**Effects Of Cellular Entry On The Efficacy Of Adenovirus Vector-Based Vaccine**

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**Background and aims.** Adenoviral (Ad) vectors are among the most promising next-generation vaccine modalities currently under development, owing to their high gene delivery efficiency. Adenoviruses are composed of three major capsid proteins—fiber, penton base, and hexon—which have been shown to play critical roles in determining the tissue tropism of Ad vectors. For the widely used adenovirus serotype 5 (Ad5) vector, three principal cellular entry pathways have been identified:
(1) binding of the fiber knob domain to the coxsackie and adenovirus receptor (CAR),
(2) interaction between the RGD motif on the penton base and αv integrins,
(3) binding of coagulation factor X (FX) to the hexon, which facilitates attachment to heparan sulfate proteoglycans on the cell surface.

However, the extents to which these receptor-mediated entry pathways influence the immunogenicity and vaccine efficacy of Ad5-based vaccines are not well understood. In this study, we investigated the effects of cellular uptake pathway on the vaccine efficacies of Ad vectors by using the capsid-modified Ad vectors which utilize the different cellular entry routes for transduction.

**Methods.** In this study, we used Ad5 vectors with mutations in the fiber, penton base, and hexon. Each capsid-modified Ad vector expressing -galactosidase (-gal) as a model antigen was administered to mice either intramuscularly or intranasally. Anti--gal antibody titers in serum, bronchoalveolar lavage fluid (BALF), and nasal wash were subsequently measured.

**Results.** Following intramuscular administration, no significant differences in the vaccine efficacies were observed among the capsid-mutant Ad5 vectors, and all the Ad vectors induced the comparable levels of anti--gal antibodies to those of the conventional Ad5 vector. In contrast, after intranasal administration, the Ad vectors with mutations in the penton base or hexon mediated anti--gal antibody titers in the BALF, serum, and nasal wash at levels comparable to those induced by the conventional Ad5 vector. On the other hand, the CAR-binding-ablated Ad5 vector exhibited a significant reduction in the anti--gal IgG titers in the serum and BALF, and anti--gal IgA titers in the BALF and nasal wash.

**Conclusion.** These results suggest that while the specific cellular entry pathway of the Ad vector does not affect the vaccine efficacy following intramuscular administration, CAR-mediated cellular entry is critical for inducing an efficient vaccine effect of the Ad vector following intranasal immunization. Therefore, these findings highlight that unmodified fibers are preferable for intranasal Ad vector-based vaccines and offer valuable insights for the development of intranasal vaccine platforms.