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Development of Blood Brain Barrier Penetrating Antibody Therapeutics for Encephalitic Alphaviruses

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Abstract

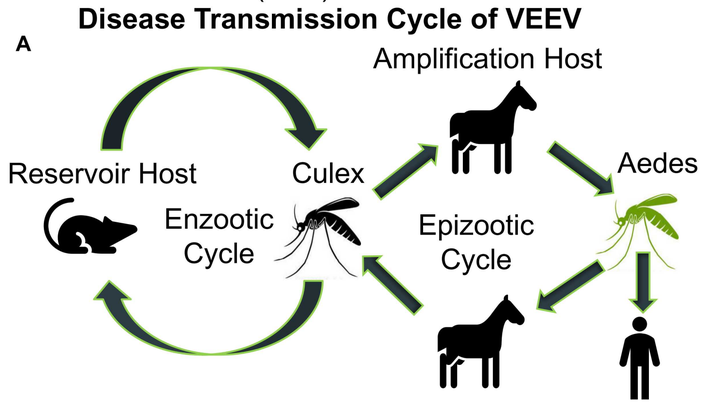
New World alphaviruses, including Venezuelan equine encephalitis virus (VEEV), cause highly pathogenic diseases in humans that exhibit overt encephalitis in a significant percentage of cases. VEEV is considered a potential biological weapon and is identified as a Category B pathogen. There are currently no FDA-approved vaccines or drugs to prevent or treat neurotropic infections caused by VEEV and similar encephalitic viruses. In the last few decades there have been several innovations in antibody (Ab) discovery, design, and characterization which have resulted in the introduction of highly effective therapeutic monoclonal Abs (mAbs). To produce Ab therapeutics that can protect against CNS infection with VEEV we are using Ab engineering to optimize BBB penetration, plasma half-life, and Ab function. For this project, Parental neutralizing mAbs that are effective prophylactically against VEEV infection are being engineered with several modifications to improve their efficacy. Parental Abs include F5, a highly potent neutralizing anti-VEEV mAb and highly potent neutralizing Abs generated by Dr. Michael at Washington University School of Medicine. Engineered recombinant Abs with and without these modifications are generated using both recombinant methods and the heavy and light chain sequences from the parental Abs. Changes in avidity and affinity is evaluated for each modification in comparison to the parental Abs by ELISA, plaque neutralization assay (PNA) and other biochemical methods. Recombinant Abs are then screened for enhanced BBB penetrance using an *in vitro* BBB transwell assay followed by biodistribution analyses in our mouse model to determine localization of our Abs in the brain, blood and other organs over time. Pharmacokinetics (PK) and biodistribution of the recombinant Abs are compared to that of the parental anti-VEEV Abs and the parental BBB-penetrating ligands. In conclusion we describe the production and *in vitro* and *in vivo* characterization of VEEV-targeting neutralizing Abs with improved PK profile and efficacy.

Background

Venezuelan Equine Encephalitic Virus (VEEV)

- VEEV is a neurotropic virus communicable via aerosol or vector exposure
- There is no therapeutic intervention for VEEV
- VEEV causes encephalitis & other debilitating neurological sequelae
- Encephalitis is particularly challenging to address because the brain is an immune-privileged site - extraneous immune cells & molecules are kept out by the action of the blood brain barrier (BBB)

Disease Transmission Cycle of VEEV



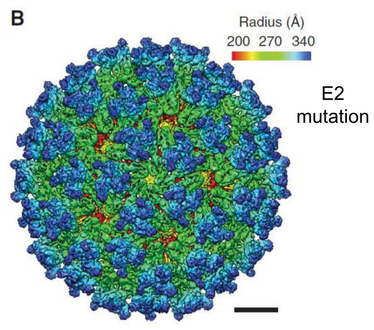
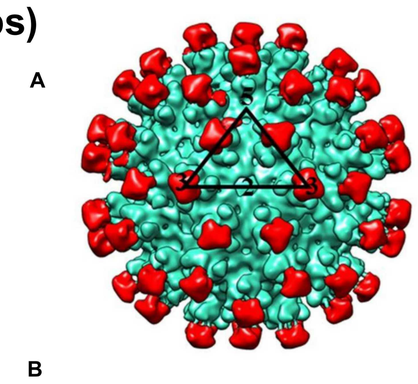


Figure 1: A. Enzootic VEEV subtypes are maintained efficiently in transmission cycles involving rodents and Culex mosquitoes, which live in sunny humid environments. Mutations in the E2 glycoprotein are selected by equines because they generate high titer viremia leading to amplification. The resultant epidemic strains are transmitted by abundant floodwater mosquitoes (*Aedes)* that have wider host ranges including equines and humans. Spillover to humans who live in proximity to infected equines results in epidemics involving up to hundreds of thousands of people. **B.** Radially colored 3D reconstruction of VEEV, showing the E1 basal triangle (green) and the E2 central protrusion (blue) for each spike. Scale bar: 10nm.

