

The AJ521 antibody detects the human CD1b protein by flow cytometry

Alexandra Laszlo¹, Eliott Bosshard¹, Orlane L. Maxit¹, Sébastien Spedaliero¹, Maxime Richard¹, Monica Bulla¹, Ali Sassi¹, Jean-Pierre Aubry-Lachainaye², Cyril Guilhen¹

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

²Flow Cytometry Core Facility, University of Geneva, 1211 Geneva 4, Switzerland

Abstract

The AJ521 antibody detects the human CD1b protein by flow cytometry.

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 flow cytometry in CD1b-transfected HEK293 cells.

Materials & Methods

Antibodies: ABCD_AJ521 antibody (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2019) and IgG produced by BCD1b3.1 hybridoma were produced by the Geneva Antibody Facility (www.unige.ch/antibodies/). AJ521 antibody was produced 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 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 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 β 2 microglobulin subunit (Mercanti *et al.*, 2010).

Protocol: The whole procedure was carried out at 4°C. 500'000 transfected cells were pelleted and washed once with washing buffer (PBS + 0.2% BSA (w/v)). Cells were then incubated for 20 minutes with either the original mouse hybridoma BCD1b3.1 supernatant (dilution 1:2 in PBS-BSA) or with the reformatted scFv antibody AJ521 (undiluted). After two washes in washing buffer, cells were incubated for 20 minutes with either secondary goat anti-mouse or anti-rabbit IgG conjugated to Alexa Fluor

488 (dilution 1:400, Molecular Probes #A11029 and #A11034 respectively). After two washes in washing buffer, cells were resuspended in 500 μ L of washing buffer and analyzed with a flow cytometer (BD Accuri™ C6).

Results

Both the IgG produced by the mouse hybridoma BCD1b3.1 and the reformatted scFv AJ521 detect the CD1b protein at the cell surface of CD1b transfected cells. No signal was detected in mock transfected cells (Fig. 1).

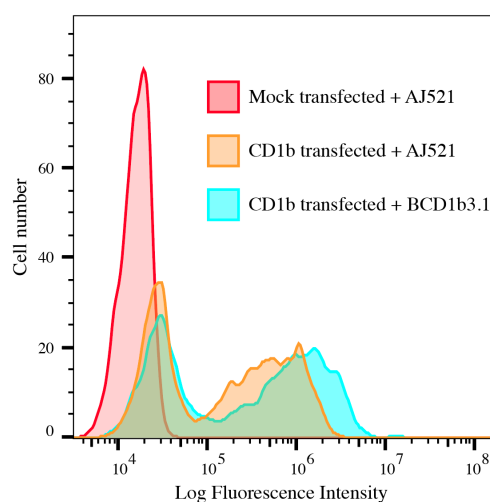


Fig. 1. Both the IgG produced by the mouse hybridoma BCD1b3.1 (blue) and the reformatted scFv AJ521 (orange) label HEK293 cells overexpressing the CD1b protein. No signal was detected in mock transfected cells incubated with the AJ521 antibody (red).

References

- Behar SM, Porcelli SA, Beckman EM, Brenner MB. A pathway of costimulation that prevents anergy in CD28- T cells: B7-independent costimulation of CD1-restricted T cells. *J Exp Med.* 1995; 182(6):2007-18. PMID:7500046
- 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
- Mercanti V, Marchetti A, Lelong E, Perez F, Orci L, Cosson P. Transmembrane domains control exclusion of membrane proteins from clathrin-coated pits. *J Cell Sci.* 2010; 123:3329-35. PMID:20826467
- Porcelli S, Morita CT, Brenner MB. CD1b restricts the response of human CD4⁺ T lymphocytes to a microbial antigen. *Nature.* 1992; 360(6404):593-7. PMID:1281285

Conflict of interest

The authors declare no conflict of interest.