A direct comparison of intramuscular antibody expression from vectors delivered by five AAV capsids

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Abstract

Use of adeno-associated virus (AAV) vectors in non-human primates is complicated by host immune responses that can limit transgene expression. Here we compared the expression of and anti-drug antibody (ADA) responses against the rhesus macaque derived, anti-SIV antibody ITS01 when delivered by five different AAV capsids. We first observed that ITS01 expressed three-fold more efficiently in mice from AAV vectors in which heavy and light-chain genes were separated by a P2A ribosomal skipping peptide, compared with those bearing F2A or T2A peptides. We then measured the preexisting neutralizing antibody responses against three traditional AAV capsids in 360 rhesus macaques and observed that 8%, 16%, and 42% were seronegative for AAV1, AAV8, and AAV9, respectively. Finally, we compared ITS01 expression in seronegative macaques intramuscularly transduced with AAV1, AAV8, or AAV9, or with the synthetic capsids AAV-NP22 or AAV-KP1. We observed at 20 weeks after inoculation that AAV9- and AAV1-delivered vectors (2.5 x 10^{12} vg/kg) expressed the highest concentrations of ITS01 (247 µg/mL, n=3, and 162 µg/mL, n=3, respectively), whereas the remaining capsids expressed an average of 35-47 µg/mL. Notable, ADA responses against ITS01 were only observed in one of the 15 animals. Overall, our data suggest that the AAV9 capsid and the P2A peptide can improve expression of antibodies from muscle tissue.

AAV transfer plasmid encoding anti-SIV antibody, ITS01

- ITS01 is anti-SIV antibody isolated from a rhesus macaque infected with SIVmac251 (Mason RD et al., PLOS Pathogens, 2016).
- Transfer plasmid encoding ITS01 utilizes a CASI promoter (Balazs AB et al., Nature, 2011), a codon-optimized coding region of ITS01, a 2A peptide to generate both ITS01 heavy and light chains, a shortened WPRE, and an SV40 polyadenylation signal sequence (**Fig. 1A**).
- ITS01 was encoded to use the rhesus macaque IgG1 Fc with the M428L/N434S amino acid substitutions to improve FcRn affinity and half-life.

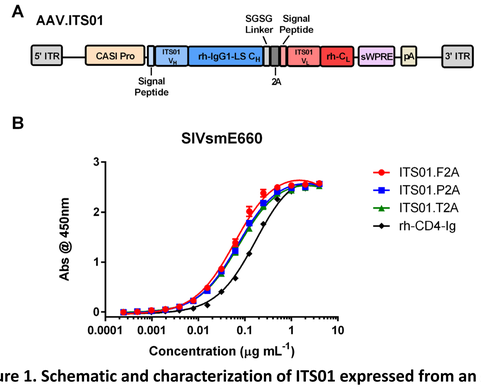

Figure 1. Schematic and characterization of ITS01 expressed from an AAV transfer plasmid. (**A**) Schematic of an the AAV transfer plasmid encoding ITS01. ITS01 expression is driven by a CASI promoter and utilizes a 2A peptide to generate both the heavy and light chains. (**B**) Purified recombinant ITS01 antibody binds SIVsmE660 with similar affinity regardless of which 2A peptide is used for making both light chains. ITS01 antibody made using F2A, P2A, and T2A peptide is indicated. rh-CD4-Ig is a positive control protein.

- Recombinant ITS01 antibody utilizing an F2A, P2A, or T2A peptide was produce through transient transfection of HEK293T cells and purified using Protein A columns.
- All versions of the ITS01 antibody bound SIVsmE660 gp120 with similar affinity, regardless of which 2A peptide was used (**Fig. 1B**).

Higher concentration of ITS01 observed in vivo from design using P2A peptide

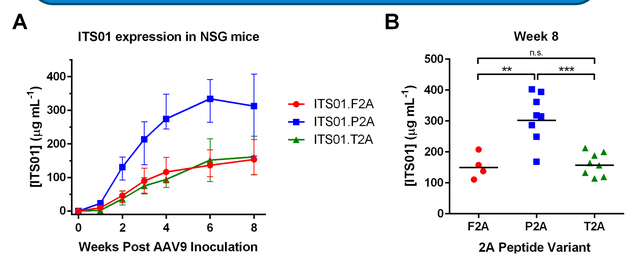

Figure 2. ITS01 expression from AAV9 vectors using different 2A peptides in NSG mice. (**A**) Three groups of four (F2A) or eight (P2A and T2A) NSG mice each were inoculated with 25 uL of 5×10^{10} vector genomes (vg) AAV9 vectors encoding ITS01 using the indicated 2A peptide (F2A – red, P2A – blue, T2A – green) in the left gastrocnemius muscle. Blood draws were performed over eight weeks and plasma samples were analyzed by gp120 ELISA to determine ITS01 concentrations. (**B**) Comparison of ITS01 concentrations at week 8 post AAV inoculation. Each dot represents an individual animal. Mean is represented by the black bar. ** indicates p-value < 0.01; *** indicates p-value < 0.001; n.s. indicates not significant based on unpaired t-test.

