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Defining the role of CD46, CD80 and CD86 in mediating adenovirus type 3 fiber interactions with host cells

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ABSTRACT

Attachment of human adenoviruses (Ads) to host cells is mediated by the interaction of the fiber protein of the capsid with specific cell-surface molecules. For one of the species B adenoviruses, Ad3, the mechanism of binding to cells remains to be defined. Several previous reports have proposed CD46, CD80 or CD86 as possible Ad3 fiber attachment molecules. In this study, CD80 and CD86 were not found to mediate Ad3 fiber binding or Ad3-EGFP transduction of cells. Low levels of Ad3-EGFP transduction of a CHO cell line expressing relatively high levels of CD46 were detected which might suggest a role for CD46 in facilitating Ad3: cell interactions, in the absence of other attachment molecules. Anti-CD46 antibodies and siRNAs had almost no effect on Ad3 fiber binding or Ad3-EGFP transduction of HeLa cells. However, treatment of A549 cells with CD46 siRNA resulted in some decrease of transduction with Ad3-EGFP.

Adenoviruses (Ads) are non-enveloped viruses that contain a linear double-stranded DNA genome of approximately 30–38 kb. At least 51 human adenovirus serotypes have currently been identified and classified into six species, A–F, on the basis of their oncogenicity in rodents, genome organisation and hemagglutination properties (Russell, 2009). The icosahedral adenovirus capsid is composed of 252 capsomers of which 240, the hexon protein trimers, are arranged in facets and 12 capsomers, the pentons, are located at each of the twelve vertices of the capsid (Fabry et al., 2005). The penton consists of a pentameric penton base protein from which projects a trimeric fiber protein. The fiber comprises an amino-terminal domain which is anchored in the penton base, a variable length shaft domain and carboxy-terminal globular knob domain (van Raaij et al., 1999; Russell, 2009).

The entry of adenoviruses into human cells is a two-step process in which an initial attachment to the cell surface is followed by a secondary interaction, which initiates internalisation of the virus. The knob domain of the fiber protein mediates the primary interaction with the cell, effectively tethering the virus particle to the cell surface via a cellular attachment protein (Zhang and Bergelson, 2005). The interaction of the fiber protein with the primary attachment molecule appears to be important in determining the tropism of adenoviruses. A further interaction occurs between the conserved RGD motif present in the penton base of most of the human Ad serotypes and cell-surface integrins, principally $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (Nemerow et al., 2009). Binding of integrins by the virus induces changes in the actin cytoskeleton, promoting endocytic uptake of the virus through clathrin-coated vesicles to endosomes (Meier and Greber, 2004). Escape from the endosome then enables delivery of partially disassembled viruses to the nucleus via nuclear pore complexes (Greber and Way, 2006).

Adenoviruses from all species, except species B and certain serotypes of species D (Ad8, Ad19, Ad30 and Ad37), utilise the coxsackie B and adenovirus receptor (CAR) as their primary cellular attachment protein (Bergelson et al., 1997; Roelvink et al., 1998; Arnberg et al., 2002; Law and Davidson, 2002; Wu et al., 2004; Zhang and Bergelson, 2005). The species B Ads have been classified into two groups, the B1 viruses (serotypes 3, 7, 16, 21 and 50) and the B2 viruses (serotypes 11, 14, 34 and 35) (Wadell et al., 1980). This division has been made on the basis of genetic similarity and correlates with the tropism of these viruses. The B1 viruses cause infection of the upper respiratory tract whereas the B2 viruses are associated with infection of the kidneys and urinary tract. Competition studies of virus binding to cells have suggested the existence of two different cell-surface molecules capable of interaction with the species B adenoviruses (Segerman et al., 2003a). It has been proposed that one of them, sBAR (species B Adenovirus Receptor), could

Abbreviations: Ad, Adenovirus; Ad3F, Ad3 fiber; Ad11F, Ad11 fiber; APC, Allophycocyanin; BHK, Baby Hamster Kidney; CAR, Coxsackie B and Adenovirus Receptor; CHO, Chinese Hamster Ovary; CMV, Cytomegalovirus; FITC, Fluorescein isothiocyanate; EGFP, Enhanced Green Fluorescent Protein; moi, multiplicity of infection; PE, Phycoerythrin; sBAR, species B Adenovirus Receptor; sB2AR, species B:2 Adenovirus Receptor; SCR, Short consensus repeat; siRNA, Small interfering RNA; STP, Serine/threonine/proline.

