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Sandia National Laboratories

Development of Blood Brain Barrier Penetrating Antibody Therapeutics for Encephalitic Alphaviruses

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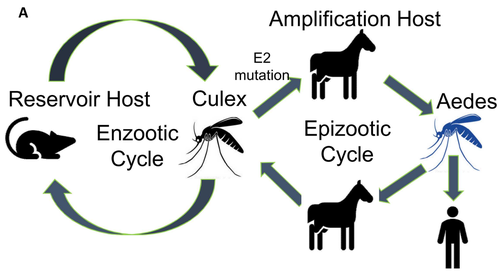
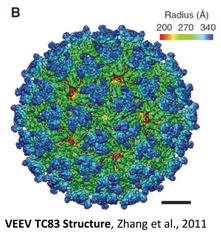
Background

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



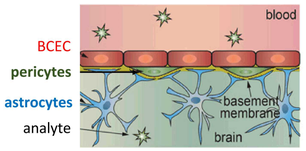


VEEV TC83 Structure, Zhang et al., 2011

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.

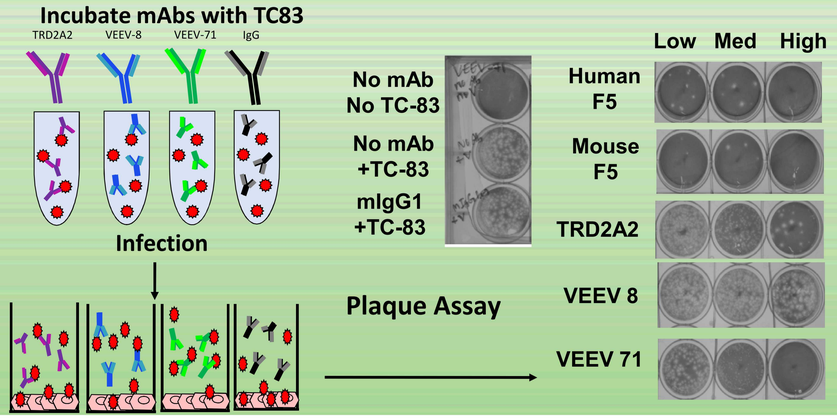
The Blood Brain Barrier (BBB)

- 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



Neutralizing Monoclonal Antibodies (mAbs)

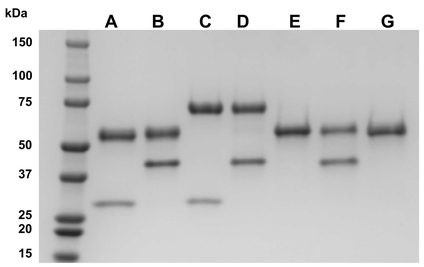
- Neutralizing mAbs bind the virus surface protein epitopes required for host cell binding and subsequent infection
- mAbs shown (TRD2A2, VEEV 8, and VEEV71) were generated by Diamond Lab and top candidates were selected based on binding affinity (ELISA), Plaque Neutralization capacity, & *in vivo* activity



Developing Bispecific Antibodies

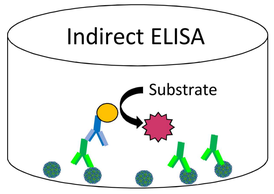
BBB-Penetrating Bispecific Antibodies

- 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 FPLC

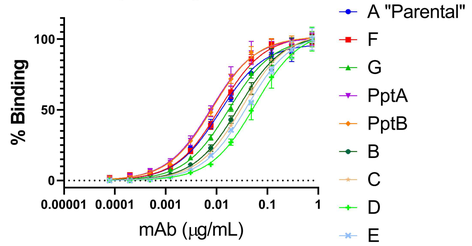


Bispecific Antibody *in vitro* Characterization

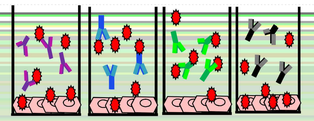
- All bsAbs produced maintain therapeutic potency by ELISA and retain their ability to neutralize viral entry via plaque neutralization assay

