 36 h post infection. Transduction efficiencies of BAd-GFP, HAd-GFP, and PAd-GFP were approximately 55, 40 and 32%, respectively. The differences in transduction efficiencies of BAd-GFP, HAd-GFP, and PAd-GFP in MDBK cells will not significantly impact interpretation of our results because the relative transduction of various treatment groups was calculated taken into consideration the transduction efficiency of each vector.

CAR-and integrin-independent transduction by BAd-GFP

Our choice of conducting various transduction experiments in MDBK cells was based on the fact that BAd3, HAd5 and PAd3 efficiently transduce these cells [11], MDBK cells express detectable levels of CAR as demonstrated by Western blot analysis [11], and they express very high levels of $\alpha_v\beta_3$ integrin as detected by flow cytometry (data not shown).

The CAR-specific mouse monoclonal antibody, RmcB, is proven to occupy CAR receptors on the cell surface and thereby inhibiting infection of CAR-binding adenoviruses [14;27]. We noticed a dose-dependent inhibition of HAd-GFP transduction of MDBK cells treated with CAR-specific antibody, but antibody-mediated blocking of CAR molecules did not significantly ($P>0.05$) affect the transduction efficiency of BAd-GFP or PAd-GFP (Fig. 2) suggesting a CAR-independent internalization of BAd3 or PAd3. In a previous study, we also observed a drastic reduction in HAd-GFP transduction of MCF-10A cells (non-transformed human breast epithelial cells) preincubated with CAR-specific antibody [22]. These results corroborated the pre-established model of CAR-mediated entry of HAd5 [14;27].

In addition to CAR, integrins have been implicated as secondary receptors in HAd5 internalization [13]. We have previously demonstrated that preincubation of MCF-10A cells with function-blocking antibody against $\alpha_v\beta_3$ or $\alpha_v\beta_5$ integrin resulted in significant inhibition of HAd5 transduction [22]. Similarly, here we observed that the treatment of MDBK cells with an anti- $\alpha_v\beta_3$ integrin antibody diminished HAd5 infectivity and the effect was dose-dependent (Fig. 3). Whereas, preincubation of MDBK cells with an anti- $\alpha_v\beta_3$ integrin antibody did not significantly ($P>0.05$) inhibit transduction efficiencies of BAd3 or PAd3 (Fig. 3). We did not attempt blocking assays using an anti- $\alpha_v\beta_5$ integrin antibody mainly because MDBK seems to express very low level of $\alpha_v\beta_5$ integrin (data not shown). These observations and the absence of the RGD or LDV motif in the BAd3 penton base indicate that CAR and $\alpha_v\beta_3$ integrin may not play a major role in BAd3 internalization into MDBK cells.

Inhibition in adenoviral vector internalization in the presence of vector-specific fiber knob

Since the primary receptor-binding domain of adenovirus is located in the fiber knob [28], the purified adenoviral fiber or knob domain has been effectively used in competitive inhibition assays to investigate distinct receptor usage by various HAd serotypes [14;24;29;30]. For our homologous and heterologous competitive inhibition assays, we needed purified BAd3, HAd5, and PAd3 trimeric fiber knobs. Purification of HAd5 and PAd3 trimeric fiber knobs is described elsewhere [22] and the successful expression and purification of BAd3 trimeric knob was conducted for this study (data not shown).

MDBK cells were preincubated with various concentrations of recombinant B3, H5, or P3 knobs prior to infection with BAd-GFP, HAd-GFP, or PAd-GFP. The B3 knob significantly ($P<0.05$) inhibited BAd-GFP transduction in a dose-dependent manner, but there was no significant ($P>0.05$) inhibition in HAd-GFP or PAd-GFP transduction even at the highest concentration of the B3 knob (Fig. 4A). Similarly, the H5 knob significantly ($P<0.05$) reduced HAd-GFP transduction in a dose-dependent manner, whereas there was no significant ($P>0.05$) inhibition in BAd-GFP or PAd-GFP transduction even at the highest concentration of the H5 knob (Fig. 4B). The P3 knob significantly ($P<0.05$) diminished PAd-GFP transduction in a dose-dependent fashion, whereas there was no significant ($P>0.05$) reduction in BAd-GFP or HAd-GFP transduction even at the highest concentration of the P3 knob (Fig. 4C). Most striking inhibition in transduction of homologous vector was observed in case of HAd-GFP. We observed low levels of inhibition of homologous vector transduction in case of BAd-GFP as well as PAd-GFP. This may be due to the variability in quality of these three recombinant knob preparations and/or the variability in the number of primary receptor/s for BAd3, HAd5, or PAd3 on the surface of MDBK cells. Since knob domain is the primary receptor-seeking moiety of most *Mastadenovirus* species [28], we attempted blocking studies with recombinant knob domain only and not with the entire fiber of BAd3. BAd3 has a uniquely long fiber with unusual kinks in the shaft region [15]. Therefore, it is quite possible that fiber domain/s other than the knob play more important role in BAd3 internalization. Knob domain of BAd3 does, however, play some role in virus attachment and entry as suggested by a recent report

