The AJ516 antibody does not detect the human CD1a protein by flow cytometry

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Abstract

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Introduction

CD1a (Uniprot #P06126), a protein displayed at the surface of antigen-presenting cells, is involved in the presentation of lipid antigens to T cells (Hunger *et al.*, 2004). Here, we describe the inability of the AJ516 antibody, a single chain fragment (scFv) derived from the 10H3.9 hybridoma, to successfully detect the CD1a protein by flow cytometry in CD1a-transfected HEK293 cells.

Materials & Methods

Antibodies: ABCD_AJ516 antibody (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2019) and IgG produced by 10H3.9 hybridoma were produced by the Geneva Antibody Facility (www.unige.ch/antibodies/). AJ516 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 10H3.9 hybridoma (Olive *et al.*, 1984) joined by a peptide linker $(GGGGS)_3$. The sequencing of the 10H3.9 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. AJ516 supernatant (10 mg/L) was collected after 4 days.

Antigen: The 10H3.9 hybridoma was originally raised against human thymocytes in BALB/c mice (Olive *et al.*, 1984). 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 CD1a protein.

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 10H3.9 supernatant (dilution 1:2 in PBS-BSA) or with the reformatted scFv antibody AJ516 (1 µg/ml). 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 µL of washing buffer and analyzed with a flow cytometer (BD Accuri™ C6).

Results

IgG produced by the mouse hybridoma 10H3.9 detects the CD1a protein at the cell surface of HEK293 transfected cells. The reformatted scFv AJ516 does not recognize CD1a. No signal was detected in mock transfected cells (Fig. 1).

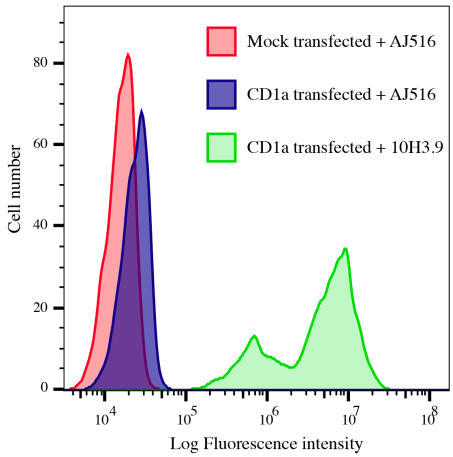

Fig. 1. The AJ516 antibody does not detect the CD1a protein in CD1a-transfected cells (blue). IgG produced by the mouse hybridoma 10H3.9 clone detects cell surface CD1a (green). No signal was detected in mock transfected cells incubated with the AJ516 antibody (red).

References

Hunger RE, Sieling PA, Ochoa MT, *et al*. Langerhans cells utilize CD1a and langerin to efficiently present nonpeptide antigens to T cells. J Clin Invest. 2004; 113(5):701-8. PMID:14991068

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

Olive D, Dubreuil P, Mawas C. Two distinct TL-like molecular subsets defined by monoclonal antibodies on the surface of human thymocytes with different expression on leukemia lines. Immunogenetics. 1984; 20(3):253-64. PMID:6205990

Conflict of interest

The authors declare no conflict of interest.

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