The AJ519 antibody labels the human TAC/IL2RA protein by immunofluorescence

David Arsimoles, Alessia G. D'Esposito, Vincent Gaspoz, Cyril Pirek, Alexandra Laszlo, Mélissa Longepierre, Teresa M. Giusti, Monica Bulla, Ali Sassi, Cyril Guilhen

Bachelor in Biomedical Sciences, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The recombinant antibody AJ519 detects by immunofluorescence the human TAC surface protein in paraformaldehyde-fixed cells.

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 antigen by immunofluorescence 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 a 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 (10 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: The whole procedure was carried out at room temperature. Transfected HEK cells were fixed with PBS + 4% paraformaldehyde (w/v) (Applichem, #A3013) for 30 min, and blocked with PBS + 40 mM ammonium chloride (NH_4Cl) (Applichem, #A3661) for 5 min. Cells were then permeabilized in PBS + 0.2% saponin (w/v) (Sigma, #S7900) for 5 min, washed once (5 min) with PBS + 0.2% (w/v) BSA (PBS-BSA), and incubated for 30 min with the antibody-containing supernatants (dilution 1:2). After 3 washes (5 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-rabbit IgG conjugated to AlexaFluor-488 (1:400, Molecular Probes, #A11034). After 3 washes (5 min) with PBS-BSA, cells were mounted on slides (Menzel-Gläser, 76x26 mm) with Möwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka, #33480). Pictures were taken using a Zeiss LSM700 confocal microscope, with a 63x Neofluar oil immersion objective.

Results

The antibody AJ519 recognizes the TAC protein at the cell surface of TAC transfected cells. No signal was detected in mock transfected cells (Fig. 1).

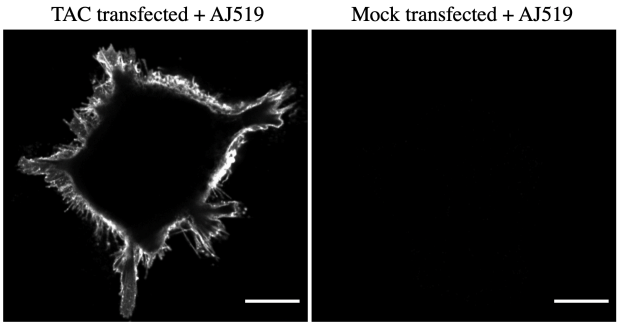

Fig. 1. The AJ519 antibody labels the TAC cell surface protein in TAC-transfected cells. No staining was observed in mock transfected cells. Scale bar: 10 μm.

References

Lima WC, Gasteiger E, Marcatili P, Duek P, Bairoch A, Cosson P. The ABCD database: a repository for chemically defined antibodies. Nucleic Acids Res. 2019; pii:gkz714. PMID: 31410491

Malek TR, Castro I. Interleukin-2 receptor signaling: at the interface between tolerance and immunity. Immunity. 2010; 33(2):153-65. PMID: 20732639

Rubin LA, Kurman CC, Biddison WE, Goldman ND, Nelson DL. A monoclonal antibody 7G7/B6, binds to an epitope on the human interleukin-2 (IL-2) receptor that is distinct from that recognized by IL-2 or anti-Tac. Hybridoma. 1985; 4(2):91‑102. PMID:2408992.

Uchiyama T, Broder S, Waldmann TA. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. J Immunol. 1981; 126(4):1393‑7. PMID:6970774.

Conflict of interest

The authors declare no conflict of interest.