describing altered tropism of BAd3 when its fiber knob was exchanged with the HAd5 knob [18].

Putting together the results of homologous and heterologous competitive inhibition assays, it is clearly evident that significant inhibition in vector transduction was only observed in the homologous system suggesting that the primary receptor/s for BAd3, HAd5 and PAd3 are distinct. These findings are a further extension of our earlier work where we have shown that the primary receptor/s for PAd3 was/were distinct from that of HAd5 [22]. Furthermore, in vivo tropism of BAd3 in a cotton rat model appear to be distinct from that of HAd5 [31]. Therefore, it will be important to investigate whether usage of distinct primary receptor/s by BAd3 vectors will also affect vector biodistribution or vector toxicity in experimental animal models. In addition to CAR, several other cell surface molecules have been shown to play a role in adenovirus binding and internalization. Heparan sulfate glycosaminoglycans (HSG) are involved in fiber-mediated entry of HAd type 2 and 5 [32;33]; sialic acid has been shown to mediate attachment and infection of subgroup D adenoviruses [30]; membrane cofactor protein (CD46) has been implicated as a receptor for subgroup B [34] as well as subgroup D [18] adenoviruses. Similarly, CD80 and CD86 have been shown to be the attachment receptors for HAd3, a subgroup B adenovirus [35]. The role of any of these cell surface molecules as the primary BAd3 receptor/s is yet to be explored.

We have previously shown that BAd3-, PAd3-, or HAd5-specific neutralizing antibody response was not cross-neutralizing [10]. In addition, we have also shown that human serum samples having HAd-neutralizing antibodies did not cross-neutralize BAd3 or PAd3 [4;11]. The study of comparative transduction efficiencies of BAd3, HAd5 and PAd3 vectors in a number of human, murine, bovine and porcine cell lines clearly indicated that the repertoire of cell types transduced by these vectors was quite different [11]. These observations and the results of the present study clearly demonstrate the usage of distinct receptor/s by BAd3. Additional experiments are needed to further understand the nature of BAd3 primary receptor/s.

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HAD5 knob TLWTTPAPSPN CR L N - -AEKDAKLTL VLTKCG- SQI LA TVSVLAVKGSLA 446
BAd3 knob TLWTGTGSNAN VT WRGYTAPG SKLFLSLTRF S TGLVLGN- - - MTIDSNAS 848
PAd3 knob TLWTGASPT AN VI LT N TTPNGTFFLCLTRVG -GLVLGS - - - F ALKSS I D 303
      **** . . . . * : . . . : * ** : . . : * . . : : . . .

HAD5 knob P - - ISGTVQSAH LI I RFD ENGV LLNNSFLDPE YWNFRNGD -LTE GTAY - 491
BAd3 knob FGQYINAGHEQIE CFI LL DNQGNLKEGSNLQGT -WEVKNN - - - -PSA - - 890
PAd3 knob - - - -LTSMTKKVN -FI - FDGAGR LQSDSTYK GR- F GFR SN DSV IEPTAAG 349
      . . . . : * : * * * . . . : . . . : *

HAD5 knob TNAVGFM PNLSAYP- - -KSHGKTAKSNIV S - QVYLNGDKTKP VTL T I TLN 537
BAd3 knob - SKAAFL PSTAL YPILNESRGSPLGKNLVGMQAI LGGGGT - - CT V I ATLN 937
PAd3 knob LSPAWLMPST F I YP- -RN - - - -TSGSSLT S - FVYIN QT YV- - - HVD IKVN 389
      . . : : * * * : . . . . . : . . : : *

HAD5 knob GTQETGDTTPSA Y SMSFSWDWSG HNY I NE I FA T SSY TFS Y I AQE - - - - 581
BAd3 knob GRR - - SNNYPAGQS I I FVWQ - E F N T I ARQPLNH STL TFS YWT - - - - 976
PAd3 knob TLS - - - - -TNGY S LEFN F Q - - - NMSFSA PFS T SYG TFCYV PRRTTHRPR 430
      . . * : * : . : : * * * .

