The AJ521 antibody labels the human CD1b protein by immunofluorescence

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Abstract

The recombinant antibody AJ521 detects by immunofluorescence the human CD1b surface protein in paraformaldehyde-fixed cells.

Introduction

Human CD1b (Uniprot #P29016), a protein displayed at the surface of antigen-presenting cells, is involved in the presentation of lipid antigens to T cells (Porcelli *et al*., 1992). Here, we describe the ability of the AJ521 antibody, a single chain fragment (scFv) derived from the BCD1b3.1 hybridoma, to successfully detect the CD1b protein by immunofluorescence in CD1b-transfected HEK293 cells.

Materials & Methods

Antibodies: ABCD_AJ521 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 BCD1b3.1 hybridoma (Behar *et al*., 1995) joined by a peptide linker $(GGGGS)_3$. The sequencing of the BCD1b3.1 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. AJ521 supernatant was collected after 4 days. Production of AJ521 was undetectable in this system, indicating a low production yield (<5 mg/L).

Antigen: The BCD1b3.1 hybridoma was originally raised against human $CD1^+$ monocytes in BALB/c mice (Behar *et al*., 1995). HEK293 suspension cells (growing in serum-free FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected 3 days before the experiment with the vector coding for the human CD1b protein fused to its β2 microglobulin subunit (Mercanti *et al.*, 2010).

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 AJ521 recognizes the CD1b protein addressed at the cell surface of CD1b transfected cells. No signal was detected in mock transfected cells (Fig. 1).

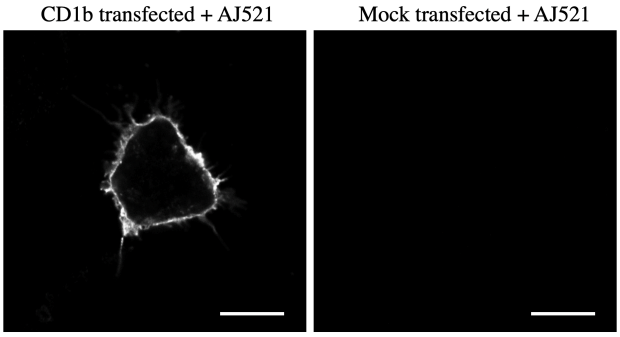

Fig. 1. The AJ521 antibody labels the CD1b cell surface protein in CD1b-transfected cells. No staining was observed in mock transfected cells. Scale bar: 5 μm.

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Conflict of interest

The authors declare no conflict of interest.