Rapamycin immune tolerization enables gene transfer following subcutaneous delivery of AAV6 but not CD4-retargeted AAV6 vectors in AAV-seropositive rhesus macaques

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Introduction

- Adeno-associated virus (AAV) vectors are a candidate gene delivery platform for anti-HIV therapeutics due to their strong safety record, ability to be produced at high titer, and low immunogenicity.
- Local administration of AAV has been used to deliver soluble therapeutics such as broadly HIV neutralizing antibodies, and AAV6 has the potential to transduce HIV+ CD4+ T cells based on its *in vitro* tropism.
- Unfortunately, sequestration in non-target organs following systemic delivery, and pre-existing immunity, hinder the *in vivo* performance of AAV.
- To overcome these challenges, we investigated: 1) if display of a CD4-binding designed ankyrin repeat protein (DARPin) on the surface of AAV6 (AAV6-CD4) would reduce vector sequestration in non-lymphoid tissues when compared to unmodified AAV6, and 2) whether rapamycin immunosuppression could facilitate efficient gene delivery via AAV6 or AAV6-CD4 vectors in AAV6 seropositive rhesus macaques.

CD4-specific AAV vectors

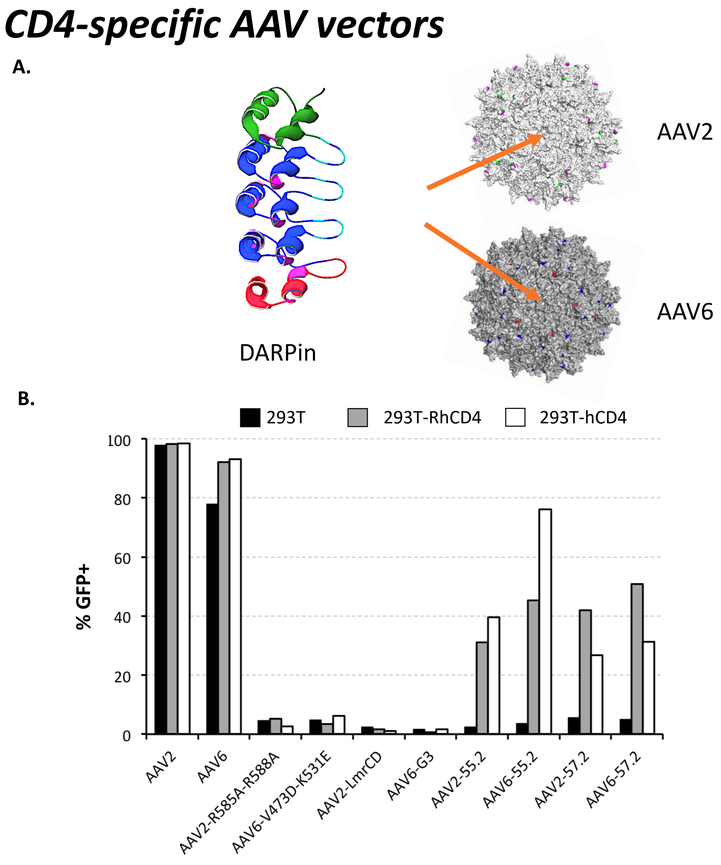


Fig 1. DARPin re-targeted AAV vectors. A, Retargeted AAV vector structures. Crystal structure ribbon diagram of a consensus DARPin containing 3 internal ankyrin repeat modules (left - pdb: 2QYJ), with N-terminal cap (Green), C-terminal cap (red), and 3 internal modules (blue) shown along with divergent amino acids within modules (pink) and connecting loops (cyan). Crystal structures of the AAV2 (upper - pdb: 1LP3) and AAV6 (lower - pdb: 3OAH) capsids showing locations for the R585A (green) and R588A (magenta) mutations that ablate heparin binding for AAV2, and the V473D (red) and K531E (blue) mutations that respectively ablate heparin and sialic acid binding for AAV6. The likely site at the 5-fold axis of symmetry where DARPins are displayed as VP2 N-terminal protrusions is indicated (orange arrow). **B,** GFP expression in human or rhesus CD4 expressing 293T cells transduced with AAV2 or AAV6 based scAAV vectors that express GFP from the MND promoter after transduction at MOI 200,000 genomes/cell. Flow cytometry was performed at 48 hours post AAV transduction. Structures were generated using PyMol 2.1.1.

