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antibodies MDPI

Evaluation of anti-drug antibody formation in response to AAV-mediated monoclonal antibody expression in sheep

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INTRODUCTION & AIM

Vectored Immunoprophylaxis (VIP)
- Delivery of genetic instructions for expression of a monoclonal antibody (mAb) toward a pathogen using a viral vector [1]
- Shown to be effective in preventing HIV, malaria, dengue, hepatitis C and influenza in animal models [1]
- Promising platform for diseases which do not have traditional vaccines and for immunocompromised or immunosenescent individuals [1]

Adeno Associated Virus (AAV)
- High-efficiency in vivo gene transfer, low pathogenicity, low levels of genome integration [2]

Roadblocks in VIP Research
- Short-lived antibody expression concurrent with Anti-drug antibodies (ADA) (present in most VIP studies) and anti-capsid immune responses [1]

Guelph Sheep: A Success
- **AAV6.2FF**-mediated expression of a **Marburg virus mAb** persisted for more than **1,100 days** following administration to 2-week-old sheep [2]
- Low ADA and anti-capsid response [2]

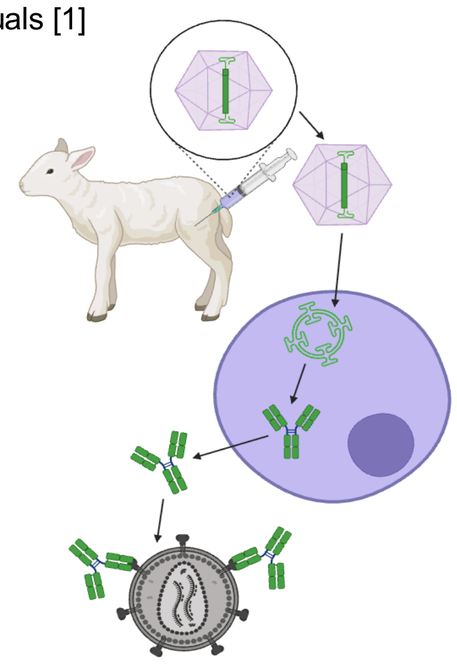

Fig 1. Visual depiction of how VIP provides immunity to the host.

Potential Contributing Factors To Success
- AAV capsid used
- Age of animals used
- Level of sequence divergence of mAb used
  - Antibodies that have gone through more somatic hypermutation will be more divergent from their closest germline sequence and may therefore be more immunogenic [3]

Aim: Investigate effect of animal age, AAV serotype and level of mAb divergence from germline on ADA response to AAV-VIP using a sheep model

METHOD

The outcomes of eight experimental groups will be compared. Groups will be composed of; 2-week-old lambs or 6-month-old sheep; AAV6.2FF or AAV8; mAbs VRC07 (isolated from chronic HIV infection) or MR191 (isolated from a survivor of an acute Marburg Virus infection).

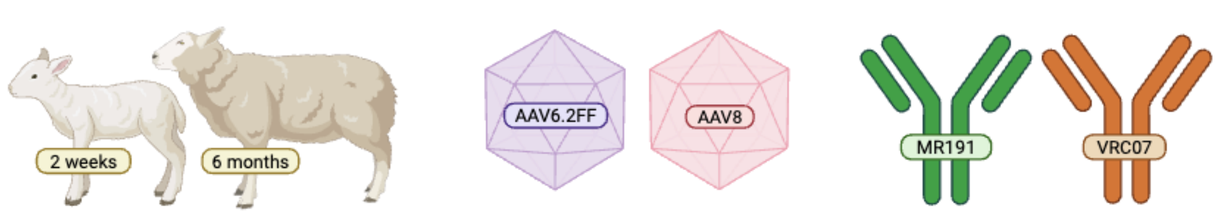

Fig 2. Visual depiction of independent variables being evaluated in this study.

Antibody Design
mAbs are expressed from a single expression cassette containing; ubiquitous CASI promoter, human IgG1 heavy chain and lambda (MR191), or kappa (VRC07) light chain, F2A self-cleaving peptide, WPRE, miR-142-3p microRNA binding site, and poly-A signal, flanked by ITRs.

Fig 3. mAb expression constructs for MR191 (left) and VRC07 (right).

Virus Production
Recombinant AAV are produced in HEK293 cells, purified via Heparin Sulfate (AAV6.2FF) or POROS CaptureSelect AAVX (AAV8) columns. Coomassie and alkaline gels are performed for QC and titre is obtained via qPCR.

