Chimpanzee adenovirus CV-68 adapted as a gene delivery vector interacts with the coxsackievirus and adenovirus receptor

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A replication-defective form of chimpanzee adenovirus type 68 (C68) has been developed to circumvent problems posed by widespread preexisting immunity to common human adenovirus vectors. To investigate the determinants of C68 tropism, its interaction with the coxsackievirus and adenovirus receptor (CAR) was studied. Although CHO cells were resistant to transduction by C68 as well as by adenovirus type 5 (Ad5), CHO cells expressing either human or murine CAR were transduced readily. C68 transduction, like Ad5 transduction, was blocked when cells were exposed to anti-CAR antibody or when virus was exposed to a soluble form of the CAR extracellular domain. These results indicate that gene delivery by C68 occurs by a CAR-dependent mechanism.

Introduction

The tropism of adenovirus is influenced greatly by the expression of specific virus receptor molecules. For a number of human adenovirus serotypes, including adenovirus types 2 (Ad2) and 5, virus attachment is mediated by the coxsackievirus and adenovirus receptor (CAR), a cell surface protein with a molecular mass of 46 kDa (Bergelson et al., 1997; Roelvink et al., 1998; Tomko et al., 1997). Susceptibility of particular cell lines to Ad2- and Ad5-mediated gene delivery has been found to correlate closely with CAR expression, and the expression of CAR on a variety of resistant cells has been found to enhance transduction by adenovirus vectors, both in cultured cells and in the tissues of transgenic mice (Hidaka et al., 1999; Li et al., 2000; McDonald et al., 1999; Nalbantoglu et al., 2001; Schmidt et al., 2000; Turtorro et al., 2000; Wan et al., 2000).

Efficient gene delivery is inhibited by preexisting immunity to virus vectors (Xiang et al., 1999) and most adults have measurable titres of antibodies capable of neutralizing Ad2 and 5 (Farina et al., 2001). To circumvent this problem, several nonhuman adenoviruses have been adapted as vectors for gene delivery (Soudais et al., 2000; Tan et al., 2001). A replication-defective vector based on chimpanzee adenovirus type 68 (C68) has been developed recently (Farina et al., 2001). Experiments in animals have demonstrated that peptides delivered in the C68 vector can elicit both humoral and cellular immune responses (Xiang, Z. Q., Gao, G., Reyes-Sandoval, A., Cohen, C. J., Li, Y., Bergelson, J. M., Wilson, J. M., Ertl, H. C. J., unpublished results). Because neutralizing antibodies to common adenovirus serotypes do not cross-neutralize C68 (Bansignt et al., 1971) and because human adults have no preexisting immunity to C68 (Farina et al., 2001), this chimpanzee-derived vector may have significant advantages both as a vaccine vector and for therapeutic gene delivery.

Clinical applications of C68 vectors will depend on understanding the interactions between the virus and its target cells and tissues. Identification of the receptor for C68 can provide important information about how this vector may behave in vivo. We have used several approaches to demonstrate that CAR is the primary receptor for C68 on a number of cell lines. Thus, it is likely that knowledge obtained about Ad2 and -5 tropism will, at first approximation, be relevant to understanding the behaviour of C68.
Methods

CHO cells transfected with cDNA constructs encoding human CAR (CHO-hCAR cells) or murine CAR (CHO-mCAR cells), or cells transfected with vector alone (CHO-pcDNA cells), were cultured in nucleoside-free α-minimal essential medium (MEM) with 10% dialysed foetal calf serum (FCS), as described previously (Wang & Bergelson, 1999). HeLa cells were cultured in MEM with 5% FCS.

For infection with adenovirus, 5 × 10^5 cells were seeded into 6-well tissue culture dishes and cultured overnight. The next day, tissue culture medium was removed and replaced with 1 ml Hanks’ balanced salt solution containing 10 mM MgCl₂, 10 mM HEPES buffer, 4 mM CaCl₂, and 4% FCS. Cells were incubated with C68, Ad5 or -7, all encoding green fluorescent protein (GFP), at an m.o.i. of 1 p.f.u. per cell for 1 h at room temperature with gentle rocking. Virus was removed, cells were washed once with PBS, medium was replaced and cells were incubated at 37 °C. After 48 h, GFP expression was determined either by flow cytometry or by examination with a Nikon Eclipse 800 fluorescent microscope. All experiments were performed at least three times.

The hCAR extracellular domain was produced as a soluble immunoglobulin Fc fusion protein, as described previously (Martino et al., 2000). To test if soluble CAR blocked infection of CHO-hCAR cells by Ad5 or C68, virus was incubated with or without 5 µg of soluble CAR for 1 h at room temperature before the addition of virus to the cells. Polyclonal anti-hCAR rabbit serum was raised against the His-tagged CAR extracellular domain produced in a baculovirus system. CHO-hCAR, CHO-mCAR or HeLa cells were preincubated with a 1:100 dilution of either the rabbit polyclonal anti-hCAR antiserum or preimmune serum for 1 h at 37 °C prior to the addition of virus.

Results

C68, like Ad5, did not efficiently transduce CHO cells. To determine if CAR is a receptor for C68, we tested whether CHO cells expressing hCAR or mCAR were transduced by C68 encoding GFP. GFP expression was seen easily in CHO-hCAR or CHO-mCAR cells exposed to Ad5 or C68, but little or no expression was seen in mock-transfected cells (CHO-pcDNA cells; Figs 1 and 2). This indicates that both hCAR and mCAR can function as receptors for C68.