HAD5 knob -----
BAd3 knob -----
PAd3 knob HGPFSLRERRHLFQLLQQ 448

```

Fig. 1.

Homology comparison of the BAd3 fiber knob with the corresponding region of PAd3 and HAd5 fibers using CLUSALW alignment software. Identical, similar, and related residues are depicted by asterisks (and highlighted), colons, and dots, respectively. The HAd5 fiber knob residues that are known to be important for CAR-binding or that peripherally involved in CAR-binding are underlined or shown in bold, respectively.

Fig. 2.

Comparison of green fluorescent protein (GFP) expression in MDBK cells infected either with BAd-GFP, HAd-GFP or PAd-GFP. **A)** MDBK cells were infected either with BAd-GFP, HAd-GFP or PAd-GFP at an m.o.i. of 4, 20, or 100 p.f.u. per cell. At 36 h post-infection, cells were observed under a fluorescence microscope. GFP expressing cells appear green. **B)** Mock-infected MDBK cells under a fluorescence microscope (left) or a light microscope (right) are shown as a negative control. $\times 100$

Fig. 3.

Inhibition of CAR-mediated internalization using a CAR-specific antibody. MDBK cells in monolayer cultures were treated with 1, 5, or 25 $\mu\text{g/ml}$ of CAR-specific antibody followed by infection either with BAd-GFP, HAd-GFP, or PAd-GFP at an m.o.i. of 20 p.f.u. per cell. At 36 h post-infection, cells were harvested by trypsinization and the cells expressing GFP were sorted by flow cytometry. For positive controls, cell monolayers without the CAR-specific antibody treatment were infected similarly with BAd-GFP, HAd-GFP, or PAd-GFP. The transduction efficiencies of positive controls were taken as 100% for calculating the relative transduction of various treatment groups. The results are presented as the mean $\pm$ standard deviation of the percent relative transduction of minimum of three independent observations. *, $P < 0.05$.

Fig. 4.

Transduction efficiencies of BAd-GFP, HAd-GFP, or PAd-GFP in the presence of $\alpha_v\beta_3$ integrin-specific antibody. MDBK cells in monolayer cultures were treated with 1, 5, or 25 $\mu\text{g/ml}$ of $\alpha_v\beta_3$ integrin-specific antibody followed by infection either with BAd-GFP, HAd-GFP, or PAd-GFP at an m.o.i. of 20 p.f.u. per cell. At 36 h post-infection, cells were harvested by trypsinization and the cells expressing GFP were sorted by flow cytometry. For positive controls, cell monolayers without the $\alpha_v\beta_3$ integrin-specific antibody treatment were infected similarly with BAd-GFP, HAd-GFP, or PAd-GFP. The transduction efficiencies of positive controls were taken as 100% for calculating the relative transduction of various treatment groups. The results are presented as the mean $\pm$ standard deviation of the percent relative transduction of minimum of three independent observations. *, $P < 0.05$.

Fig. 5.

Inhibition in adenoviral vector internalization in the presence of vector-specific fiber knob. MDBK cells in monolayer cultures were treated with 1, 5, or 25 µg/ml of the recombinant **A)** BAd3 (B3), **B)** HAd5 (H5), or **C)** PAd3 (P3) fiber knob followed by infection either with BAd-GFP, HAd-GFP, or PAd-GFP at an m.o.i. of 20 p.f.u. per cell. At 36 h post-infection, cells were harvested by trypsinization and the cells expressing GFP were sorted by flow cytometry. For positive controls, cell monolayers without any fiber knob treatment were infected similarly with BAd-GFP, HAd-GFP, or PAd-GFP. The transduction efficiencies of positive controls were taken as 100% for calculating the relative transduction of various treatment groups. The results are presented as the mean $\pm$ standard deviation of the percent relative transduction of minimum of three independent observations. *, $P < 0.05$.