MULTI-SEROTYPE NANOBODY-ENGINEERED AAV VECTORS FOR CD4 TARGETED GENE THERAPY

Martin V. Hamann[1], Daniel Foth[1], Priti Kumar[2], Ulrike C. Lange[1,3]

[1]Leibniz Institute of Virology (LIV) - Genomics of Retroviral Infections, Hamburg, Germany, [2]Yale School of Medicine - Infectious Diseases, New Haven, Connecticut, [3]University Medical Center Hamburg-Eppendorf (UKE) - Institute for Infection Research and Vaccine Development, Hamburg, Germany

HIV Obstruction by Programmed Epigenetics

0425

BACKGROUND

HIV cure approaches involving in vivo gene therapy have the potential to target prominent tissue-resident HIV reservoirs. Virus-based vectors are most common in clinical studies, with Adeno-associated Virus (AAV) being the vector of choice. Effectiveness of in vivo gene therapy largely correlates with high vector doses, but must be balanced to avert unwanted effects including immune responses and transgene activity in non-target cells. Modulating receptor binding specificities of therapeutic vectors increases on-target effectivity while reducing off-target effects. So far, no robust viral gene therapy vector specific for CD4-positive HIV target cells has been described. We here show that coupling of AAV capsid surfaces with CD4-specific nanobodies mediates retargeting of AAVs to CD4-positive cells. These CD4-AAVs could provide an invaluable vector for anti-HIV therapeutic approaches.

Figure 1: Schematic outlines of the Nanobody-engineered AAV capsids. Illustrated is the Nanobody genetic fusion within VP1 CDS.

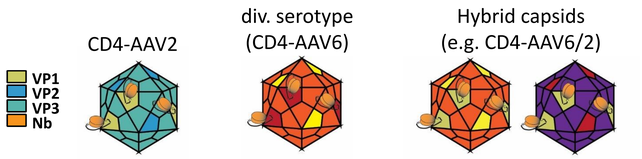
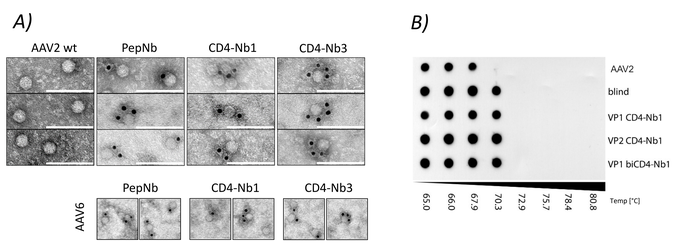


Figure 2: Morphological and thermal stability characterization of engineered AAV capsids. A) Immuno-gold electron microscopy of purified AAV preparations. B) Thermal stability assay using an antibody recognizing intact AAV capsids.



METHODS

We used structure-based rational design to genetically engineered CD4-specific nanobodies into AAV capsid proteins VP1 and VP2. We examined optimal nanobody/insertion-site combination for targeted reporter transgene delivery in vitro and in vivo. We applied our engineering platform to multiple AAV serotypes, proving system versatility. Electron microscopy (EM) and biochemistry analyses were used to investigate particle characteristics.

- using rational design we engineered CD4-specific nanobodies onto AAV capsids
- AAV transduction show high specificity for CD4 positive cells in vitro and in vivo
- beneficial AAV vector characteristics preserved
- amenable to multiple AAV serotypes
- platform technology – exchangeable and expandable

- these CD4-AAV's are a valuable addition to the limited toolbox of CD4 specific delivery systems

RESULTS

Functional competition assays with CD4-AAV vectors revealed highly specific transduction of CD4-positive cells in vitro. Isolated human primary CD4-positive T cells from multiple donors are effectively transduced compared to wild-type AAV serotypes. CD4-positive cells were transduced with 5-fold higher specificity compared to CD4-negative cells in vitro. CD4-specific transduction was also observed in humanized mice after intravenous delivery. EM and biochemical characterization of CD4-AAV particles revealed overall beneficial features: regular morphology, increased capsid stability, nanobody presentation on the capsid outer shell, exceeding titers and unaffected genome packaging compared to wild-type AAVs.

Figure 3: In vitro AAV2 CD4-specificity mixed culture assay. A) Experimental outline of HeLa mixed culture assay. B) Flow cytometry analysis 3dpi.