Neutralizing Monoclonal Antibodies (mAbs)

- Neutralizing mAbs bind the virus surface protein epitopes required for host cell binding and subsequent infection
- mAbs shown (TRD2A2, VEEV 8, and VEEV71) were generated by Diamond Lab and top candidates were selected based on binding affinity (ELISA), Plaque Neutralization capacity, & *in vivo* activity



The Blood Brain Barrier (BBB)

- A tightly regulated vascular network of endothelial cells, pericytes, & astrocytes, & separates the brain from the rest of the body
- The stringency of the BBB is essential to preventing unnecessary exposure to exogenous agents that can cause damage or inflammation
- The BBB is also the greatest obstacle to delivering potentially life-saving drugs to circumvent infection & ensuing brain damage

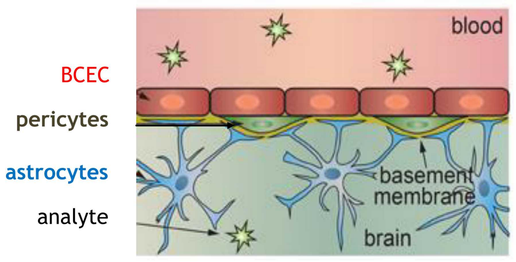


Figure 2: A. Cryo-EM structure of VEEV TC83 with F5 Fab bound to the surface of E2 glycoprotein, *Journal of Virology*, **2014**, 88, 9616-9623. **B.** Cellular organization of the BBB.

Developing Bispecific Antibodies

BBB-Penetrating Bispecific Antibodies

- BBB-shuttling bispecific antibodies (bsAbs) have both a therapeutic function (viral neutralization) and a transport capability, via receptor mediated transcytosis
- Multiple BBB-penetrating moieties and configurations were explored for their ability to facilitate BBB penetration
- Recombinant Abs were expressed in Expi-CHOs and purified using both affinity and size-exclusion chromatography

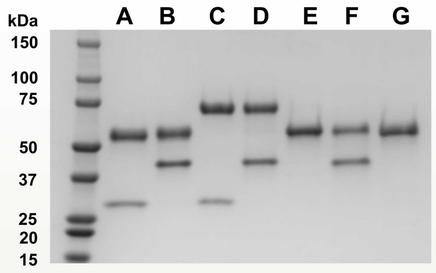
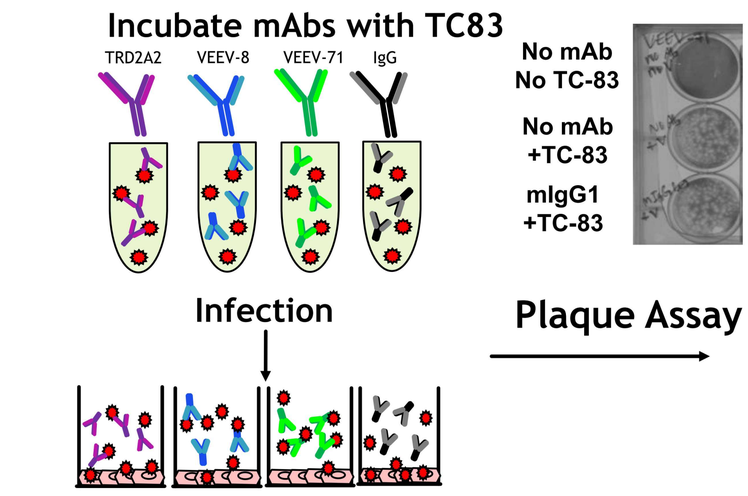


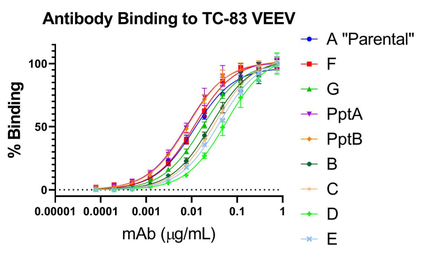
Figure 3: SDS-PAGE of recombinant mAb, and bsAb produced in house.