Rhesus macaque selections for NHP study based on pre-existing neutralizing antibodies

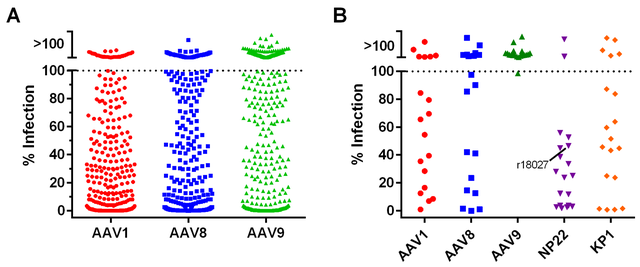

Figure 3. Pre-screening rhesus macaques for AAV neutralizing antibodies. (**A**) To screen for neutralizing antibodies, we developed an assay similar the HIV-1 TZM-bl neutralization assay. 360 rhesus macaque serum samples were diluted 1:5 in cell culture medium and mixed at a 1:1 ratio with AAV vectors encoding the firefly luciferase reporter. After 60 minutes, 3×10^4 cells were added to the serum/virus mixture and incubated for 24 hours. Neutralization was determined as the absence of luciferase production. We observed that 8.9%, 16.1%, and 42.4% of the samples were negative for neutralizing antibodies against AAV1, AAV8, and AAV9 capsids, respectively. (**B**) 19 rhesus macaque samples for the NHP study were screened for neutralizing responses as in (A) except that AAV-NP22 and AAV-KP vectors were included. Three macaques that were negative for each capsid were selected to be used in the study, except for AAV-NP, which only had two macaques without pre-existing neutralizing antibodies. The third macaque selected, r18027, is identified with about a 50% neutralizing response to AAV-NP22.

AAV vectors for NHP study express ITS01

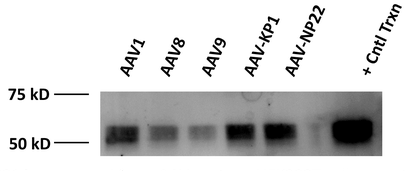

Figure 4. ITS01 is expressed from AAV vectors. HEK293T cells were transduced with 2×10^{11}(AAV8, AAV9), 2×10^{10} vg/mL (AAV1), or 2×10^9 (AAV-KP1, AAV-NP22) vg/mL. Cells were washed with PBS after a 16-hour overnight incubation and medium was replaced with an FBS-free medium. Supernatants were harvested at 96 hours post transduction. A Western blot was performed to detect ITS01 expressed in the supernatants using an HRP-conjugated, anti-human IgG antibody.

NHP study to compare 5 AAV capsids for intramuscular inoculation

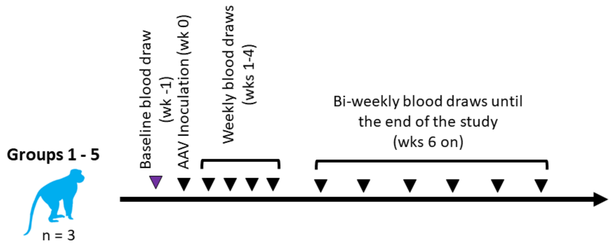

Figure 5. Diagram of NHP study. Timeline of events for the NHP study evaluating different capsids for ITS01 expression. Five groups of rhesus macaques were intramuscularly inoculated with 2.5×10^{12} vg/kg of an AAV vector encoding ITS01. Vectors were inoculated in 8 sites: 2 per quadriceps muscle; 1 per deltoid; 1 per biceps muscle. Blood collections took place weekly through the fourth week after inoculation followed by bi-weekly blood collections.

