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Generate the peptide-specific AQP4 monoclonal antibody to compete with human NMOSD NMO-IgG in vitro

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Abstract

Neuromyelitis Optica Spectrum Disorder (NMOSD) is one of the CNS autoimmune disorders characterized by optic neuritis and acute myelitis. The NMO-IgG has been proven as a pathologic autoantibody to target a subtype of the water channel protein, known as aquaporin-4 (AQP4). Herein, we successfully produced an extracellular Loop C peptide-specific AQP4 monoclonal antibody named A001. We confirm the binding affinity of A001 with AQP4 protein. Furthermore, we approved that A001 preserves competitive competence to NMO-IgG and can ameliorate complement-derived cytotoxicity and protect intact AQP4 protein from NMO-IgG attack in vitro. We believe our results provide the therapeutic potential of A001 in the human NMOSD in the future.

Introduction

Neuromyelitis Optica (NMO) is a CNS autoimmune disorder with acute inflammatory disease in CNS, principally affecting the spinal cord and optic nerves. An IgG autoantibody (called NMO-IgG, also named AQP4-IgG) is identified as a specific marker for NMO. NMO spectrum disorders (NMOSD) are stratified further by serologic testing (seropositive NMOSD with AQP4-IgG or seronegative NMOSD without AQP4-IgG) and contain typical NMO. NMO-IgG initiates two potentially competing consequences, AQP4 endocytosis/degradation and complement activation. Meanwhile, binding of NMO-IgG to AQP4 triggers ADCC on astrocytes, which causes eosinophil binding and degranulation producing complement-independent and -dependent astrocyte damage. Loop C is the largest epitope peptide of AQP4, Loop C peptide contributes to NMO rAb binding to AQP4 and abolishes binding of NMO Patient Sera. Accordingly, we assume while generating competitive loop C peptide specific monoclonal antibodies may block NMO-IgG or impede the functions of NMO-IgG.

Materials & Methods

Experimental Animals: C57BL/6J female mice aged 6-8 weeks, used to induce immune responses.

Intrasplenic immunization the mouse myeloma cell line (NS-1) was fused with spleen lymphocytes from Loop C peptide immunized mice based on the protocol.

Immunized Spleen Lymphocyte Isolation and Cell Fusion and Selection of Hybridoma Clone:: On the 4th and 7th days post-immunization, the mice were sacrificed, and splenic lymphocytes were collected. Subsequently, NS-1 myeloma cells and the lymphocytes were mixed in a 1:1 ratio of cell numbers.

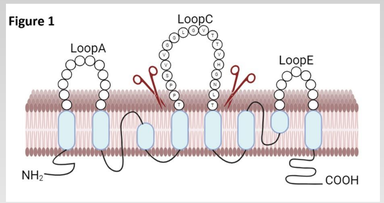
Specificity and affinity assay of antibody: cDNA of mouse AQP4 M23 extracted from mouse brain. HEK293 cell lines were transfected with pFUW-eGFP or pFUW-eGFP-mAQP4 or antibodies purified from B cell line supernatant.

Antibody and complement preparation and Competitive assay: To compare the affinity of anti-mouse AQP4 antibodies to NMO-IgG acting on human AQP4 in both dead and live cell states.

Complement-mediated cytotoxicity assay: after HEK293 cells were transfected with eGFP-hAQP4 plasmid for 24 hours at 37°C with 5% CO2, the cells were incubated with normal IgG, NMO-IgG, A001, or both with NMO-IgG and A001 combine with 10% human or mouse complement.

Results

Figure 1: Loop C peptide: the largest epitope of mouse AQP4 as the candidate for immunogen. (photo Created in BioRender.com)



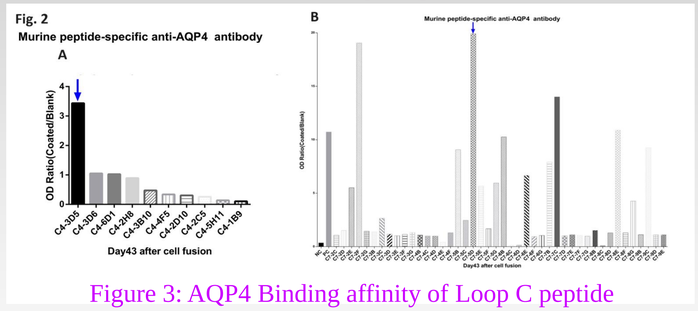


Figure 2: Selected hybridoma cell lines with high efficiency to produce Loop C peptide-specific monoclonal antibodies in either day-4 (C4-3D5) or day-7 immunization (C7-5D)

Figure 3: AQP4 Binding affinity of Loop C peptide monoclonal body (from C7-5D clone, name d A001)

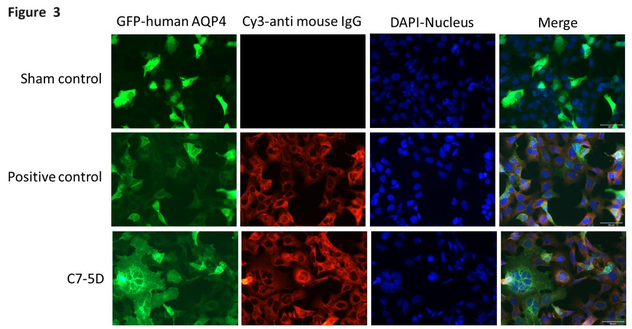
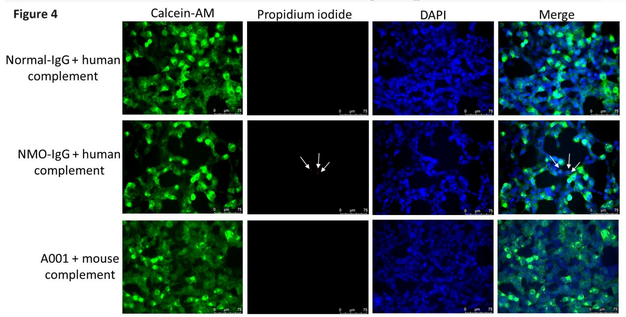


Figure 4: The effect of Complement-dependent cytotoxicity (CDC) noted in the NMO-IgG group but not found in the A001 group



Discussion and Conclusion

1. Most cases of NMO-IgG (AQP4-IgG) seropositive NMOSD have relapsing courses. Rituximab, eculizumab, inebilizumab, and satralizumab all have class 1 evidence for use in AQP4-IgG seropositive NMOSD approved by the US FDA, but long-term adverse effects should be a concern.
2. Strikingly, we generate Loop C peptide-specific monoclonal antibody named A001 that successfully bind with AQP4 and protects cells from CDC attack in vitro.
3. The limitation of the study is no in vivo data, that is future work.

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