Proteome Remodeling in a Peptide Immunization Animal Model of Anti-NMDAR Encephalitis

UNIVERSITY of MARYLAND SCHOOL OF MEDICINE

Andres Chavez-Barragan, Yesenia Enriquez-Gonzalez, Timothy Zhang, and David R. Benavides

Department of Neurology, University of Maryland School of Medicine, Baltimore, MD 21201

Background

Anti-NMDAR encephalitis is the most common subtype of autoimmune encephalitis (AIE) and is characterized by GluN1 antibodies. Disease is associated with substantial morbidity and mortality, highlighting critical unmet therapeutic needs. Most animal models of anti-NMDAR encephalitis depend on intracerebroventricular transfer of patient-derived antibodies or cerebrospinal fluid (CSF), or immunization with structurally intact NMDARs. However, recent studies have demonstrated the induction of anti-NMDAR encephalitis in rodent models through GluN1 peptide immunization, with differing effects.

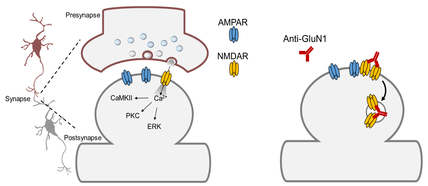


Figure 1: Schematic of synapse and molecular pathophysiology of anti-NMDAR encephalitis.

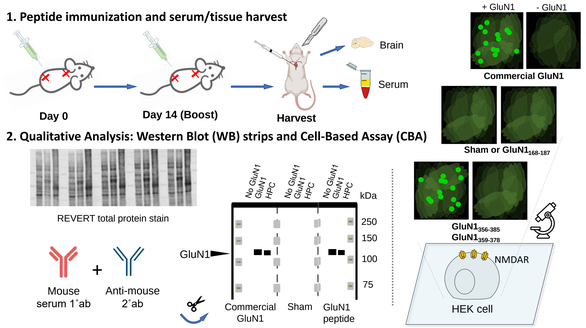
Anti-NMDAR antibodies target the amino terminal domain of the GluN1 subunit, and cause receptor loss and associated neuropsychiatric symptoms such as memory impairment, psychosis, and seizures with high morbidity and mortality. The immunopathogenesis remains unclear and therapeutic management of anti-NMDAR encephalitis have been limited by incomplete knowledge of its pathogenesis.

Goals

1. To characterize immune responses in mice immunized with GluN1$_{359-378}$, GluN1$_{168-187}$, or GluN1$_{356-385}$ peptides.
2. To investigate the molecular effects of GluN1 peptide immunization on post-synaptic NMDAR expression.

Methods

Immunogenic peptides: GluN1$_{356-385}$, GluN1$_{359-378}$, GluN1$_{168-187}$



Immunization with GluN1$_{359-378}$ shows absent signal on WB and CBA for GluN1

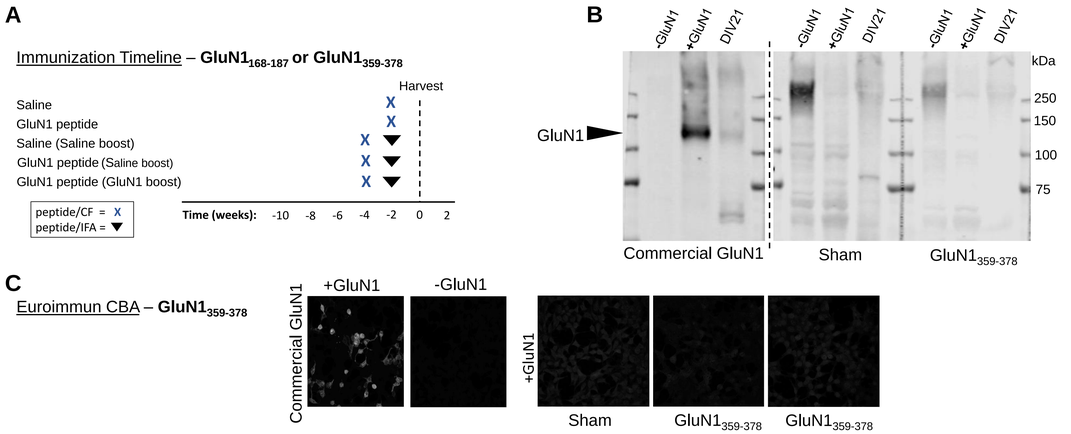


Figure 2: Immunization of mice with GluN1$_{168-187}$ or GluN1$_{359-378}$ peptide shows absent signal on Western blot and Cell based assay for GluN1. **(A)** Immunization strategy and timeline for GluN1$_{359-378}$, GluN1$_{168-187}$, and saline (Sham). Mice cohorts were immunized at either 2 weeks before harvest, or 4 weeks before harvest with a peptide boost at 2 weeks in some groups. Immunization and boost injections consisted of 200 ug of total peptide. Complete Freund's Adjuvant (CFA) was used with initial immunization, while Incomplete Freund's Adjuvant (IFA) was used with boost injections. **(B)** Sera from mice immunized with Sham and GluN1$_{359-378}$ were screened by WB membrane strip against 3 sample targets: HEK-GluN1 cell lysate, HEK+GluN1 cell lysate, and DIV21 neuron cell lysate. A signal for GluN1 is expected at 120 kDa, as shown in the WB strip treated with Commercial GluN1 antibody. **(C)** Sera derived from mice immunized with saline (Sham) and GluN1$_{359-378}$ did not produce staining signal against HEK293 cells expressing the GluN1 on CBA.

