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An Anti-VEEV BBB-penetrating bispecific provides comparable therapeutic protection in the context of VEEV-TrD infection *in vivo*

Jennifer L. Schwedler[1], Maxwell A. Stefan[1], Christopher A. Sumner[1], Christine E. Thatcher[1], Peter R. McIlroy[1], Anupama Sinha[1], Gloria E. Doudoukjian[1], Devon G. Welborn[1], Ashlee M. Phillips[2], Dina R. Weilhammer[2], and Brooke Harmon[1]

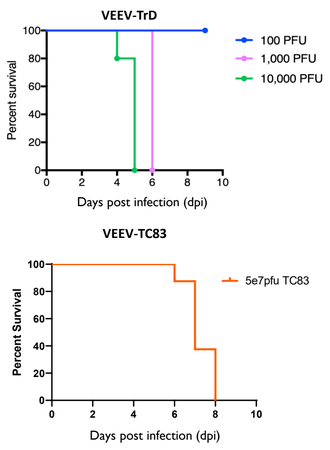
[1]Sandia National Laboratories, Livermore CA , [2]Lawrence Livermore National laboratory, Livermore CA

Abstract

The Blood Brain Barrier (BBB) provides a unique challenge for delivering therapeutics to the central nervous system due to high stringency, regulated access, and selective transport of ions, molecules, cells, toxins, and pathogens. The recent pandemic illustrates the interconnected nature of the global population, and as such, the seriousness of emerging neurotropic chemical and biological agents that can severely harm global health and security if effective medical countermeasures remain lacking. The quality of a therapeutic traversing the blood brain barrier (BBB) is highly sought after for medical countermeasures against diseases and infections afflicting the Central Nervous System (CNS), but has had limited success delivering therapeutically relevant doses to the brain with high-affinity targeting of endogenous receptors for receptor mediated transcytosis, or RMT (e.g. transferrin receptor, TfnR, insulin receptor, INSR, or low density lipoprotein receptor-related protein, LRP1). A previously identified brain-targeting single-domain antibody, "BrNb", has shown promise in BBB-penetrating (BBBP) delivery in monovalent and bivalent formats, but never before in combination with an anti-encephalitic alphavirus therapeutic Ab. Venezuelan equine encephalitis virus, (VEEV) is a category B select agent that can induce febrile illness, body aches, and inflammation of the CNS, leading to severe neurological sequela and even death in equines and humans. In addition, VEEV can be easily produced in large volumes and can spread by aerosol or transdermal inoculation, making it an ideal bioweapon due to its scalability and transmissible nature. Although several promising neutralizing and non-neutralizing mAbs have been identified, there are no approved vaccines or therapeutics to combat encephalitic alphaviruses. Furthermore, upon VEEV seeding the brain and subsequently establishing a replicative viral niche, the infection is difficult to contain without incurring severe neuropathological consequences due to an unbridled inflammatory immune response. We engineered a multivalent anti-VEEV therapeutic which not only rapidly crosses the BBB and is retained for up to 72 hours post injection, but also exhibits neutralization capacity and *in vivo* protection efficacy comparable to the parental antibody, human F5 (hF5).

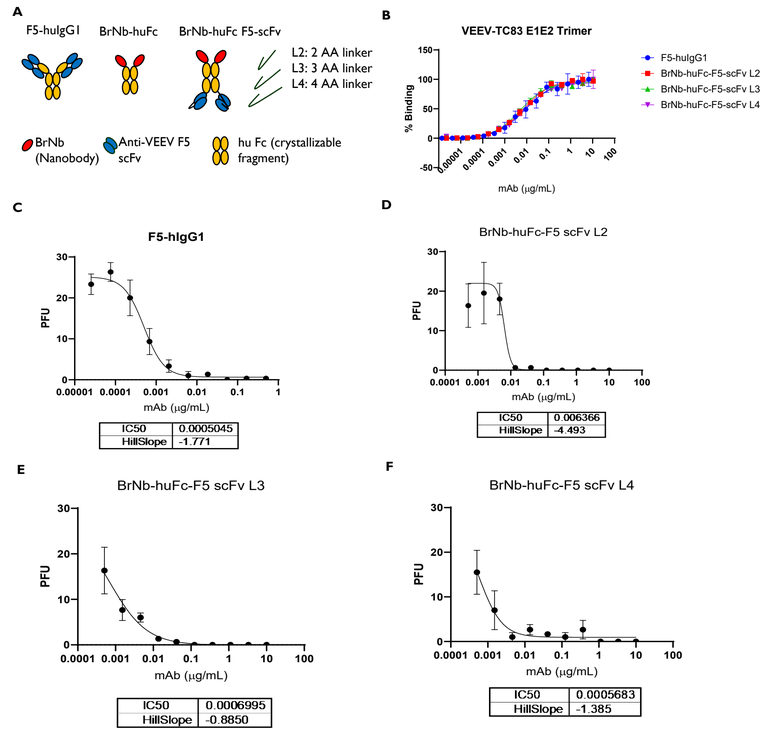
By designing and testing a BBBP bsAb against VEEV, we begin to address the approach for working within the confines of a delicate balance between viral neutralization and neuroimmune engagement. We describe here, our method of successfully generating a BBB-targeting, VEEV-neutralizing bispecific antibody (bsAb) therapeutic, and report on the functional changes in immune effector engagement observed in this bsAb. Our work highlights these crucial elements needed for the thoughtful engineering and execution of brain-targeting therapies to reduce incidence of harmful neurological sequelae, and greatly improve disease outcomes, allowing us to better treat and protect the nation and world.

