The influence of adenovirus species C hexons on vector properties in vitro and in vivo

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Abstract

The majority of adenovirus (Ad) vectors are based on human Ad type 5, which is a member of human Ad species C. Species C also includes the closely-related types 1, 2, 6 and 57, but much less is known about the properties of vectors derived from these non-Ad5 types. We evaluated vectors based on these non-Ad5 types both in vitro and in mice, and we also constructed chimeric Ad5 vectors that contain the hexon hypervariable regions from these non-Ad5 types. Our goal was to understand how hexon influences liver transduction by Ad vectors after intravenous injection in mice. In addition, we studied how coagulation factors and natural antibodies affect these vectors.

We found that Ad5 and Ad6 vectors have the highest ability to transduce mouse liver. Vectors with Ad6 hexon have strikingly different properties from Ad5, showing increased resistance to host natural antibodies and a reduced dependence on coagulation factors for liver transduction. In normal mice, vectors with Ad1 or Ad2 hexon were relatively poor at transducing liver. However, vectors with Ad1 or Ad2 hexon had high liver transduction in antibody-deficient mice, showing that these vectors do not lack liver tropism, but rather that these vectors are highly sensitive to inhibition by natural antibodies in vivo.

Ad5 hexon is known to bind strongly to coagulation factor X (FX), but binding of coagulation factors to other Ad types has been studied to only a limited extent. We found that all species C vectors had the ability to bind FX, but they also could bind another highly-abundant coagulation factor: prothrombin (FII). Ad5 vectors bound FII relatively poorly, but other vectors had much higher ability to bind FII. We found that vectors based on Ad1, Ad2, Ad6 and Ad57 shared a similar pattern of coagulation factor binding, which was different from the pattern seen with Ad5.

In sum, hexons from different human Ad species C viruses confer diverse properties on vectors, including differences in coagulation factor binding and liver transduction.

Materials and Methods

- **Ad production and characterization:** Wild-type viruses, E1-deleted vectors, and helper-dependent vectors were constructed and grown according to previously-published methods. All vectors expressed luciferase. Wild-type viruses were originally obtained from ATCC.
- **Animal experiments:** Animal experiments were performed according to institutional guidelines and were approved by the FDA/CBER Animal Care and Use Committee. For i.v. injection, groups of 4-7 mice were restrained and injected via the lateral tail vein with vector at a volume of 8 ml/g. Mice were injected with 4.0×10^{11} vp/kg of vector and analyzed for liver transduction 48 h later. Approximately 10 min before they were killed, mice were anesthetized by intraperitoneal injection of ketamine and xylazine. Livers were collected and luciferase activity was measured (Promega). Luciferase expression data were normalized to total protein concentration (Bio-Rad). To deplete vitamin K-dependent coagulation factors, mice were subcutaneously dosed with 133 µg of warfarin suspended in peanut oil on days -1 and -3 before injection with vector. 24 h later, mice were then injected i.v. with 4.0×10^{11} vp/kg of vector, and liver luciferase was assayed at 48 h.
- **Coagulation factor binding:** Human FX (HCX-0050), mouse FX (MCX-5050), bovine FX (BCX-1050), human FII (HCP-0010), mouse FII (MCP-5010) and bovine FII (BCP-1010) were purchased from Haematologic Technologies Inc. Surface plasmon resonance experiments were performed on a Biacore T200 system (GE Healthcare). To inhibit serine protease activity from FXa that may be present as a contaminant in FX, the competitive FXa inhibitor GGACK (Haematologic Technologies Inc.) was added to FX at a final concentration of 125 µM. After incubating on ice for 30 min, GGACK was removed by dialysis. All vectors and coagulation factors were dialyzed at 4°C against 10 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.4. Approximately 300 to 5,000 reference units of Ad vectors were immobilized on a CM5 Biacore sensor chip. Running buffer was 10 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.1% (wt/vol) bovine serum albumin and 0.05% (vol/vol) polysorbate 20, pH 7.4. All coagulation factors were diluted in running buffer at four concentrations (one of which was run in duplicate). The flow rate was 50 µL/min, and in some experiments an extended dissociation time was used for one run to aid in obtaining an accurate off-rate. After each cycle, sensors were regenerated for 2 min with running buffer in which the $CaCl_2$ and $MgCl_2$ were replaced with 3 mM EDTA. Regeneration was followed by equilibration for 1 min in running buffer. Data were acquired at 1 Hz and were globally fitted to a 1:1 kinetic model using Biacore T200 Evaluation Software 3.0, with double referencing.
- **Effects of coagulation factors on cell transduction:** The day before each experiment, SKOV3 cells were plated in 96-well plates at 5×10^4 cells per well. On the day of each experiment, coagulation factor mixtures were prepared at physiological concentrations (10 µg/mL for human FX and 100 µg/mL for human FII) in PBS containing calcium and magnesium. Ad vectors were added at a concentration of 2×10^8 vp/mL and incubated on ice for 30 min. Negative control samples (baseline transduction) consisted of vector at 2×10^8 vp/mL in PBS containing calcium and magnesium. SKOV3 cells were rinsed once with PBS, and then 100 µL of each mixture (containing 2×10^7 vp of vector) was added to triplicate wells. Following incubation at 37°C and 5% CO_2 for 2 h, the inoculum was replaced with McCoy's 5A medium containing 10% FBS. After approximately 18 h, cells were rinsed with PBS once and then lysed. 20µL of lysate for each reaction was then transferred to a white-walled 96-well plate and mixed with 100 µL of Luciferase Assay System Reagent (Promega, E1501) using the manufacturer's protocol for a plate reading luminometer (Glomax, Promega). Protein concentrations of lysates were determined by diluting 15 µL of lysate in 135 µL of water and then performing a Micro BCA Protein Assay (ThermoFisher, 23235) according to the manufacturer's instructions.