Highest serum concentrations of ITS01 observed in rhesus macaques inoculated with AAV9 vectors

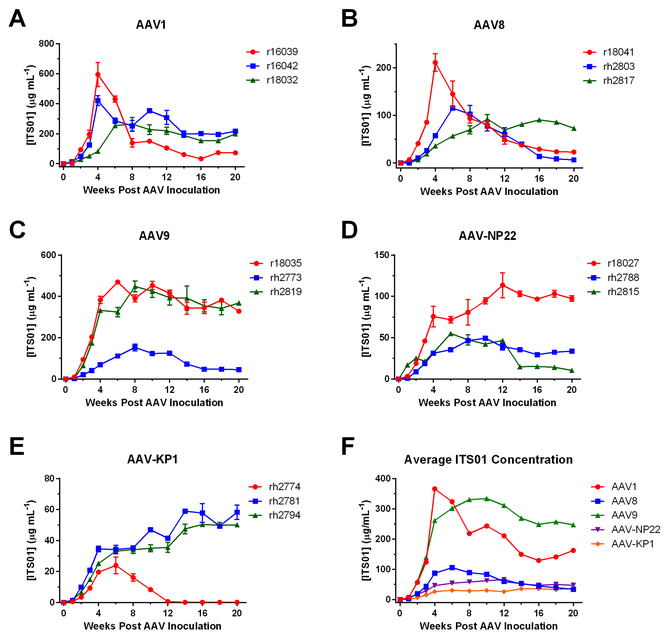

Figure 6. Comparison of ITS01 concentrations from rhesus macaques with different AAV vectors. Evaluation of ITS01 expression from AAV vectors with AAV1, AAV8, AAV9, AAV-NP22, and AAV-KP1 capsids. Three groups of three macaques each were inoculated with 2.5×10^{12} vg/kg of the indicated AAV vector in eight inoculation sites per macaque. ITS01 concentrations for the AAV1 (A), AAV8 (B), AAV9 (C), AAV-NP-22 (D), and AAV-KP1 (E) groups were measured over the course of 20 weeks by gp120 ELISA. The average ITS01 concentrations for each group are in (F). Out of the five groups, the AAV8, AAV-NP22, and AAV-KP1, had the lowest week 20 concentrations (34,47, and 36 µg/mL, respectively). The AAV1 group had the highest peak concentrations (366 µg/mL), which dropped to 162 µg/mL at week 20. We observed peak ITS01 concentrations in the AAV9 group at week 10 (334 µg/mL) and had only a slight decrease at week 20 (247 µg/mL). Based on these data, we can conclude that the AAV9 capsid is suitable for intramuscular inoculations in rhesus macaques.

Low anti-drug antibody (ADA) responses against ITS01 observed in all but 1 rhesus macaque

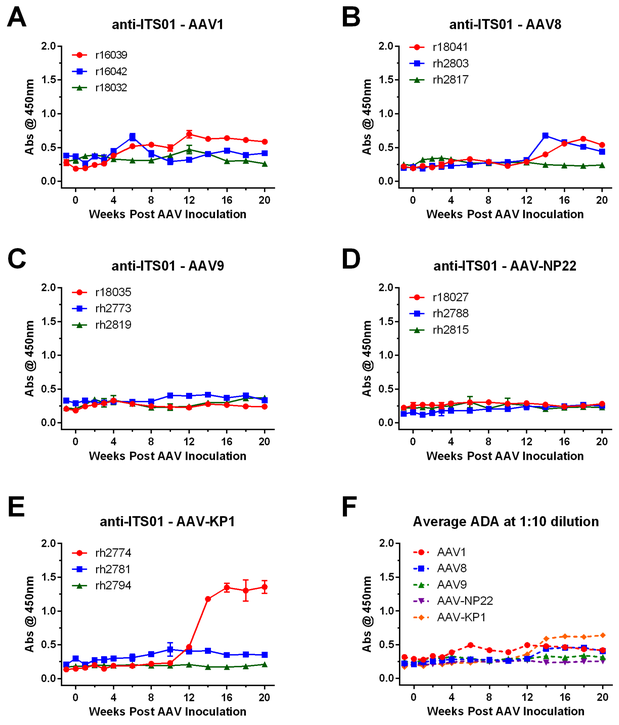

Figure 7. Low ADA responses against expressed ITS01 in all groups. ADA responses were measured by ELISA. ITS01-coated were incubated with diluted serum samples from the incubated timepoints. Anti-ITS01 antibodies were detected using a human, anti-lambda secondary. (**A-E**) The absorbance values for each macaque from the study are graphed. Note that only rh2774 is the only macaque with an ADA response higher than the samples pre-AAV inoculation and is the animal with the lowest ITS01 serum concentrations. (**F**) Average absorbance values for the five groups. Overall, 14 of 15 macaques have ADA responses around the absorbance values of the pre-inoculation time points.

Summary & Recommendations

Based on this study, we recommend the following for intramuscular inoculations in NHP studies:

- Codon optimize the transgene
- Use a P2A peptide when encoding an antibody
- Inoculate with a dose around 2.0-5.0 × 10^{12} vg/kg
- Spread the inoculation over at least 8 sites
- An AAV9 capsid is suitable replacement for AAV1

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