Venezuelan Equine Encephalitis Virus (VEEV) is a Neuroinvasive Pathogen

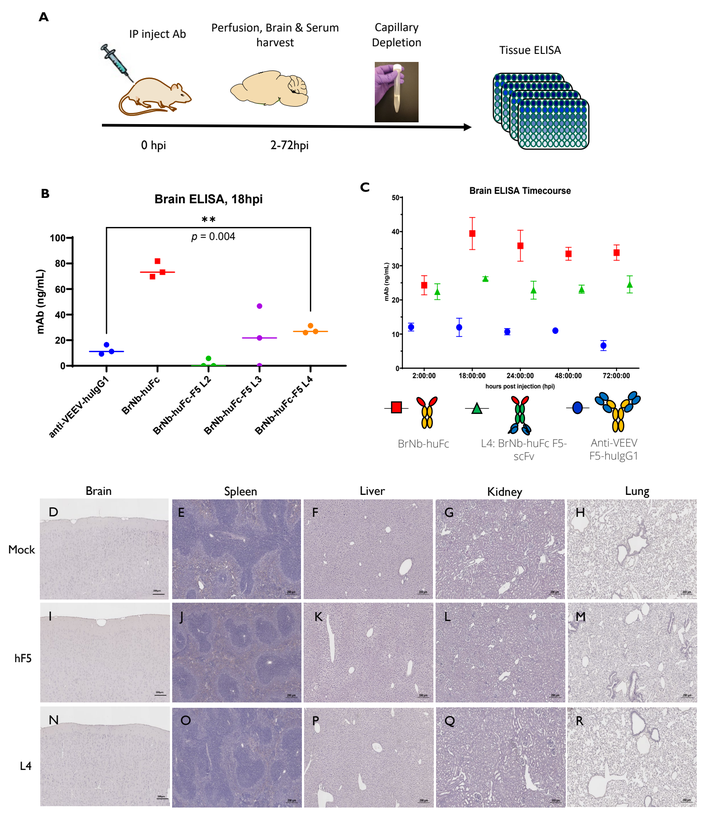


- Mosquito-borne virus affecting humans, equines, and other mammals
  In humans: can cause deadly inflammation of the brain
- Top priority biodefense pathogen (aerosol transmission, no vaccines or therapeutics)
- Endemic throughout Central and South America, but climate change is expanding the geographic range of the mosquitoes that harbor VEEV[1]
- Mouse infection models include an attenuated strain VEEV-TC83 & fully-virulent VEEV-TrD[3]

