

# Modification of Ad5 Hexon Hypervariable Regions Circumvents Pre-existing Ad5 Neutralizing Antibodies



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The development of an effective malaria vaccine is a high global health priority. Adenovirus vectors are capable of generating robust and protective T cell and antibody responses in animal models and are considered a leading viral vector platform for vaccines. To date the most potent adenovirus vectors for use as vaccines are based on the subgroup C serotype, Ad5, and early clinical data conclusively show that Ad5 vectors can induce potent CD8+ and CD4+ T cell and antibody responses. However, the high prevalence of neutralizing antibodies to Ad5 in human populations, especially in sub-Saharan Africa, has the potential to limit the effectiveness of an Ad5-based malaria vaccine. Our hypothesis is that modification of the determinants of neutralizing antibodies on the Ad5 virion will enhance vaccine-induced immunity by circumventing Ad5 neutralizing antibody responses.

The hexon protein is the most abundant capsid protein and is the major target for adenovirus neutralizing antibody. The targets of serotype-specific neutralizing antibodies on the hexon are the hypervariable regions (HVR) contained within exposed loops at the surface of the capsid. We generated new Ad5-based vectors that precisely remove the hypervariable regions from the Ad5 hexon and replace them with those derived from Ad43, a subgroup D serotype with a low prevalence of neutralizing antibodies in humans. We demonstrated that these hexon-modified adenovectors are not neutralized by Ad5 neutralizing antibodies *in vitro* using sera from mice, rabbits and human volunteers. Moreover, we generated a hexon-modified adenovector that expresses PyCSP and demonstrated that it is as immunogenic as an unmodified vector in naive mice. In contrast to the unmodified vector, the hexon-modified adenovector induced robust T cell responses in mice that contained high levels of Ad5 neutralizing antibodies (NAB). To map the epitopes for adenovirus neutralizing antibodies and determine the structural constraints of various hypervariable region substitutions, we have generated chimeric vectors containing substitutions of a subset of the hypervariable regions. Results from the analysis of these vectors are presented.

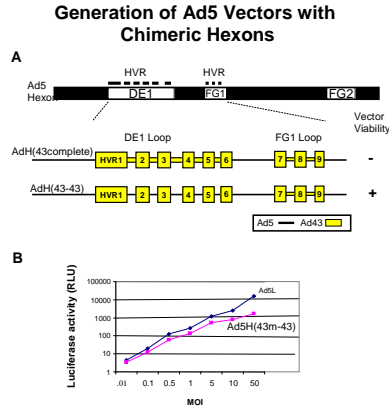


Figure 2. A. Chimeric vectors were generated by retaining the Ad5 sequences that flank the hypervariable regional (HVR) and exchanging only the Ad43 HVR for the Ad5 HVR sequences. B. Analysis of luciferase activities showing hexon-modified Ad5 expresses reporter gene efficiently.

## Hexon modified vectors Express PyCSP Efficiently

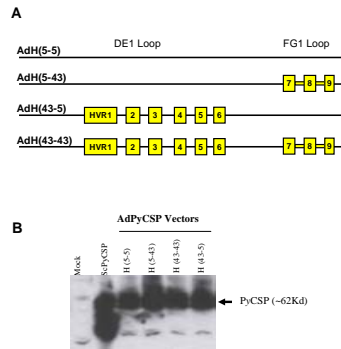


Figure 3. A. Diagram of viable hexon modified vectors. B. Western blot that shows the expression of the PyCSP antigen from each of the hexon-modified vectors. Cell lysates were harvested 24 hours after infecting A549 cells with 200 pucell of purified vectors. Immunoblots were probed with antibody specific for PyCSP.

## Adenovirus Structure

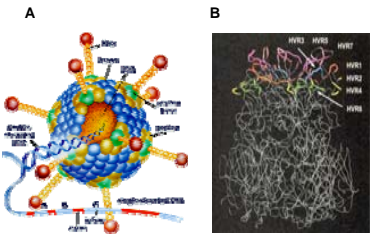


Figure 1. A. Schematic representation of an adenovirus, showing the hexon as the most abundant protein on the surface of the capsid. B. The crystal structure of the hexon trimer, showing the hypervariable regions (HVR).

A. The Nobel Prize in Physiology or Medicine, 1952. An Oronator, Frank Ulfner, Computer Graphics, Olofsholm Strand, Statia Frank, Stockholm, Sweden; The Nobel Committee for Physiology or Medicine at the Karolinska Institute, S-171 77 Stockholm, Sweden  
 B. Ross JJ, Burnett RM. Mol Ther. 2000 Jan; 1(1):3-4.