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interact with all species B adenoviruses and a distinct molecule, termed sB2AR (species B:2 Adenovirus Receptor) could only be utilised by the B2 adenoviruses. Subsequently, a molecule involved in the primary attachment of Ad11 was shown to be CD46, a complement regulatory protein, which is expressed on all human nucleated cells (Segerman et al., 2003b) and these observations were extended to other species B adenoviruses, with the exception of Ad3 (Gaggar et al., 2003). In contrast to these studies, CD46 was proposed to mediate Ad3 attachment to non-permissive baby hamster kidney (BHK) cells (Sirena et al., 2004) and a human glioma cell line (Ulasov et al., 2006). Adding a further complexity in understanding of the interactions of species B Ads with human cells, it has been shown that Ad3 and several other members of B1 and B2 subspecies interact with the co-stimulatory molecules CD80 and CD86 (Short et al., 2004, 2006; Ulasov et al., 2007).

The interaction of Ad3 with human cells is clearly complex. It is possible that Ad3 can utilise one or several of these three attachment molecules or other, as yet unidentified cell-surface molecules, perhaps depending upon the target cell type. Ad3 is a serotype of growing importance, as Ad5 vectors modified to carry Ad3 fibers show significantly higher transduction efficiency when compared to Ad5 in several cell systems (Stoff-Khalili et al., 2007; Tsuruta et al., 2008; Volk et al., 2003). As a result of the lack of clarity regarding the identity of the molecules used by Ad3 to enter cells and the importance of this serotype in terms of improving vectors designed for gene therapy, we have investigated the roles that CD46, CD80 and CD86 may play in mediating Ad3 attachment to a range of human cells, as well as rodent cells engineered to over-express each of these cell-surface molecules.

Results

Interaction of Ad3 fiber with cell-surface CD46, CD80 and CD86

To assess whether there was a relationship between the levels of cell-surface CD46, CD80 and CD86 and the extent of Ad3 fiber (Ad3F) binding, expression of each molecule was determined by flow cytometry in several human and transfected rodent cell lines. Fiber binding assays were performed using recombinant His-tagged fiber. The use of purified fiber enabled the primary interaction of Ad3 with target cells to be studied, separate from the penton base interaction with integrins.

Recombinant Ad3F was purified from *E. coli* using an immobilised metal affinity chromatography resin. SDS-PAGE followed by Coomassie Blue staining showed that the Ad3F consisted mainly of fiber trimer with some monomer in the absence of heat treatment; following boiling of the sample prior to electrophoresis, all of the Ad3F migrated as a monomer (Supplementary Fig. 1A). Densitometric analysis revealed an approx. 3:1 ratio of Ad3 fiber trimer to monomer (results not shown). Western blotting using an anti-penta-his antibody confirmed the presence of the his tag in both trimers and monomers (Supplementary Fig. 1B). In addition to Ad3 fiber attachment, Ad11 fiber (Ad11F) binding was also performed for comparison, since these fibers are derived from species B1 (Ad3) and species B2 (Ad11) and there is a consensus from several studies that CD46 interacts with Ad11 (Gaggar et al., 2003; Marttila et al., 2005; Segerman et al., 2003b). Ad11F was also purified from *E. coli* as a trimer, converted to a monomer after boiling of the sample prior to electrophoresis (Supplementary Fig. 1B). Thus both Ad3 and Ad11 fibers adopted a native conformation. The biological activity of recombinant Ad3F was determined by its ability to block the entry of Ad3-EGFP virus into HeLa cells in a concentration-dependent manner (Supplementary Fig. 1C).