PRELIMINARY RESULTS & DISCUSSION

Preliminary Mouse Study
Recombinant AAV6.2FF and AAV8 carrying expression cassettes for MR191 or VRC07 mAbs were administered to 6-week-old Balb/C mice (n=4). Serum was collected weekly up to 28 days, then biweekly up to 70 days. Transgene levels were quantified by human IgG ELISA.

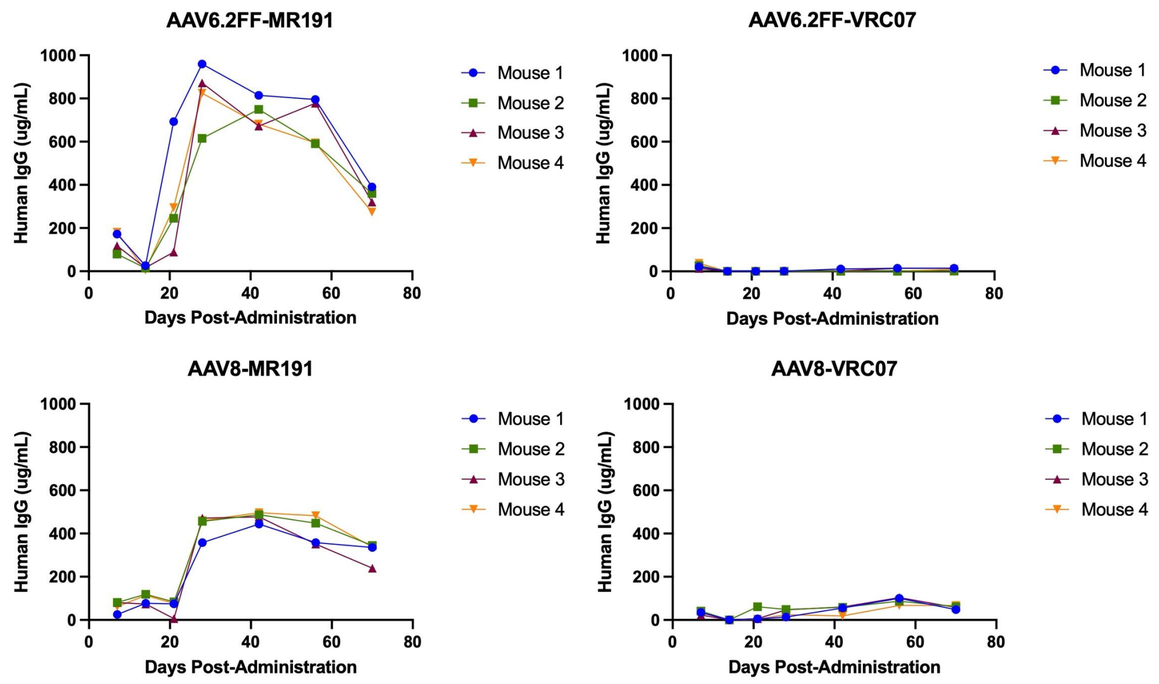

Fig 4. Human IgG expression levels in mice over 70 days. AAV6.2FF-MR191 (top left), AAV6.2FF-VRC07 (top right), AAV8-MR191 (bottom right), AAV8-VRC07 (bottom right).

Results
- AAV6.2FF-VRC07 and AAV8-VRC07 had lower transgene expression when compared to AAV6.2FF-MR191 and AAV8-MR191, respectively
  - VRC07 was derived from a patient with a chronic HIV infection and would have gone through more somatic hypermutation, resulting in 44% (VH) and 29% (VL) divergence from germline [4], which may have resulted in a higher immune response to this mAb compared to MR191, which has 25% (VH) and 26% (VL) divergence from germline
- AAV6.2FF-MR191 showed higher transgene expression than AAV8-MR191
  - AAV6.2FF had mutations introduced that led to higher transgene expression and may have resulted in higher expression of the less-immunogenic MR191 mAb than when delivered by AAV8 [5]
- AAV6.2FF-VRC07 showed lower transgene expression than AAV8-VRC07
  - AAV8 has tissue tropisms for the liver, which processes antigens in a more tolerogenic manor and may have reduced the host immune response to VRC07 compared to AAV6.2FF delivery [6]

CONCLUSION

Antibodies that have undergone more somatic hypermutation will be more divergent from germline and may be more immunogenic. This is a possible reason that VRC07 showed lesser transgene expression in both capsids when compared to MR191.

FUTURE WORK / REFERENCES

The expression of mAbs MR191 and VRC07 in AAV6.2FF and AAV8 capsids will be evaluated in immune deficient mice to confirm if the decreased VRC07 expression is a result of an immune response. Additionally, all four constructs will be tested in 2-week-old and 6-month-old sheep.

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