Rhesus macaque AAV seroprevalence

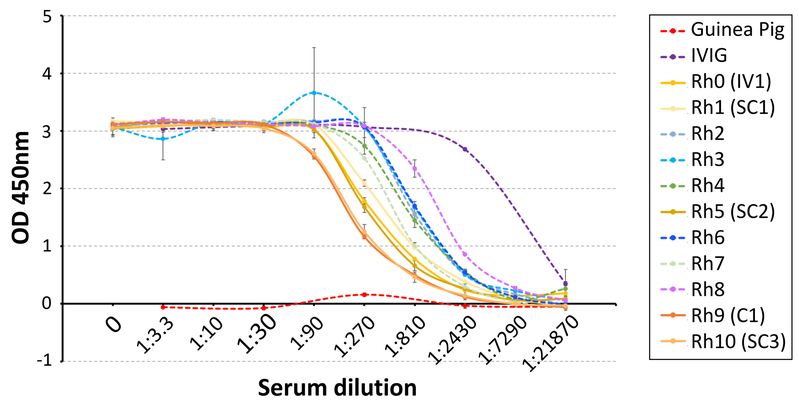


Fig 2. AAV6 seropositivity. Serum from 11 rhesus macaques was screened for the presence of AAV6 binding antibodies using an anti-AAV6 antibody ELISA. AAV6 coated maxisorp plates were incubated with heat-inactivated serum and specific binding was detected using a rhesus cross-reactive anti-human IgG Fc HRP conjugated antibody, and TMB substrate. Solid lines indicate animals selected for AAV administration. Animals selection for inclusion in our study were renamed for clarity: control PBS injected (C1), AAV intravenously injected (IV1) and AAV subcutaneously injected (SC1-SC3) animals. C – control; IV – intravenous; SC – subcutaneous.

Experimental setup

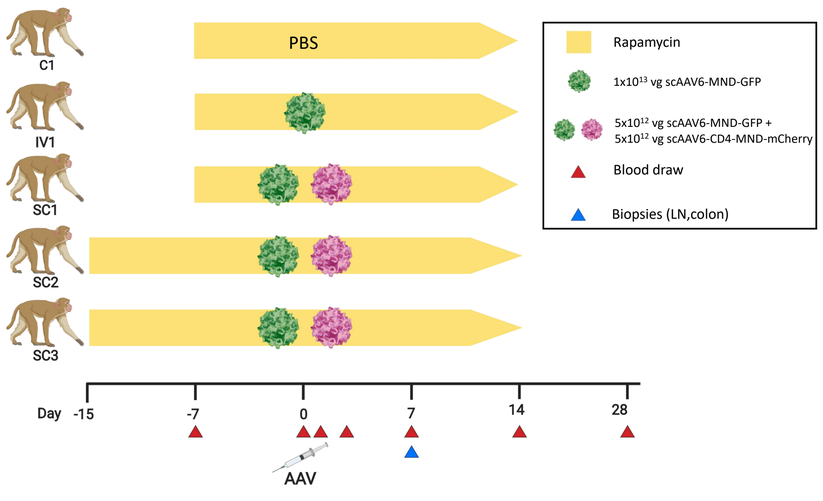
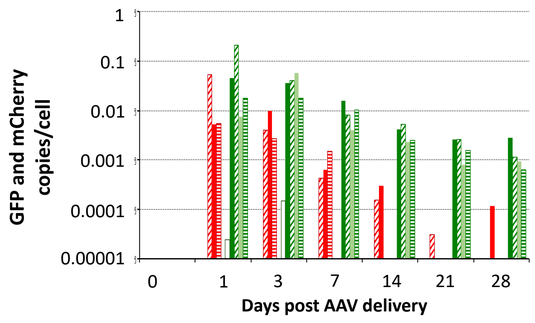