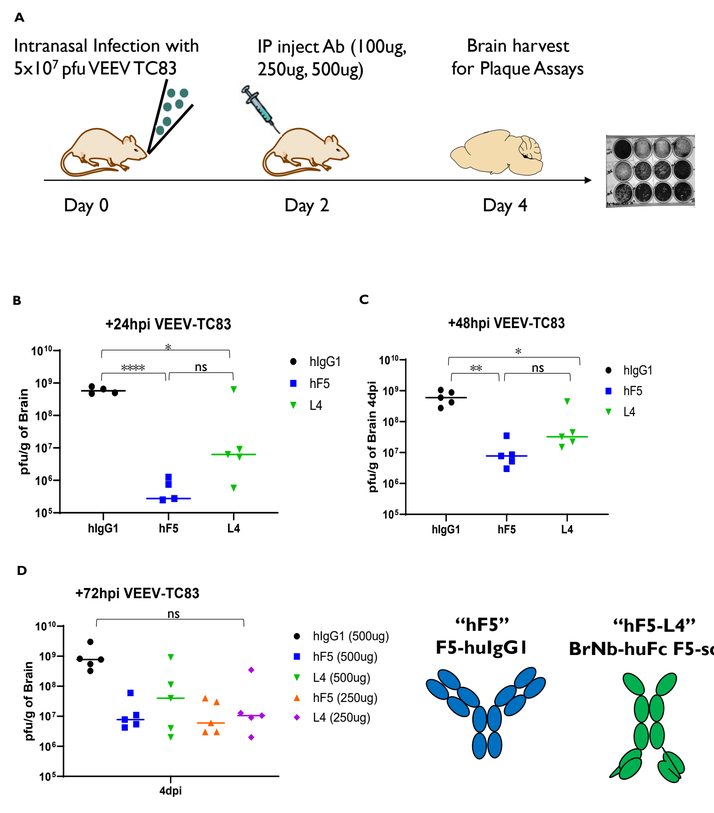
Bispecific BBBP anti-VEEV antibody design & validation of neutralization capacity



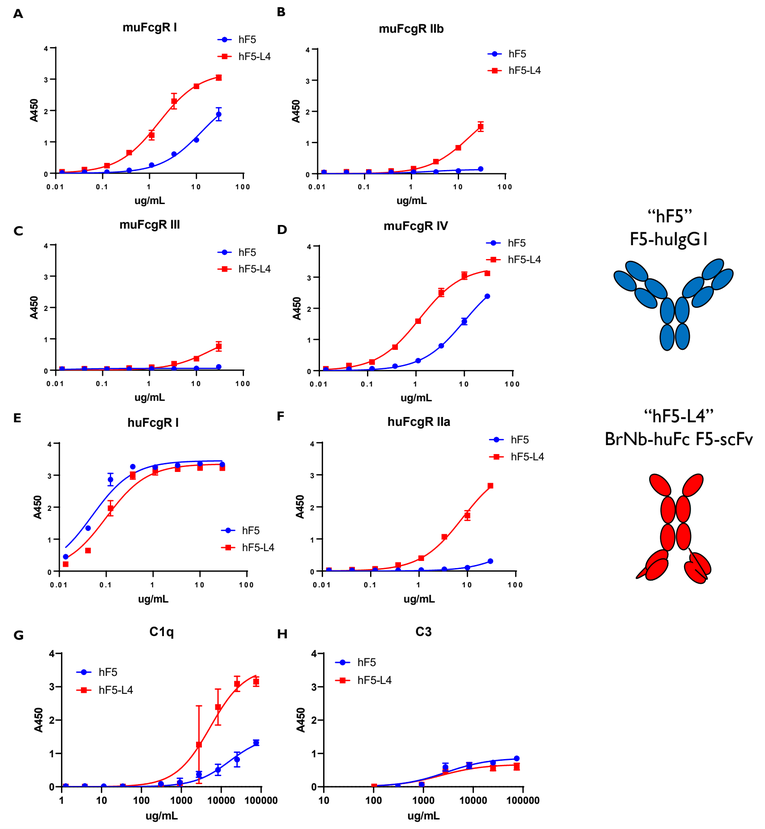
Engineered bispecific Ab L4 exhibits rapid BBB penetration and retention