The highest levels of surface CD46 were detected on HeLa (which are cervical carcinoma cells and are widely used for adenovirus propagation) and A549 cells (lung epithelial carcinoma cells which are permissive for Ad3 replication and also represent the known tropism of Ad3 for airway cells), while cell lines of lymphoid origin, Daudi and Raji, expressed significantly lower levels of this protein (Fig. 1A). In particular, Daudi cells possessed only about 10–15% of the CD46 present on either HeLa or A549 cell lines. There was considerable variation in the levels of surface CD46 on hamster CHO cell lines engineered to express human isoforms of this molecule (Fig. 1B). Notably, two clones of different origin expressing the BC2 isoform (CHO-BC2a and CHO-BC2b) differed by two-fold in the level of cell-surface CD46 (Fig. 1B). Cell-surface CD80 and CD86 were detectable on Raji and Daudi cells, but at much lower levels than on CHO cells engineered to express human forms of these proteins (Figs. 1C and D). HeLa and A549 cells did not possess cell-surface CD80 and CD86, although they displayed the highest levels of Ad3 fiber binding. Ad3F binding was not detected to Daudi and Raji cells (Fig. 2), although they expressed all three proposed attachment molecules. Ad11F strongly associated with HeLa and A549 cells and also bound to Daudi and Raji cells, albeit at much lower levels (Fig. 2). Expression of either human CD80 or CD86 on CHO cells did not result in a significant difference in Ad3F or Ad11F binding (Fig. 2). Ad11F, but not Ad3F, bound to all CHO cell lines expressing CD46 isoforms (Fig. 2).

Transduction of cell lines expressing CD46, CD80 and CD86 by Ad3-EGFP virus

To extend the fiber binding studies, transduction of cell lines was performed using a replication-deficient Ad3-EGFP. This permits Ad3 entry to be assayed by measuring EGFP expression. Entry of Ad3-EGFP was fiber-dependent, since it was greatly reduced by pre-incubation of HeLa cells with recombinant Ad3F (Supplementary Fig. 1C) and pre-treatment of Ad3-EGFP with anti-Ad3 (but not anti-Ad5) fiber serum in a concentration-dependent manner (Supplementary Fig. 1D). Cell lines which displayed the greatest level of Ad3F binding were transduced most efficiently by Ad3-EGFP, namely HeLa and A549 (Fig. 2). However, no EGFP expression was detected when Ad3-EGFP was used to transduce Daudi or Raji cells.

The efficiency of transduction of CHO cell lines expressing the major CD46 isoforms (CHO-C1, CHO-C2 and CHO-BC1), as well as two different CHO clones expressing BC2 was also compared. Very low levels of transduction of these cell lines were detected, with the exception of CHO-BC2a (Fig. 2). This cell line could be transduced with considerable efficiency, but at three- to four-fold lower level than HeLa or A549 cells, regardless of comparable expression of cell-surface CD46. Interestingly, the CHO-BC2b cell line was not transduced at the level observed for CHO-BC2a. This disparity could be due to the lower level of CD46 expressed by CHO-BC2b, compared to CHO-BC2a (Fig. 1B). Expression of CD80 and CD86 in CHO cells did not result in Ad3-EGFP transduction (Fig. 2).

The effect of polyclonal anti-CD46 antibodies on Ad3-EGFP transduction and Ad3 fiber binding to HeLa cells

To investigate the role that CD46 might play in Ad3 entry, the effect of pre-treating HeLa cells with antibodies against CD46 on Ad3-EGFP transduction and Ad3F binding was determined. To evaluate the effect on fiber binding, HeLa cells were pre-incubated with polyclonal anti-CD46 antibodies at 4 °C for 30 min, Ad3F was then added and the level of fiber binding to the cells was determined by flow cytometry using Alexa 488-labelled anti-penta-his antibody. The binding of Ad3F to HeLa cells was reduced by approximately 10% in the presence of anti-CD46 antibodies (Figs. 3A and B). This was not significant (p-value 0.5888), whereas Ad11F binding was significantly reduced by approximately 70% (p-value 0.0003). To determine the effect on Ad3-EGFP transduction, HeLa cells were pre-incubated with the same antibody for 30 min at room temperature. Ad3-EGFP was then added to the cells and incubated for 2 h at 37 °C. Cells were then washed and growth medium was added to each well. After 24 h, cells were