Results and Discussion

Liver transduction by species C adenovirus vectors

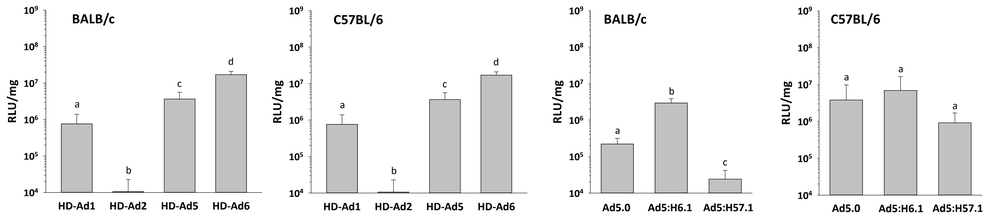

Figure 1. Groups of mice were injected i.v. with various luciferase-expressing vectors and assayed for the amount of liver luciferase expression 48 h later. Helper-dependent vectors were used in the left two panels. In the right two panels, Ad5.0 is an Ad5 vector, Ad5:H6.1 is an Ad5 vector with the hexon of Ad6, and Ad5:H57.1 is an Ad5 vector with the hexon of Ad57. Groups that do not share the same letter (a, b, c, etc.) are significantly different from each other (ANOVA and Holm-Sidak post-hoc test).

Depletion of coagulation factors severely affects liver transduction by Ad5, but not by Ad6

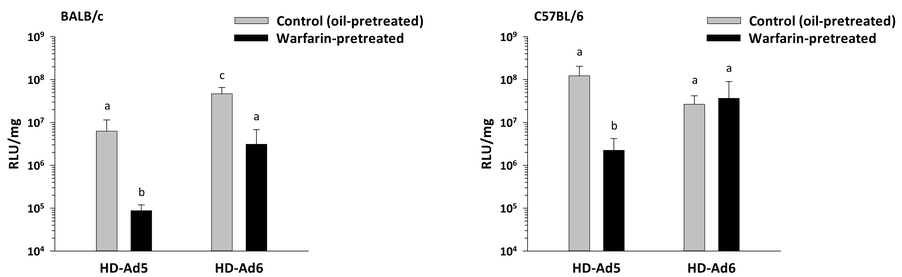

Figure 2. Groups of mice were pretreated with warfarin to deplete coagulation factors (or pretreated with oil as a control), then injected i.v. with helper-dependent Ad5 or Ad6 vector. The amount of liver luciferase expression was assayed 48 h after injection of vector. Groups that do not share the same letter (a, b, c, etc.) are significantly different from each other (ANOVA and Holm-Sidak post-hoc test).

