The AF122 antibody recognizes the AChRα subunit in murine muscle endplates by immunofluorescence

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Abstract

The recombinant antibody AF122 binds to the acetylcholine receptor (AChR) α subunit expressed in murine muscle endplates and can be detected by immunofluorescence in paraformaldehyde (PFA)-fixed *tibialis anterior* cryosections.

Introduction

The AChRα (UniProt #P04756) is a subunit of the ligand-gated nicotinic AChR ion channel expressed at the neuromuscular junction. Binding of its ligand ACh leads to ion influx, to depolarization of the postsynaptic membrane and to subsequent contraction of the muscle. Here we report that the recombinant antibody AF122 detects by immunofluorescence the AChRα subunit in cryosections of the murine *tibialis anterior* muscle.

Materials & Methods

Antibodies: ABCD_AF122 (ABCD nomenclature, https://web.expasy.org/abcd/) targets the α subunit of the AChR. It was produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies) as mini-antibody with the antigen-binding scFv fused to a human IgG1 Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions of the clone 637 (Graus *et al.,* 1997) joined by a peptide linker $(GGGGS)_4$. HEK293T cells (growing in DMEM GlutaMAX™ (Gibco, #31966) supplemented with 8% Fetal Bovine Serum (Gibco, #10270)) were transiently transfected with the vector coding for the scFv-Fc of the antibody. Supernatants (<5 µg/L) were collected after 4 days.

Antigen: The antibody AF122 was originally generated by a phage display selected for reactivity against the human AChR (UniProt #P02708). The phage display Fab library was generated from thymic lymphocytes derived from two Myasthenia gravis patients (Graus *et al.,* 1997). *Tibialis anterior* was isolated from C57BL/6 mice and rinsed in PBS. The muscle was embedded in OCT (Tissue-Tek, #4583) and cryosectioned at 10 µm using a Hyrax C60 cryostat (Zeiss).

Protocol: Cryosections were fixed with 4% (w/v) PFA (Electron Microscopy Sciences, #15710) in 0.1 M phosphate buffer, pH 7.4, for 10 min at room temperature, washed in PBS, and blocked with PBS supplemented with 0.1% Triton X-100 and 5% normal goat serum. Subsequently, sections were incubated with the following primary antibodies overnight at 4°C: Alexa Fluor 647-conjugated α-bungarotoxin (1:300, Thermo Fisher Scientific, #B35450), mouse anti-neurofilament 200 (1:300, Sigma, #N0142) and the undiluted human monoclonal antibody AF122. Sections were then washed in PBS and incubated with Alexa Fluor 488-labeled goat anti-human and Alexa Fluor 555-labeled goat anti-mouse secondary antibody overnight at 4°C (1:250 Life, Technologies, #A11013, #A28180). Counterstaining was performed using SlowFade Gold antifade reagent with DAPI (Invitrogen, #S36936). Fluorescence photomicrographs were captured with a SP5 Leica confocal laser scanning microscope (SP5; Leica, Heerbrug, Switzerland) equipped with argon and helium lasers using the 40x objective (oil immersion, NA1.25). Images were processed and merged by Imaris imaging software (Bitplane, Zurich, Switzerland).

Results

The AF122 antibody staining in the neuromuscular endplates of the murine *tibialis anterior* colocalizes with bungarotoxin (Fig. 1), which binds with high affinity to the AChRα subunit (Tzartos *et al.*, 1983). Neuromuscular endplates are identified by the presence of AChRα in the proximity of nerve fibers in the muscle. Thus, AF122 recognizes the AChRα in murine PFA-fixed muscle endplates.

References

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Conflict of interest

The authors declare no conflict of interest.

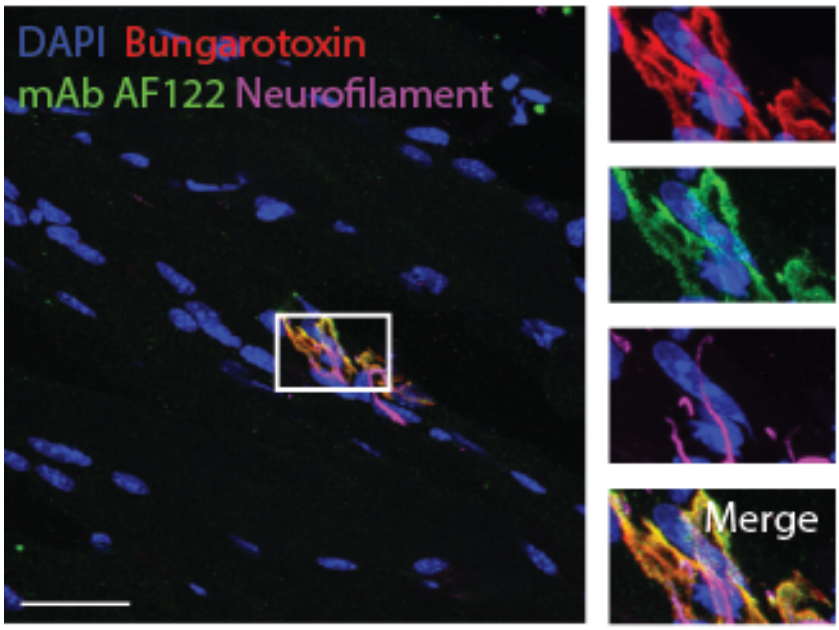

Fig. 1. The AF122 antibody recognizes the AChR in the murine *tibialis anterior* muscle. Muscle endplates are identified by neurofilament staining and AChRs are visualized by binding of bungarotoxin. AF122 in green, bungarotoxin in red, neurofilament in purple and DAPI in blue. Scale bar: 20 μm.

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