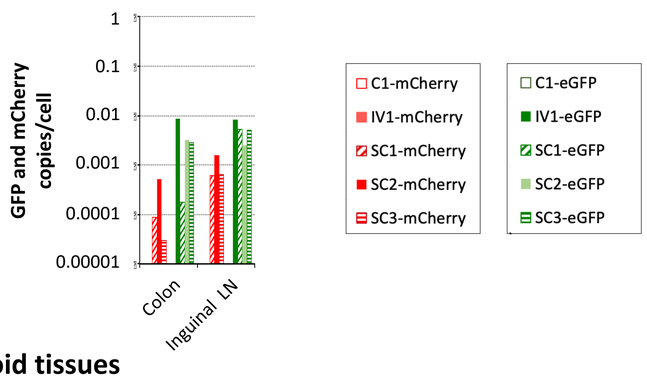
Fig 3. Experimental treatment timeline. Rapamycin was given daily (1 mg/Kg) via oral administration, with an initial dose of 2mg/Kg. Blood and tissue sampling was performed as indicated. Necropsy was performed on day 28. Kg – kilogram.

Biodistribution

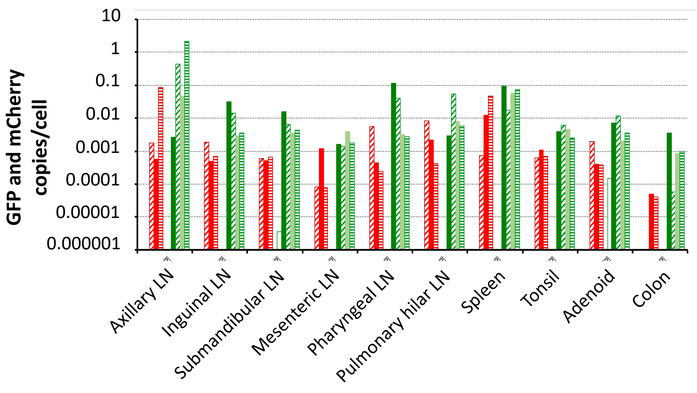
PBMCs



Biopsies



Lymphoid tissues



Non-lymphoid tissues

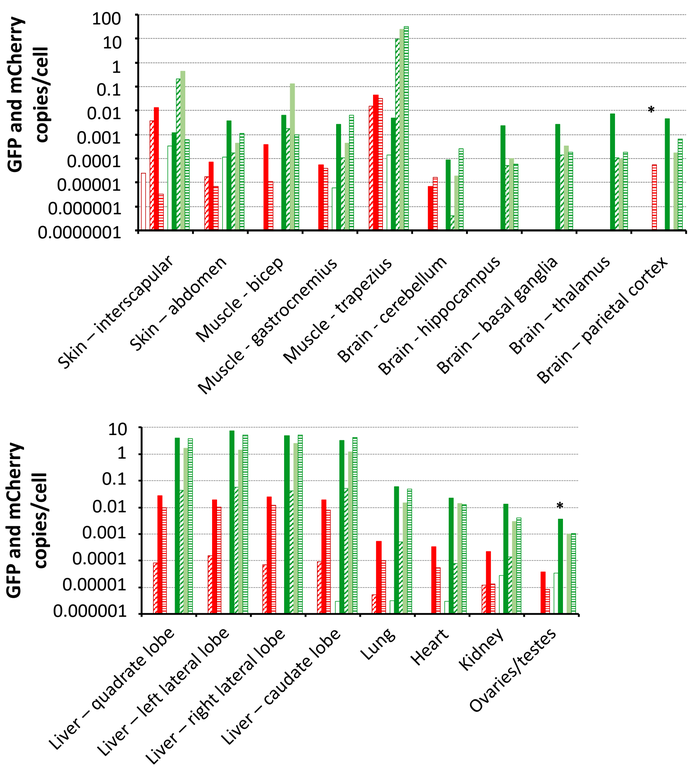


Fig 4. AAV vector biodistribution. Quantification of scAAV6-MND-GFP and scAAV6Δ-DARPin-55.2-MND-mCherry vector genomes (vg) in PBMCs at days 0-28, mononuclear cells from day 7 colon and inguinal lymph node biopsies, day 