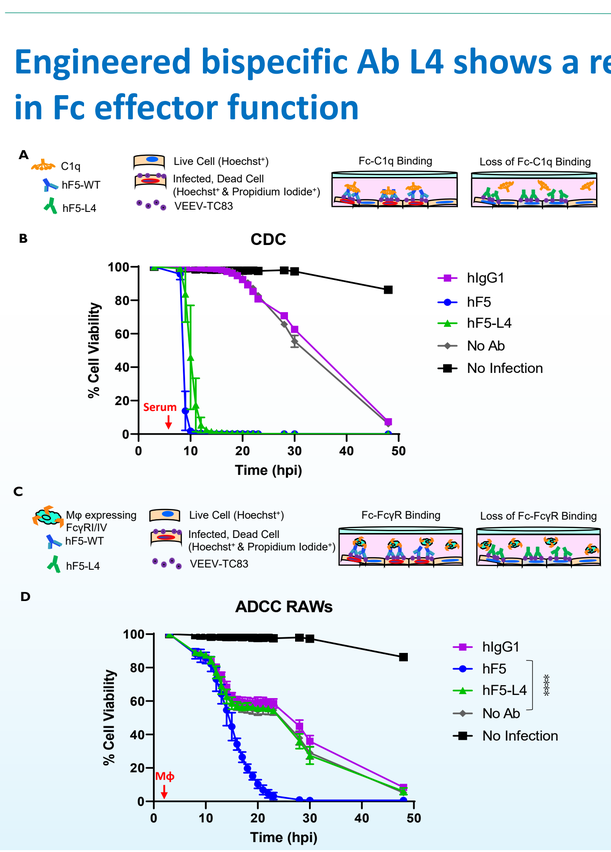
Engineered bispecific Ab L4 does not enhance protection over parental hF5



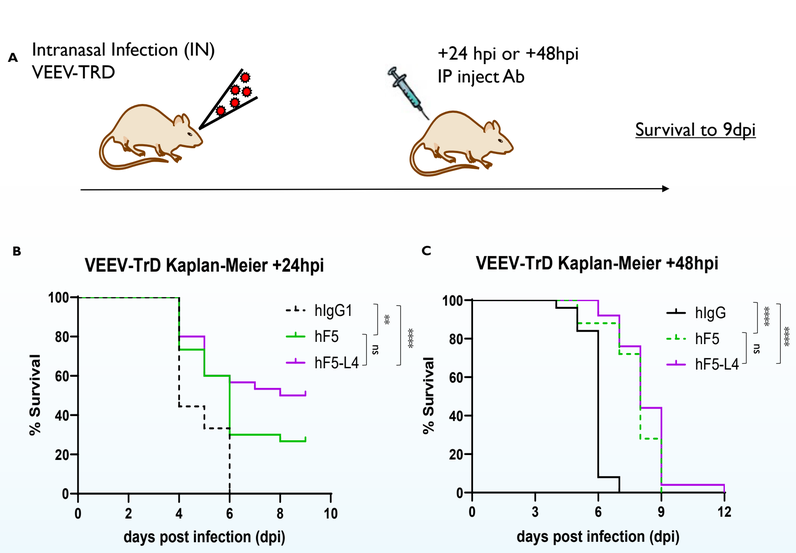
Engineered bispecific Ab L4 shows comparable or enhanced binding to FcγR and complement



Engineered bispecific Ab L4 shows a reduction in Fc effector function



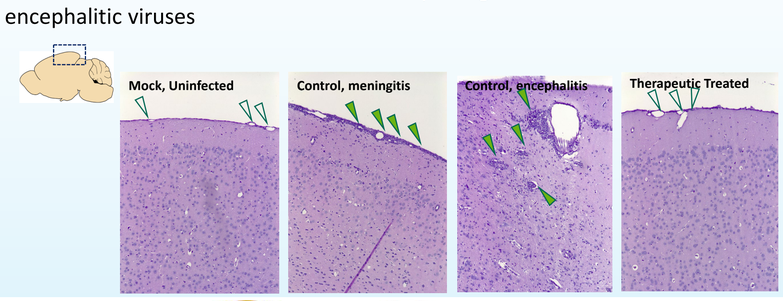
L4 shows comparable therapeutic protection to parental hF5 against neurovirulent VEEV-TrD



Conclusions & Future Work

- An anti-VEEV brain-targeting bispecific antibody (hF5-L4) retains binding and neutralization potency against VEEV E1E2 antigen and VEEV-TC83 virus
- hF5-L4 rapidly penetrates the intact BBB, is detectable from 2-72hpi, and does not elicit abnormal histology at a 4mg/kg dosage
- hF5-L4 did not enhance protection over parental hF5 in the context of infection with attenuated VEEV-TC83
- hF5-L4 exhibits comparable or enhanced binding to FcγRs and compliment, but functional engagement to facilitate ADCC is lost, which is important for therapeutic efficacy[4]
- hF5-L4 confers therapeutic protection comparable to hF5 against WT VEEV-TrD, but does not provide significantly improved efficacy

- It will be important going forward to evaluate intact Fc effector function with different bispecific Ab assemblies
- Particularly, we can assess the effect of Fc effector function within the confines of the BBB as it relates to neuroinvasive pathogens such as VEEV and other encephalitic viruses



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