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Using Empty AAV Capsids to Reduce Inhibitory Effect of Neutralizing Antibodies on AAV Transduction

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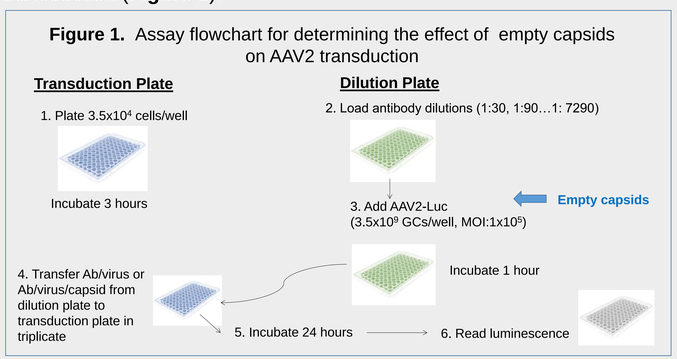
PURPOSE

Currently, gene therapy is becoming more promising for treating numerous genetic disorders. The non-pathogenic adeno-associated viruses (AAVs) are among the most frequently used viral vectors for gene therapy. However, patients with pre-existing immunity against AAVs have neutralizing antibodies (NAb) that may hinder viral transduction; thus, they are less likely to benefit from this type of therapy. Since the prevalence of NAb in the human population can reach up to 60%-70% for some serotypes such as AAV2, systematic exclusion of seropositive patients from treatment would greatly limit the potential impact of gene therapy in humans. For this reason, there is a need to understand how NAbs can interact with AAVs and explore strategies to allow expansion in the application of gene therapy in individuals with NAb. The purpose of this study was to investigate, in an *in vitro* cell-based assay, the potential utility of empty capsids of AAV2 in overcoming the negative effect of NAb on AAV2-mediated transduction.

METHODS

Transduction efficiency was measured using HEK293 cells transduced with AAV2-luciferase (AAV2-Luc). The assay procedure is as follows. HEK293 cells were plated into 96-well plate (3.5x10^4 cells/well) and incubated at 37°C for 3 hrs, then cells were transduced with AAV2-Luc at 1x10^5 MOI, if not specified, for 24 hr. Subsequently, cells were equilibrated at room temperature for 30 min and, after addition of the luciferase substrate the reaction was allowed to proceed for 10 min at room temperature. The luminescence of the reaction product was recorded on a BMG CLARIOstar multi-mode plate reader. The neutralizing effects of NAb on AAV transduction was examined using a monoclonal antibody (MoAb) against AAV2. Prior to transduction, AAV2 was mixed with the serially diluted MoAb, and pre-incubated for 1 hour before being added to the cells. The feasibility of using empty capsids to overcome the neutralizing effect of NAb was assessed by exposing moAb to empty AAV2 capsids prior to viral transduction (**Figure 1**).

Figure 1. Assay flowchart for determining the effect of empty capsids on AAV2 transduction



RESULTS

Figure 2. Effect of capsids on inhibition of AAV2 transduction of HEK293 cells by NAb

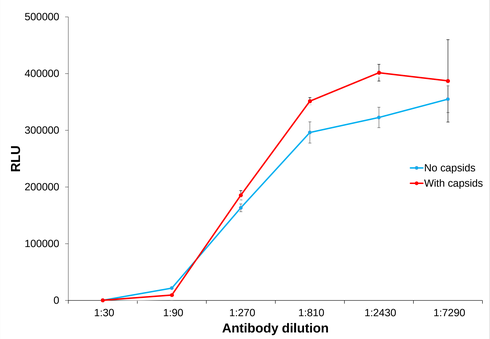
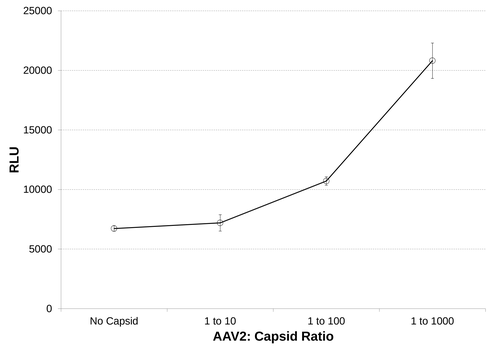


Figure 3. Effect of AAV2/Capsid Ratio on NAb inhibition of transduction



As shown in **Figure 2**, AAV2 transduction on HEK293 cells was completely inhibited by MoAb at 1:30 and 1:90 dilutions; this inhibitory effect decreased as moAb was further diluted and was minimal at 1:7290 dilution. The transduction efficiency was partially restored when MoAb was treated with empty capsids (AAV2:capsid = 1:10) at 1:270, 1:810, and 1:2430 NAb dilution, suggesting that capsids adsorbed or bound the antibodies against AAV2, which led to enhanced transduction of HEK293 cells.