28 lymphoid necropsy tissues, and day 28 non-lymphoid necropsy tissues. All tissue DNA samples were treated with Chelex to remove PCR inhibitors. eGFP and mCherry copies are shown per two copies of the rhesus RPP30 housekeeping gene. MNC – mononuclear cells; LN – lymph node. Asterisk – PCR reaction inhibited despite Chelex treatment.

CBC values

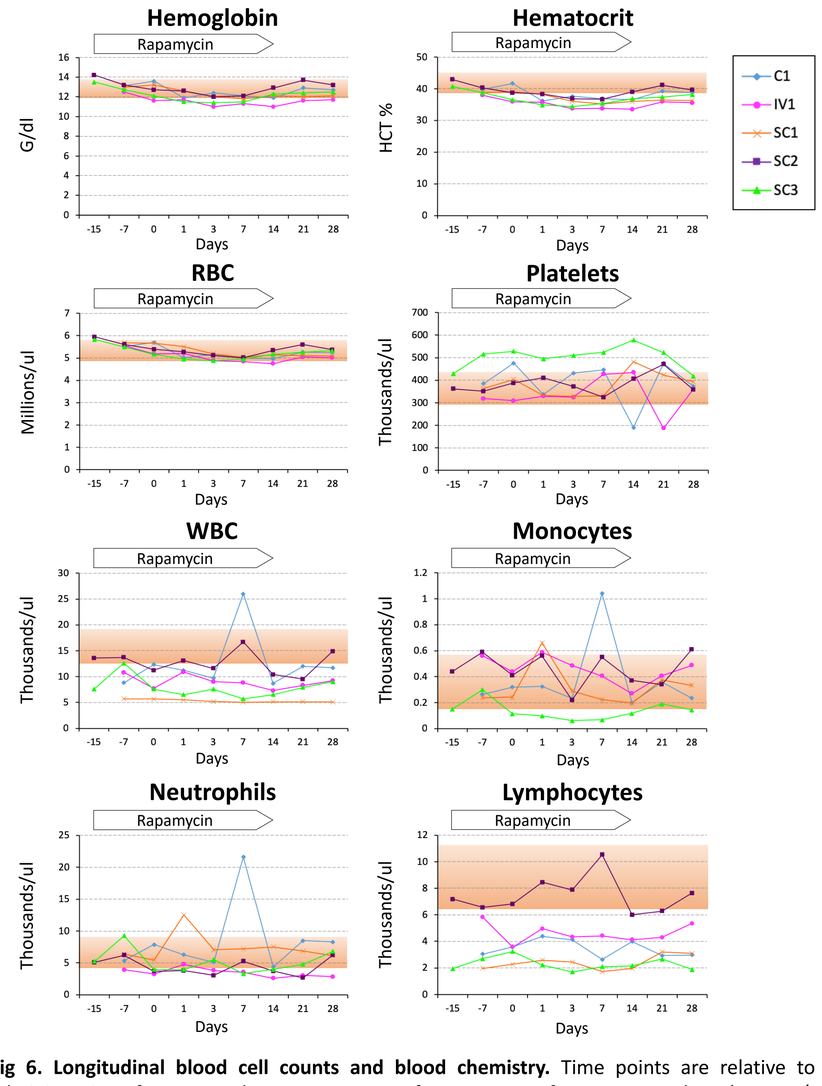


Fig 6. Longitudinal blood cell counts and blood chemistry. Time points are relative to administration of PBS control or AAV vectors. Reference ranges for *Macaca mulatta* (mean +/- SD) are indicated using data from Chen *et al.* (orange bars, PMID 20042049).