Immunization with GluN1$_{356-385}$ shows positive signal on WB and CBA for GluN1

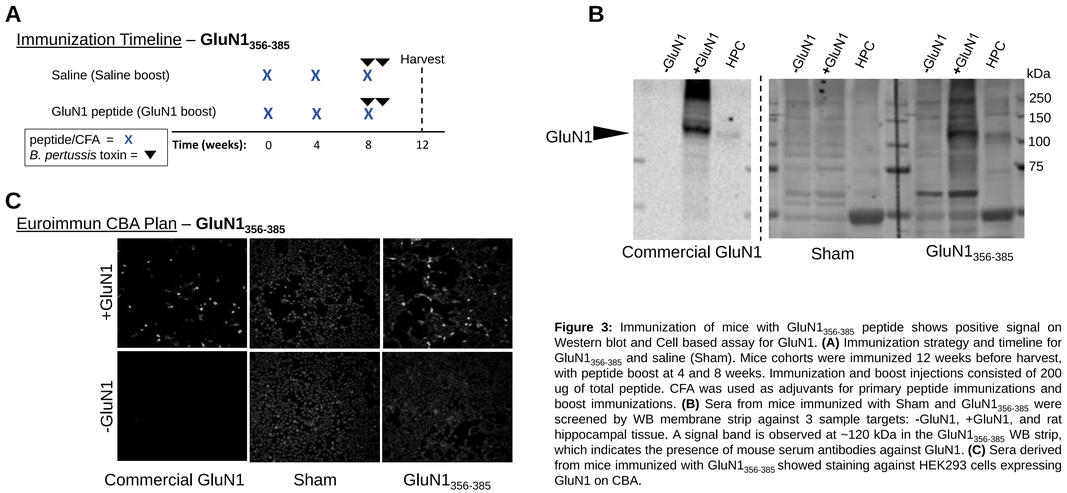


Figure 3: Immunization of mice with GluN1$_{356-385}$ peptide shows positive signal on Western blot and Cell based assay for GluN1. **(A)** Immunization strategy and timeline for GluN1$_{356-385}$ and saline (Sham). Mice cohorts were immunized 12 weeks before harvest, with peptide boost at 4 and 8 weeks. Immunization and boost injections consisted of 200 ug of total peptide. CFA was used as adjuvants for primary peptide immunizations and boost immunizations. **(B)** Sera from mice immunized with Sham and GluN1$_{356-385}$ were screened by WB membrane strip against 3 sample targets: -GluN1, +GluN1, and rat hippocampal tissue. A signal band is observed at ~120 kDa in the GluN1$_{356-385}$ WB strip, which indicates the presence of mouse serum antibodies against GluN1. **(C)** Sera derived from mice immunized with GluN1$_{356-385}$ showed staining against HEK293 cells expressing GluN1 on CBA.

Conclusions

Immunization of mice with specific pathogenic GluN1 peptides reliably produced antibodies against NMDARs. This was contrasted by the absence of antibodies observed in sera from animals immunized with sham, non-pathogenic, and some pathogenic GluN1 peptides. Here, we evaluate postsynaptic density (PSD) fractions from hippocampal brain tissue of immunized mice with the aim to quantify changes in NMDAR expression.

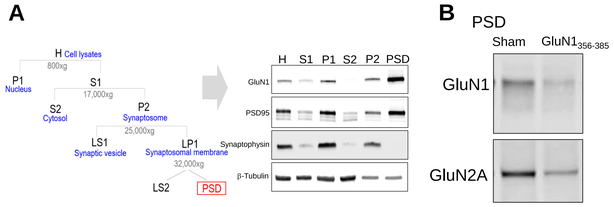


Figure 4: Western blot analysis of subcellular fractionations. (A) DIV21 neurons were used to prepare representative fractions: homogenate (H), cytosol (S2), synaptosome (P2), and PSD. (b) NMDAR levels are reduced in hippocampal PSD samples from mice immunized with GluN1$_{356-385}$ compared to sham controls.

Future Directions

Future studies will investigate the regulation of synaptic NMDAR expression and neuronal glutamate receptor signaling pathways. These studies may contribute to the identification of potential therapeutic targets for novel therapies for patients with anti-NMDAR encephalitis and disorders involving antibodies targeting cell surface antigens.

References

1. Dalmau J, Graus F. Antibody-Mediated Encephalitis. *N Engl J Med*. 2018 Mar 1;378(9):840-851.
2. Jones BE, Tovar KR, Goehring A, et al. Autoimmune receptor encephalitis in mice induced by active immunization with conformationally stabilized holoreceptors. *Sci Transl Med*. 2019;11(500):eaaw0044.
3. Wagnon I, Hélie P, Bardou I, et al. Autoimmune encephalitis mediated by B-cell response against N-methyl-d-aspartate receptor. *Brain*. 2020;143(10):2957-2972.
4. Ding Y, Zhou Z, Chen J, et al. Anti-NMDAR encephalitis induced in mice by active immunization with a peptide from the amino-terminal domain of the GluN1 subunit. *J Neuroinflammation*. 2021;18(1):53.
5. Linnoila J, Jalali Motlagh N, Jachimiec G, et al. Optimizing animal models of autoimmune encephalitis using active immunization. *Front Immunol*. 2023;14:1177672.

Acknowledgements

The authors would like to thank Yuyoung Joo, Thanh Hien Vu, Minerva Contreras, Michael Anderson and members of the Blanpied lab at UMSOM for technical support. This work is supported by funding from UMSOM Seed Funds and NIH/NINDS K08 NS114039.