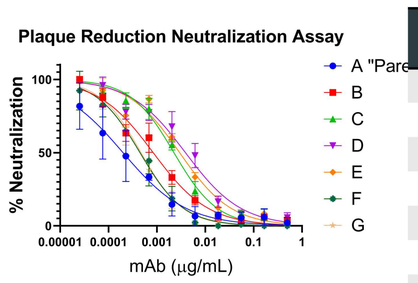
Characterizing BBB Penetration

Bispecific Antibody *in vitro* Characterization

- All bsAbs produced maintain therapeutic potency by ELISA using BPL inactivated TC83 and retain their ability to neutralize viral entry via plaque neutralization assay using live TC83



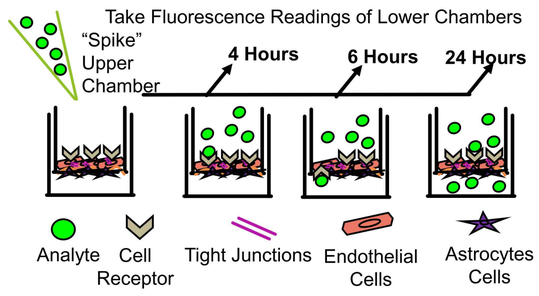


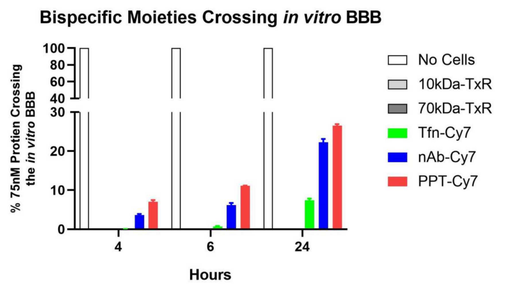


Antibody	$K_{D,App.}$ (ng/mL)	$PRNT_{50}$ (ng/mL)
A (Parental)	12	0.2
F	12	0.5
G	18	0.5
E	27	0.8
C	32	2.3
D	56	3.9
E	40	3.0
PptA	8	
PptB	8	

Modeling the Blood Brain Barrier to Screen for Active Receptor-Mediated Translocation (RMT)

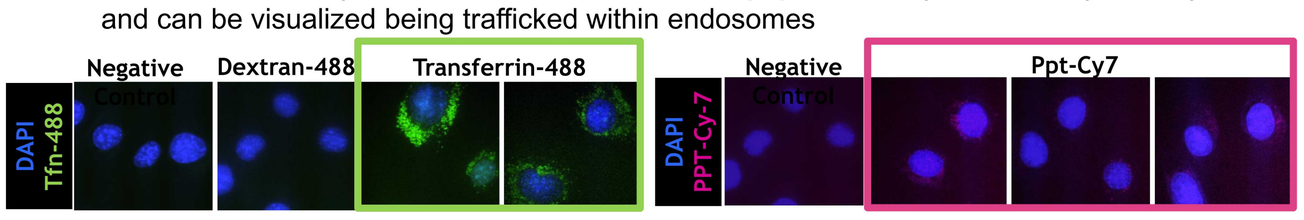
- An artificial BBB has been developed to evaluate transcytosis of each of our parental and bsAbs using co-cultured bEND.3 cells on the apical side of the transwell and C6 glioma cells on the luminal side.





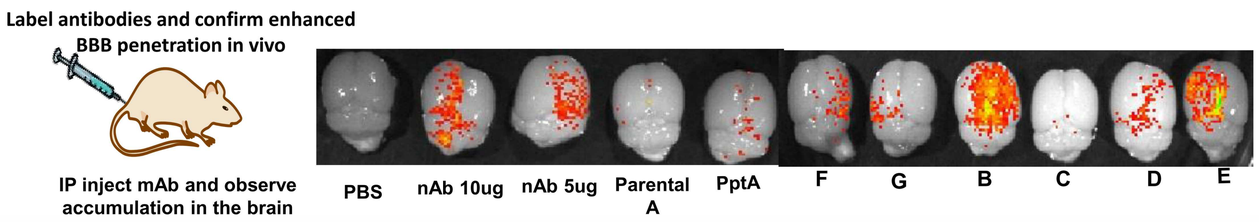
Our *in vitro* BBB Exhibits Receptor-Mediated Uptake

- Both fluorescent dye-labeled Transferrin and our peptide moiety are actively endocytosed and can be visualized being trafficked within endosomes



Bispecific Antibodies Penetrate the BBB *in vivo*

- Using *in vivo* imaging (IVIS) and Cy7-labeled controls and Cy7-bsAbs we find several of our antibodies are able to penetrate the BBB and can be observed 4 hours post-injection in the parenchyma



Conclusion and Future Work

- Our team has produced several configurations of bsAb which maintain their ability to neutralize TC83 virus and show initial penetration of the BBB in initial biodistribution studies
- Next steps include determining pharmacokinetic profiles for the bsAbs and continuation of *in vivo* efficacy studies using BSL3 VEEV Trinidad strain.

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