Antibodies strongly inhibit liver transduction by Ad1, Ad2 and Ad5, but not by Ad6

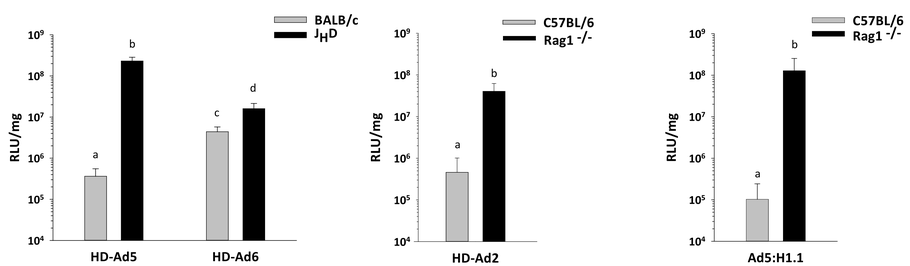

Figure 3. Comparison of liver luciferase expression in normal mice (grey bars: BALB/c or C57BL/6) and antibody-deficient mice (black bars: J_HD or $Rag1^{-/-}$). Groups of mice were injected with helper-dependent Ad5, Ad6 or Ad2, and liver transduction was assayed at 48 h. One experiment was performed using Ad5:H1.1, an Ad5 vector that contains the Ad1 hexon. Groups that do not share the same letter (a, b, c, etc.) are significantly different from each other (ANOVA and Holm-Sidak post-hoc test, or t-test).

High-affinity binding of FX and FII to species C adenoviruses

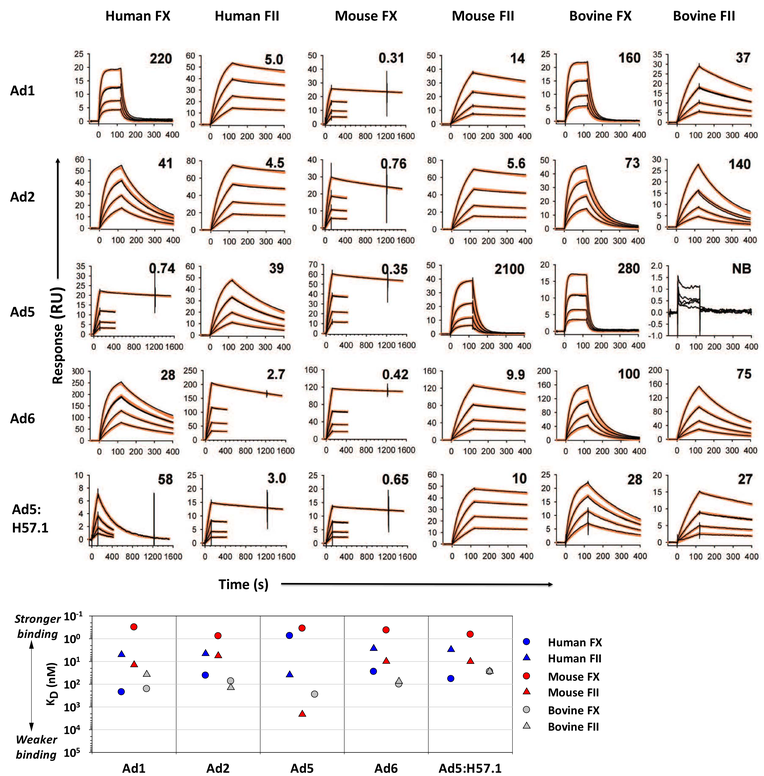

Figure 4. Surface plasmon resonance determination of kinetic binding affinities (nM) of human, mouse and bovine coagulation factors for adenoviruses. Viruses were immobilized and exposed to various concentrations of coagulation factors. Binding data are shown in black, and fitted curves are shown in orange. Wild-type Ad1, Ad2, Ad5 and Ad6 are shown, and Ad5:H57.1 is a chimeric Ad5 vector that has the hexon of Ad57. NB = no binding.

FX enhances the ability of Ad vectors to transduce cells, but FII does not

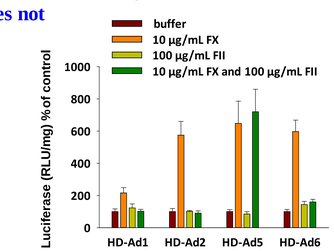

Figure 5. SKOV3 cells (human ovarian carcinoma) were assayed for luciferase expression after transduction with helper-dependent Ad vectors in the presence of human coagulation factors. The physiological concentration of FX is 10 µg/mL, and the physiological concentration of FII is 100 µg/mL. FX enhanced transduction by all vectors, but FII did not. FII competed with the ability of FX to enhance transduction of Ad1, Ad2 and Ad6, but FII did not inhibit the ability of FX to enhance transduction by Ad5.

Conclusion

Species C adenoviruses have strikingly different properties for coagulation factor binding and for liver transduction in mice. These properties are conferred by the hexon protein.

Of the five species C serotypes, Ad5 and Ad6 have the best ability to transduce liver after i.v. injection of mice