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Modulating Immune Responses To AAV By Creating AAV Capsid Specific Chimeric Antigen Receptor T-regulatory Cells

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Introduction

AAV capsid induces T cell immune response

Early gene therapy clinical, using systemic AAV delivery resulted in robust CD8 T-cells responses to AAV capsid, and loss of transgene expression. The immune responses to capsid observed in patients, had never been observed in animal models. To overcome the immune response, now most trials include robust immunosuppression to maintain transgene expression.

Regulatory T cells (Tregs) modulate capsid specific immune responses and allow for sustained transgene expression

However, concurrent clinical trials using intramuscular delivery (IM) of AAV displayed long-term transgene expression despite significant immune infiltration. This long-term expression was attributed to the induction of Tregs in the muscle of AAV-injected patients. This infiltration suggested a immune modulatory role of T-regs to AAV capsid in IM trials .

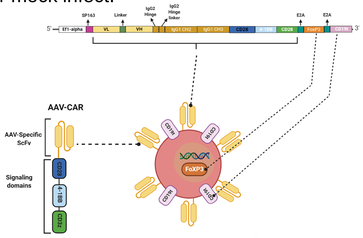
Currently there are no animal models that mimic the capsid specific immune response and loss of transgene expression observed in the clinic. Therefore we utilized chimeric antigen receptor (CAR) technology to create AAV specific CAR T-cells, to mimic T-cell immune response against AAV-capsids. In addition, to mitigate immune responses against AAV capsid, we created a AAV CAR Regulatory T-cells. These AAV CAR T-cells and AAV CAR T-regs can be used not only to study immune responses to AAV gene therapy but also as novel therapeutics for modulation of the immune response to AAV capsid for clinical applications.

Purpose

To optimize and characterize AAV-CAR Tregs for modulation of immune response to AAV gene therapy.

Materials and Methods

- We created CAR T-cells specific for AAV capsid using an ScFv from a previously published anti-AAV antibody, and a 3rd generation CAR construct. In order to create T-regulatory cells we added a 2A sequence to the CAR construct and a FoxP3 transcription factor.
- To test the functionality of AAV-CAR T cells and AAV-CAR Tregs we designed a luciferase-based killing (CAR) and inhibition of killing assay (CAR Treg). In this experiment we have created cell lines that constitutively express luciferase and use them as our target cells that we either infect with different AAV capsids or mock infect.



- To model the cellular immune response against AAV capsid, we injected AAV-CAR T cells into AAV-injected C57/BL6 mice. Mice received 5×10^{10} vector genome of AAV1-Alpha-1 antitrypsin (AAT) IM followed three weeks later by either 2.5×10^{6} AAV-CAR T cells or saline IV.
- To examine the immune modulatory ability of AAV-CAR Tregs *in vivo*, we employed the previously described immune response against AAV rh32.33 capsid. C57/BL6 mice received an intramuscular injection of 5×10^{10} vector genome of AAV-rh32.33 expressing AAT, and one week later a systemic injection of 2×10^{6} of AAV-CAR Tregs, non-specific expanded T-regs, or saline.
- To examine AAV-CAR Tregs ability to suppress transgene immune response, C57/BL6 mice were injected with AAV expressing ovalbumin(OVA) followed by either AAV-CAR T-regs, non-specific expanded Tregs or saline.

Results

AAV-CAR T cells and AAV-CAR Tregs respond to different AAV capsid variants *in vitro*

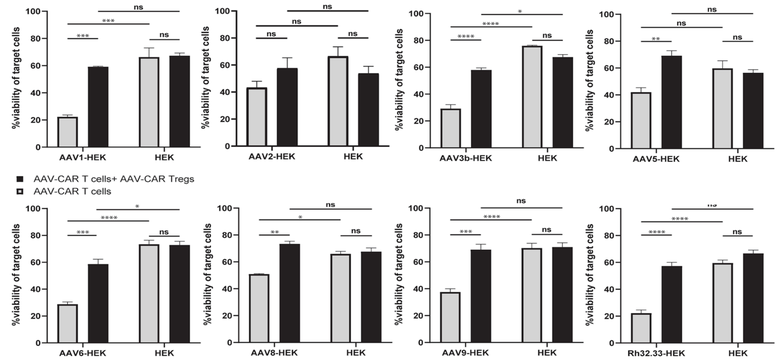


Figure 1: AAV-CAR T cells and AAV-CAR Tregs respond to antigen to functionally kill or repress killing of target cells. Luciferase expressing target cells are infected with AAV or mock infected then are co-cultured with AAV-CAR T cells or a mixture of AAV-CAR T cells and AAV-CAR Tregs. Luciferase expression is calculated to determine target cell viability.

AAV-CAR T cells clear transfected cells *in vivo*

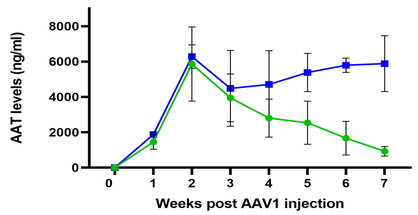


Figure 2: AAV-CAR T cells reduce expression of AAT. Animals are injected with AAV1-AAT, and AAV-CAR T cells or PBS are delivered at week 3 post AAV1 injection. Expression of AAT transgene were measured by ELISA.