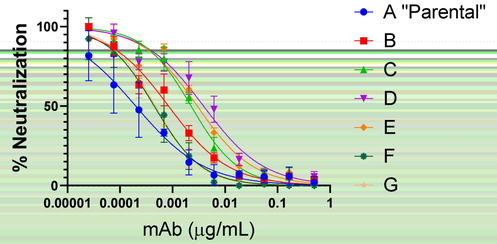


Antibody Binding to TC-83 VEEV



Plaque Reduction Neutralization Assay



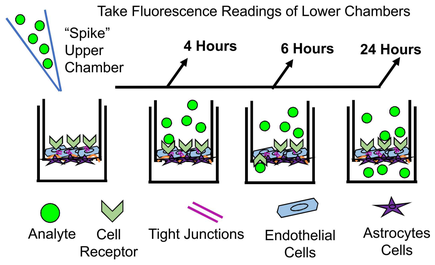


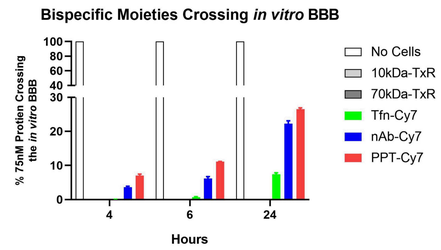
Antibody	$K_{D,App.}$ (ng/mL)	$PRNT_{50}$ (ng/mL)
A (Parental)	12	0.2
F	12	0.5
G	18	0.5
E	27	0.8
C	32	2.3
D	56	3.9
E	40	3.0
PptA	8	
PptB	8	

Characterizing BBB Penetration

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

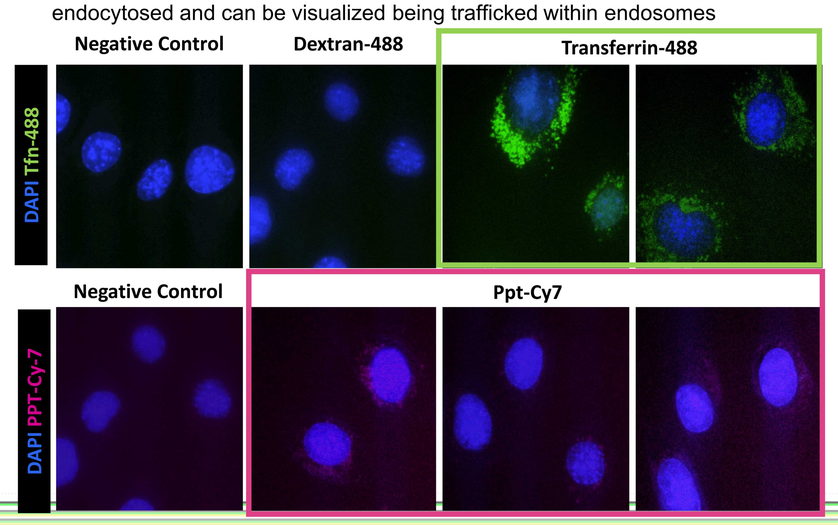
- An artificial BBB has been developed to evaluate transcytosis of each of our parental and bsAbs using co-cultured bEND.3 cells on the apical side of the transwell and C6 glioma cells on the luminal side.





Our *in vitro* BBB Exhibits Receptor-Mediated Uptake

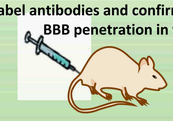
- Both fluorescent dye-labeled Transferrin and our peptide moiety are actively endocytosed and can be visualized being trafficked within endosomes



Bispecific Antibodies Penetrate the BBB *in vivo*

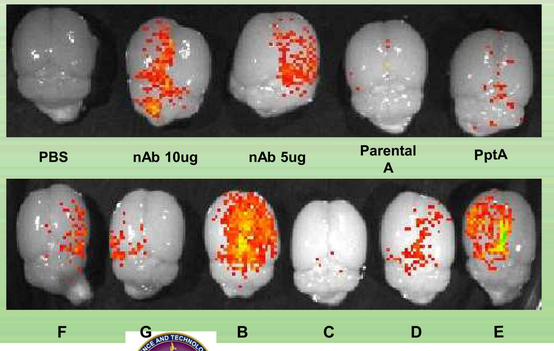
- Using *in vivo* imaging (IVIS) and Cy7-labeled controls and Cy7-bsAbs we find several of our antibodies are able to penetrate the BBB and can be observed 4 hours post-injection in the parenchyma

Label antibodies and confirm enhanced BBB penetration in vivo



IP inject mAb and observe accumulation in the brain

PerkinElmer IVIS for Imaging



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