The AJ519 antibody detects the human TAC/ILR2A protein by western blot

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Abstract

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Introduction

The alpha subunit of the interleukin 2 receptor, also known as the TAC antigen (Uniprot #P01589), is a protein displayed notably at the surface of T lymphocytes (Uchiyama *et al*., 1981; Malek and Castro, 2010). Here, we describe the ability of the AJ519 antibody, a single chain fragment (scFv) derived from the 7G7 hybridoma, to successfully detect the TAC protein by western blot in TAC-transfected HEK293 cells.

Materials & Methods

Antibodies: ABCD_AJ519 antibody (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2019) was produced by the Geneva Antibody Facility (www.unige.ch/ antibodies/) as mini-antibody with the antigen-binding scFv fused to a rabbit IgG Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions of the 7G7 hybridoma (Rubin *et al*., 1985) joined by a peptide linker $(GGGGS)_3$. The sequencing of the 7G7 hybridoma was performed by the Geneva Antibody Facility. HEK293 suspension cells (growing in serum-free FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. AJ519 supernatant (50 mg/L) was collected after 4 days.

Antigen: The 7G7 hybridoma was originally raised against human influenza virus-stimulated PBMC in BALB/cJ mice (Rubin *et al*., 1985). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected 3 days before the experiment with the vector coding for the full-length human TAC protein.

Protocol: $5x10^6$ transfected HEK cells were pelleted and lysed in PBS containing 0.5% (v/v) Triton X-100. Nucleus were pelleted by centrifugation (10 min at 12'000 g) and supernatant was recovered and mixed with reducing or non-reducing sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, +/- 6% (v/v) β-mercaptoethanol). Each sample was migrated (200 V, 30 min) in a 4-20% acrylamide gel (SurePAGE Bis-Tris, Genscript #M00655), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked overnight in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk and washed three times for 5 minutes in PBS + 0.1% (v/v) Tween 20. The membranes were then incubated with the primary antibody AJ519 (dilution 1:10 in PBS-Tween) for 1 hour at room temperature and washed three times for 5 minutes. The membranes were then incubated with horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma-Aldrich #A8275, dilution 1:3000) and washed twice for 5 minutes and once for 15 minutes in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Millipore) using a PXi-4 gel imaging systems (Syngene).

Results

The antibody AJ519 recognizes the TAC protein in both non-reducing and reducing conditions. No signal was detected in mock transfected cells (Fig. 1).

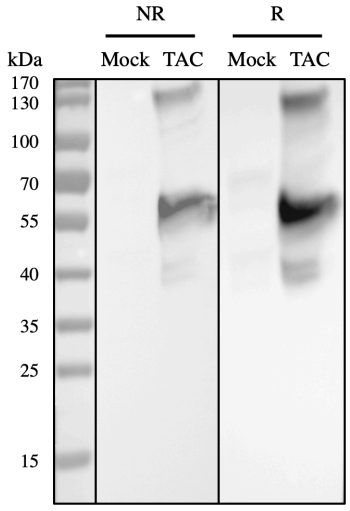

Fig. 1. Specific binding of the AJ519 antibody to TAC protein in TAC-transfected cells in both non-reducing (NR) and reducing (R) conditions. No band was observed in mock transfected cells.

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Conflict of interest

The authors declare no conflict of interest.