AAV-CAR Tregs suppress capsid specific immune response *in vivo*

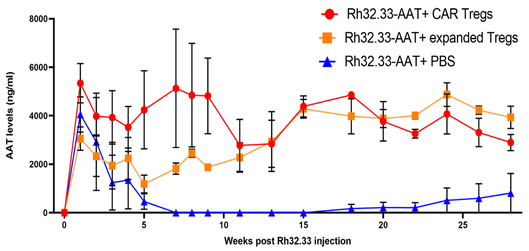


Figure 3: AAV-CAR Tregs suppress anti-capsid immune responses allowing for stable expression of AAT. ELISA for AAT expression on animals injected with rh32.33-AAT, and 1 week later injected with AAV-CAR Tregs, expanded Tregs or PBS.

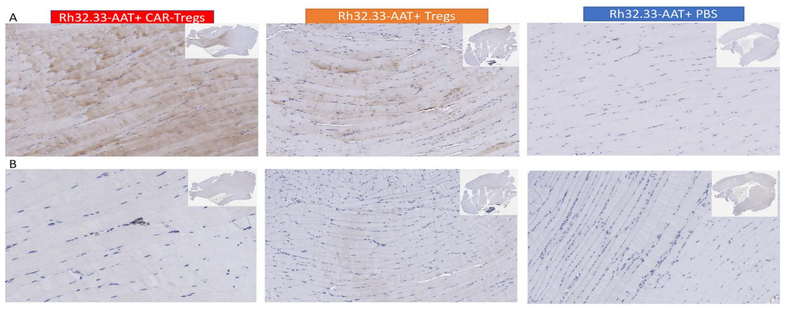


Figure 4: AAT gene expression observed after AAV-CAR Treg treatment. Animals injected with rh32.33-AAT vectors were harvested 28 weeks after AAV delivery. Immunohistochemical staining is observed in injected muscle (A) of animals treated with AAV-CAR T cells and Tregs but not PBS (B) CD4 immunohistochemical staining

Results

AAV-CAR Tregs modulate immune response against Ovalbumin

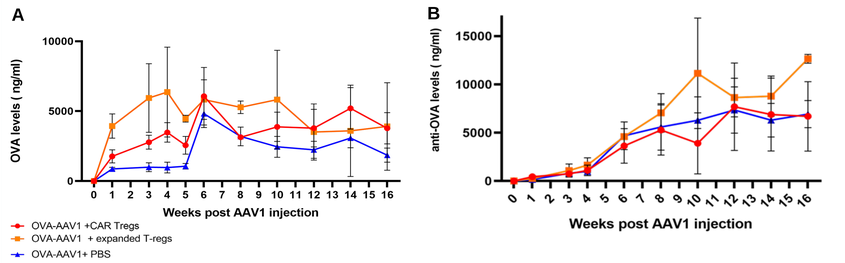


Figure 5: Stable expression of Ova after AAV-CAR Treg treatment in spite of Ova levels by ELISA (A) anti-Ova antibodies by ELISA (B). high aanti-OVA antibodies.

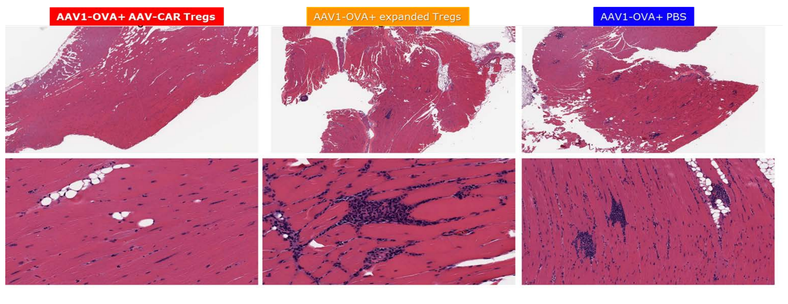


Figure 6: Severe myositis in PBS animals after AAV-Ova injection. H&E staining of injected muscle in animals treated with AAV-OVA followed by AAV-CAR Tregs, expanded Tregs or PBS. Diffuse and severe focal inflammation observed in Treg and PBS groups.

Summary and Conclusion

- In the cytotoxicity assays, AAV-CAR T cells killed infected cells with different AAV capsid variants, but not mock infected controls. Coculture with AAV-CAR Tregs significantly reduced this effect (Figure 1).
- We observed a robust reduction in AAT levels in animals that received AAV-CAR T cells, but not in saline treated animals (Figure 2).
- In mice receiving AAV-rh32.33-AAT (followed by saline), AAT levels began to increase over the first two weeks, and then dropped below the limit of detection, indicating a native effector T-cell response. However, mice receiving AAV-CAR Tregs continued to show increasing expression, indicating successful suppression of the effector T cell response (Figure 3, 4).
- In vivo, AAV-CAR Tregs were able to modulate immune responses to the immunogenic transgene OVA. Injected muscle histology revealed severe focal and diffuse myositis when untreated, significantly reduced inflammation was observed in the muscle of the animals treated with AAV-CAR Tregs despite a robust OVA expression within the muscle (Figure 5, 6).
- This study demonstrates that AAV-CAR Tregs provide a powerful new tool to model the cellular immune response against AAV capsid and ovalbumin in a mouse model.

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References

- Kuck D, Kern A, Kleinschmidt JA. Development of AAV serotype-specific ELISAs using novel monoclonal antibodies. J Virol Methods 2007;140:17-24.
- Mingozzi F, Maus MV, Hui DJ et al. CD8(+) T-cell responses to adeno-associated virus capsid in humans. Nat Med 2007;13:419-422.
- Mueller C, Chulay JD, Trapnell BC et al. Human Treg responses allow sustained recombinant adeno-associated virus-mediated transgene expression. J Clin Invest 2013;123:5310-5318.
- Allan SE, Alstad AN, Merindol N et al. Generation of potent and stable human CD4+ T regulatory cells by activation-independent expression of FOXP3. Molecular therapy : the journal of the American Society of Gene Therapy 2008;16:194-202.