Transduction of CHO-hCAR cells was blocked when Ad5 or C68 was exposed to the extracellular domain of hCAR produced as a soluble Fc fusion protein (Fig. 3). Similarly, transduction was blocked when cells were exposed to polyclonal anti-hCAR antiserum, but not when cells were exposed to preimmune control serum (Fig. 4). Transduction of CHO-mCAR cells was blocked partially by anti-hCAR serum (Fig. 4), which has a lower avidity for mCAR than for hCAR (data not shown). These results confirm that transduction of CHO-hCAR and CHO-mCAR cells is dependent on the direct interaction between C68 and CAR on the cell surface.

hCAR is expressed on HeLa cells (Bergelson et al., 1997). As was observed with CHO-hCAR cells, transduction of HeLa cells was blocked by anti-hCAR antiserum (Fig. 5A), indicating that hCAR is the primary receptor for C68 on these cells. Transduction of HeLa cells by Ad7, a virus that does not bind to CAR (Roelvink et al., 1998), was not blocked by anti-hCAR antiserum (Fig. 5B), indicating that the antiserum only blocks CAR-mediated transduction.

Adenovirus attachment to CAR is a function of the knob

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\text{Relative Fluorescence Intensity}
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\text{Cell Number}
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\text{AD5}
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\text{C68}
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\text{CHO-hCAR}
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\text{CHO-mCAR}
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\text{CHO-pcDNA}
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\text{Relative Fluorescence Intensity}
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\text{AD5}
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\text{C68}
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\text{CHO-hCAR}
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\text{CHO-mCAR}
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\text{CHO-pcDNA}
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domain of the viral fibre protein. Based on a mutational analysis of the Ad5 fibre (Roelvink et al., 1999) and the crystal structure of the Ad12 knob complexed with the CAR domain (Bewley et al., 1999), the AB loop within the fibre knob domain is known to mediate the binding of adenovirus to CAR. An examination of the C68 fibre sequence revealed that, within this region of the fibre, which is highly conserved among CAR-binding serotypes, including human Ad2, -4, -5 and -12, C68 is identical to Ad4 and highly similar to Ad2, -5 and -12 (Fig. 6). Two other fibre knob residues, Y177 and Y491 (Roelvink et al., 1999), involved in the interaction with CAR, are also conserved in C68.

Discussion

We found that expression of hCAR or mCAR on nonsusceptible cells permitted efficient C68-mediated gene delivery and that gene delivery to susceptible cells was blocked by CAR-specific antibody or by soluble CAR. These results indicate that CAR functions as a receptor for this chimpanzee adenovirus. Studies with Ad2 and -5 suggest that, in many cells and tissues, CAR is the primary – but not the sole – determinant of susceptibility to virus-mediated gene delivery. Our results suggest that CAR may have a similar importance for the tropism of C68.
A variety of other molecules may function in adenovirus attachment or entry into cells. Integrons, including αVβ3 and αVβ5, facilitate virus internalization by a mechanism that involves recognition of an RGD motif within the viral penton base protein (Wickham et al., 1993); the C68 penton base, like those of Ad2 and -5, includes an RGD motif, suggesting that integrons may play a role in internalization by C68. For some adenoviruses, or on some cell types, attachment to other receptor molecules may also be important for infection (Arnberg et al., 2000; Hong et al., 1997; Huang et al., 1996) and alternative receptors may influence the tropism of C68. However, the inhibition of transduction by a CAR-specific antibody suggests that, at least for HeLa and CHO cells, any CAR-independent entry pathways must, at best, be inefficient.

C68 is serologically distinct from all human adenoviruses tested (Basnight et al., 1971; Farina et al., 2001), but its fibro protein is closely related to that of human Ad4 and identical within the region of the knob domain implicated in fibre interaction with CAR. Thus, it is likely that the mechanism of receptor attachment by C68 is similar to that for other CAR-binding viruses. The fibres of Ad4 and C68 (426 aa) are shorter than those of Ad2, -5 and -12 (580–590 aa). It has been suggested that the very short Ad9 fibre (362 aa) may permit the direct interaction between secondary receptors, such as integrons, to recognition sites on the virus (Roelvink et al., 1996; Shayakhmetov & Lieber, 2000). However, Ad4 attachment itself depends exclusively on fibre interaction with CAR (Roelvink et al., 1998) and this is likely to be true for C68 as well.

Although a variety of factors may influence C68 interaction with cells and tissues, identification of CAR as a receptor for this virus provides an intellectual framework for empirical studies of virus tropism. The considerable literature on CAR-dependent gene delivery by Ad2 and -5 can inform experimental approaches and a variety of CAR-specific reagents can be applied to understanding the tropism of this new vector.

Supported by grants from the National Institutes of Health (HL54734, DK47757-08 and PO1 HL5907-02) and the Cystic Fibrosis Foundation. C.J.C. was supported by a Pediatric Infectious Diseases Society Fellowship Award sponsored by Glaxo–SmithKline. We would like to thank Jeffrey Faust and Lester Acosta for their work with the flow cytometry.

References


Chimpanzee adenovirus type 68 and CAR


Received 10 July 2001; Accepted 19 September 2001