## Hexon Modified Vector Avoids Neutralization by Ad5 Nab *In Vitro*

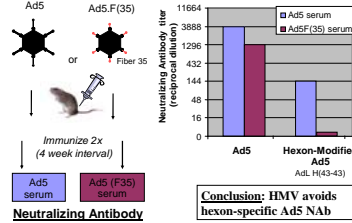


Figure 4. Mice were immunized with an Ad5 vector (blue boxes) and the serum from these mice was tested for neutralizing activity toward an Ad5 vector and the chimeric vector. This sera neutralizes the Ad5 vector efficiently, even when diluted out to 1:3888. It is much less effective at neutralizing the chimeric vector. To determine if the residual neutralizing activity is due to NAB against the Ad5 fiber, we immunized mice with an Ad5 vector that contains the Ad35 fiber (maroon boxes). Serum from these mice efficiently neutralized the Ad5 vector, but did not neutralize the chimeric vector. This suggests that the low level neutralization seen here is due to Ad5 fiber NAB.

## Both Fiber and Hexon Contain Determinants of NAB *In Vitro*

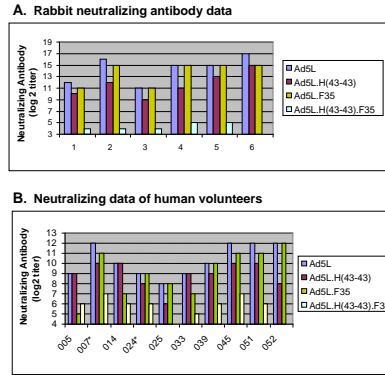


Figure 5. Sera from (A) rabbits immunized with 1x10<sup>10</sup> pu of AdNull and from (B) human volunteers were analyzed for their neutralizing antibody titers using various luciferase expressing chimeric adenovectors. Ad5L is an unmodified Ad5 vector; Ad5L.H(43-43) is a hexon-modified Ad5 vector containing HVR 1-9 from Ad43; Ad5L.F35 is a Ad5 vector carrying the Ad35 Fiber; Ad5L.H(43-43).F35 is a hexon modified vector carrying the Ad35 Fiber.

## Ad5 Hexon Modified Vector Induces Robust PyCSP-Specific T Cell Responses In Mice And is Not Inhibited by Ad5 Nab

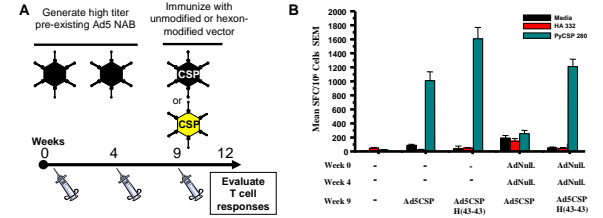


Figure 6. Ad5 hexon modified vector induces robust PyCSP-specific T cell responses in mice and is not inhibited by Ad5 NAB. A. Design of murine immunogenicity experiments. B. Naive mice (n=6/group), or mice pre-immunized with two injections 1e10 pu AdNull were immunized with Ad5CSP, or hexon modified vector Ad5CSP.H(43-43). PyCSP-specific T cell responses were assessed by IFN $\gamma$  ELISpot four weeks after immunization.

## Hexon Modified Vector Can Boost Ad5 Primed Cell Mediated Immune Responses

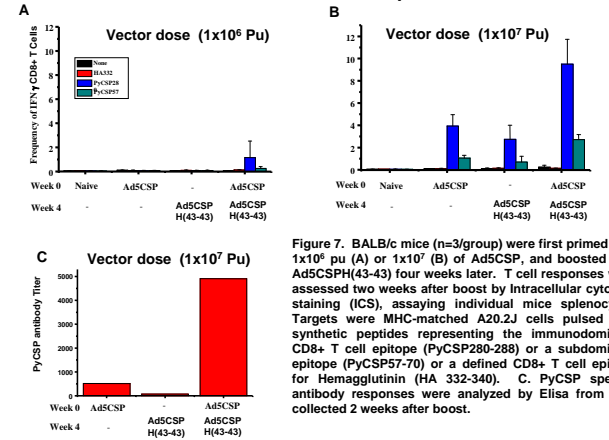


Figure 7. BALB/c mice (n=3/group) were first primed with 1x10<sup>6</sup> pu (A) or 1x10<sup>7</sup> (B) of Ad5CSP, and boosted with Ad5CSP.H(43-43) four weeks later. T cell responses were assessed two weeks after boost by intracellular cytokine staining (ICS), assaying individual spleenocytes. Targets were MHC-matched A20.2J cells pulsed with synthetic peptides representing the immunodominant CD8+ T cell epitope (PyCSP280-288) or a subdominant epitope (PyCSP57-70) or a defined CD8+ T cell epitope for Hemagglutinin (HA 332-340). C. PyCSP specific antibody responses were analyzed by Elisa from sera collected 2 weeks after boost.

## Conclusions

- We generated hexon-modified Ad5 vectors with HVR from a rare group D adenovirus (Ad43).
- These hexon-modified vectors are not neutralized efficiently by Ad5 neutralizing antibody *in vitro*.
- These vectors have Ad5 like vaccine capability and induce robust antigen-specific T cell responses in mice with high titers of Ad5 neutralizing antibody.
- Hexon-modified adenovectors can effectively boost Ad5 vector primed T cell and antibody responses.