Gene transfer

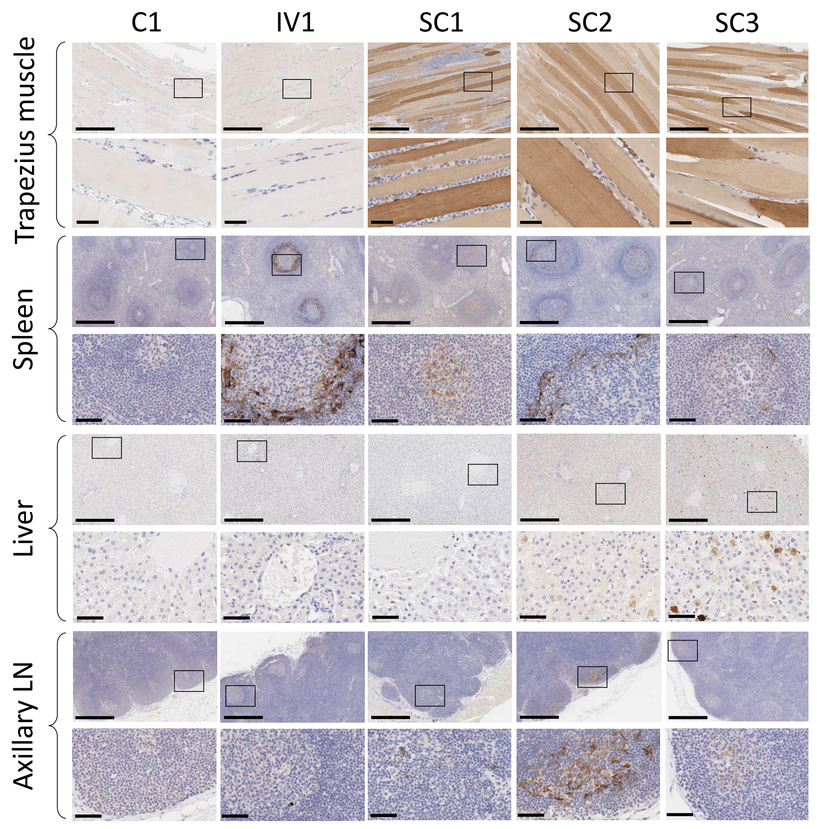


Fig 5. AAV6-mediated gene transfer. Trapezius muscle, spleen, liver and axillary lymph nodes from each animal were analyzed for GFP expression at day 28 post AAV administration. Serial sections were stained by immunohistochemistry with anti-GFP antibody. Scale bar = 500μm (upper 2 panels); 50μm (lower panels). Box inset is enlarged below.

Lymphocyte counts

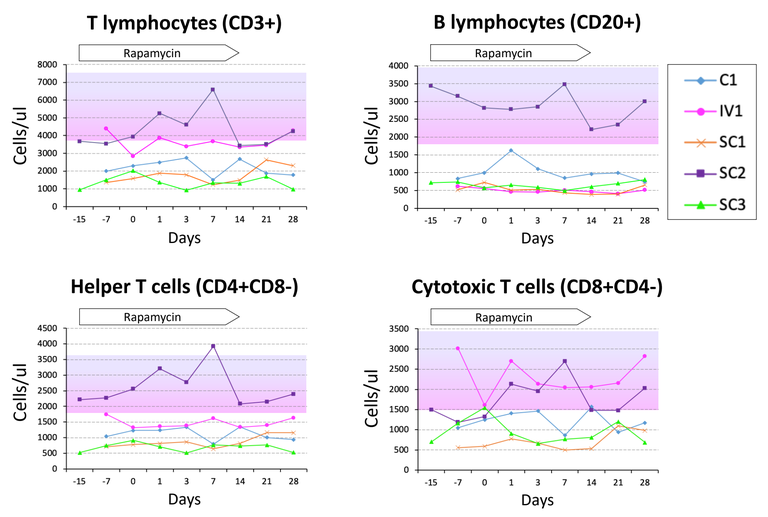


Fig 7. Longitudinal circulating lymphocyte counts. Time points are relative to administration of PBS control or AAV vectors. Reference ranges (pink bar) for *Macaca mulatta* (mean +/-SD) are indicated using previously reported data for males and females by Caldwell *et al*. 2016 (PMID 25600312).