To investigate if increasing the capsid/AAV2 ratio can further enhance the transduction efficiency, one dilution of NAb (1:270) was selected to determine the effect of the capsid/AAV2 ratio on reduced transduction caused by NAb. MOI was 1x10^4. Different AAV2/capsid ratios (1:10, 1:100, and 1:1000) tested the dosage effect of empty capsids on inhibiting AAV2 virus transduction by NAb. As shown in **Figure 3**, the effect of capsid on the transduction inhibited by NAb was dosage dependent and significant differences were observed between groups (1:10 vs 1:100: p=0.0043; 1:10 vs 1:1000: p=0.001; 1:100 vs 1:1000: p=0.005). Though the transduction efficiency could be enhanced when high dose of capsid (AAV2/capsid=1:1,000) was added, the transduction was only partially enhanced (RLU ~20,000) since the RLU was ~80,000 when infected with AAV2 and without adding antibody.

To test if pre-incubation of the capsid with antibody can enhance the transduction efficiency of HEK293 cells with AAV2, we pre-incubated the capsid (1x10^6 GC) with antibody for 10 min and 20 min, then infected with AAV2 (MOI 1x10^4). Therefore, the AAV2/capsid selected for the experiment was 1:100. As shown in **Figure 4**, the capsid significantly enhanced the transduction efficiency in all 3 groups compared to the group without the added capsid (No capsid vs capsid 0 min: p=0.0098; No capsid vs capsid 10 min: p=0.001; No capsid vs capsid 30 min: p=0.0041), while no difference was observed among these 3 groups, p>0.05).

To assess the relevance of using capsids to overcome the effect of NAb on AAV transduction efficiency, additional experiments were conducted by replacing MoAb with sera from two volunteers: one with high (1:243) and one with low (1:3) AAV2 titer. In the presence of low titer serum, the addition of capsids increased the transduction efficiency by nearly two-fold; whereas there was no appreciable effect of capsids with high titer serum (**Figure 5**).

RESULTS (CON'D)

Figure 4. Effect of pre-incubation of Ab and capsid on NAb inhibition of AAV transduction

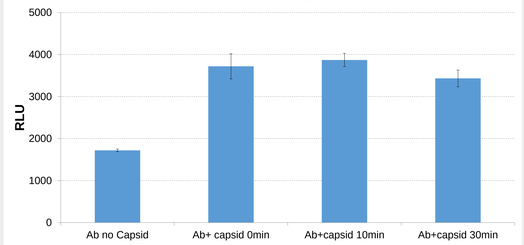
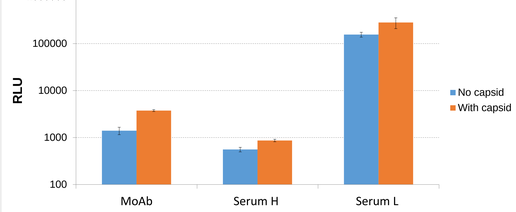


Figure 5. Effect of capsids on inhibition of AAV2 transduction by human sera with high and low titers of antibody



CONCLUSION

This study demonstrated a profound inhibitory effect of NAb on the AAV2-mediated transduction of HEK293 cells using the luciferase reporter gene. It appears that AAV2 capsids were able to interact with NAb and partially restored the AAV2-mediated transduction impeded by NAb. The lack of effect of capsids with high NAb titer serum suggests that a higher amount of capsids may be needed to 'bind' the large amounts of NAb present in this sample. This strategy is worthwhile investigating because, by establishing a direct link between NAb binding and transduction efficiency, it may provide greater impetus to the possibility of identifying approaches that lead to NAb 'binding' without the immunogenicity associated empty capsids, which, in principle, would permit to extend the number of patients who could potentially benefit from AAV gene therapy.

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