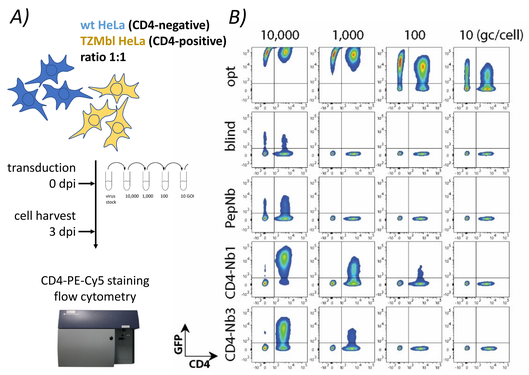


Figure 4: In vitro AAV2 transduction of Jurkat 1G5 T cells. A) CD4 expression histogram in 1G5 cells. B) Flow cytometry analysis 3dpi. C) Cumulative data from three independent experiments.

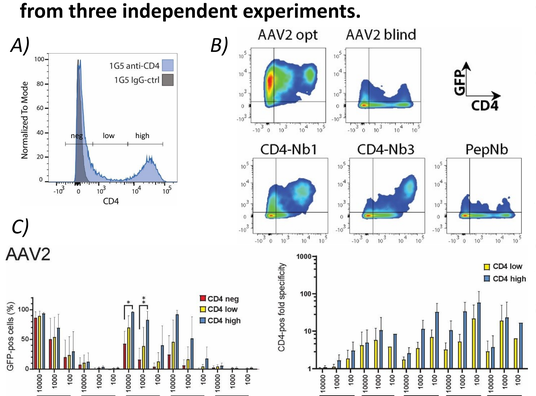
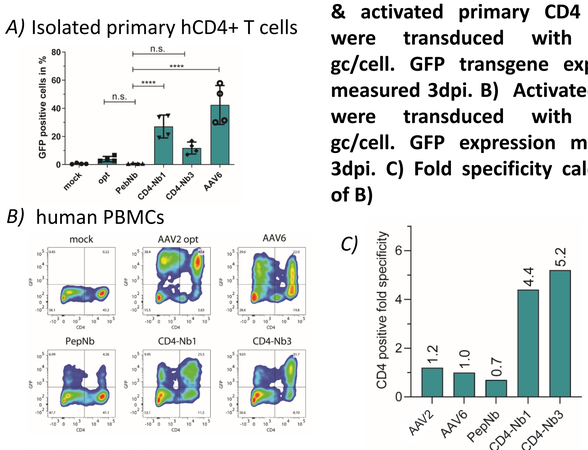


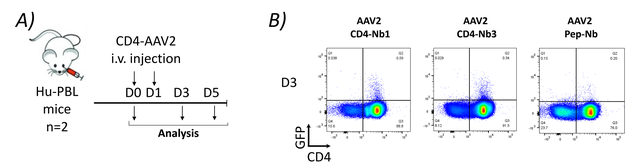
Figure 5: In vitro AAV2 transduction of primary CD4 T & PBMCs. A) Isolated & activated primary CD4 T cells were transduced with 20,000 gc/cell. GFP transgene expression measured 3dpi. B) Activated PBMC were transduced with 10,000 gc/cell. GFP expression measured 3dpi. C) Fold specificity calculation of B)



CONCLUSIONS

We provide proof-of-concept for a novel AAV-based vector platform with high specificity towards CD4-positive cells. These nanobody-engineered AAVs further preserve all beneficial AAV vector characteristic. Adaptation to other serotypes and exchange of nanobodies is straightforward. In combination with Crispr/Cas modules or HIV-specific recombinases, we proposed CD4-AAV vectors as valuable tool for safe and efficient anti-HIV in vivo gene therapy approaches.

Figure 6: In vivo AAV2 transduction in hu-mice model. A) Experimental outline. B) Representative flow cytometry analysis at 3dpi.



Acknowledgements

We thank the entire Ulrich Rothbauer lab (Tübingen University) for providing CD4 specific nanobodies, the Büning lab (Hannover Medical School) for help characterizing AAV2 particles as well as the imaging core facility at LIV for EM analysis. Furthermore, we thank all the collaborators in the HOPE MDC for their feedback and suggestions.

Funding

This research was supported by NIAID award number UM1AI164559, co-funded by NHLBI, NIDA, NIMH, NINDS, and NIDDK.

Federal Ministry of Education and Research

DZIF Deutsches Zentrum für Infektionsforschung

Presented in
Seattle, Washington 19-22 February 2023
2023 Conference on Retroviruses and Opportunistic Infections (CROI)