Liver enzymes

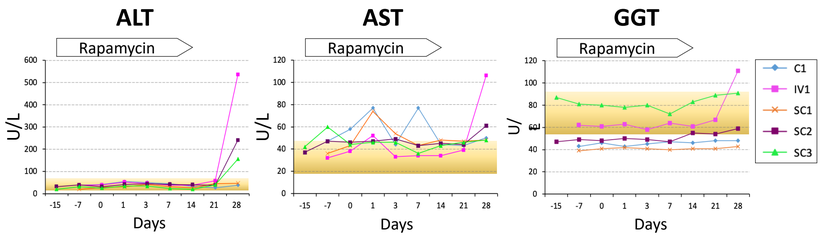


Fig 8. Longitudinal liver enzyme levels in blood. Time points are relative to administration of PBS control or AAV vectors. Reference ranges for *Macaca mulatta* (mean +/-SD) are indicated using data from Lee *et al.* (yellow bars, PMID 22909137). ALT – alanine transaminase; AST – aspartate transaminase; GGT – gamma-glutamyl transaminase.

Vector and transgene T cell responses

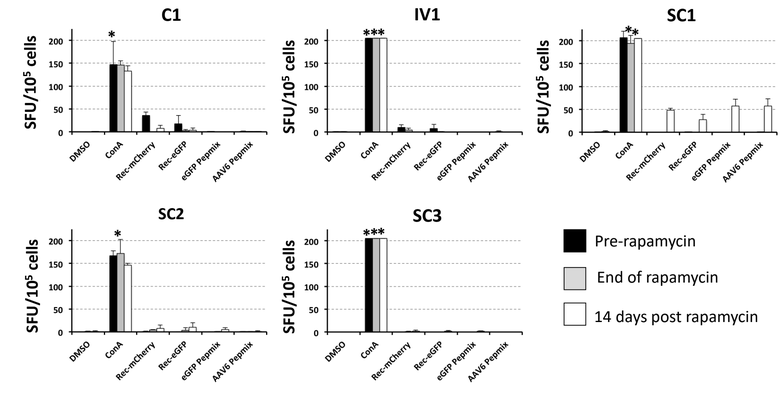


Fig 9. Capsid and transgene-specific cytotoxic T cell responses. AAV6, eGFP and mCherry specific T cell responses were measured for each animal by IFNγ ELISpot using PBMCs isolated before rapamycin treatment, at the termination of rapamycin treatment, and 14 days post cessation of rapamycin treatment. PBMCs were incubated with the indicated mitogen or antigen for 24 hr at 37°C. Asterisks = samples with at least one replicate that had too many spots to count. These spot counts were set to 205 counts per well, the maximum detected in this experiment. DMSO – Dimethyl sulfoxide; ConA – concanavalin A; Rec – recombinant; Pepmix – tiled 15mer peptides with 11 amino acid overlap.

Conclusions

- IV and SC injection resulted in detectable AAV6 and AAV6-CD4 vg in PBMCs and most organs up to 28 days post administration. The highest AAV6 and AAV6 and AAV6-CD4 vg levels were seen in liver, spleen, lymph nodes and muscle, suggesting that CD4 retargeting did not prevent vector sequestration.
- AAV6-CD4 transgene expression was not detected despite vg presence, but SC injection of AAV6 mediated efficient gene expression in muscle. Low-level gene expression was also seen in spleen, lymph nodes, and livers with both injection routes.
- Both vectors were also well tolerated. Liver enzymes were elevated in 3 of 4 treated animals, but not until two weeks after rapamycin treatment ended. SC1, the SC injected animal that received the shortest duration of rapamycin treatment, was the only animal with detectable T cell responses against transgene and AAV6 capsid at any time point.
- Our data suggest that rapamycin suppresses pre-existing anti-AAV immunity and that muscle is a potential target tissue for AAV6-based anti-HIV therapeutics.