The AJ072 full-length IgG antibody labels the surface and early endosomes of HEK cells by immunofluorescence

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Abstract

The AJ072 full-length mouse IgG antibody, against the human transferrin receptor, labels the plasma membrane and early endosomes of HEK cells by immunofluorescence.

Introduction

TfR (Transferrin receptor protein 1, CD71; Uniprot P02786) is a type II transmembrane glycoprotein that binds the iron-carrier glycoprotein transferrin (Tf) (Candelaria *et al.*, 2021). Here, we describe the ability of the AJ072 full-length mouse IgG antibody, directed against the human TfR, to label the cell membrane of HEK cells by surface immunofluorescence, as well as early endosomes by immunofluorescence in permeabilized cells. The AA841 antibody, in an scFv-Fc format, did not label any structures of HEK cells.

Materials & Methods

Antibodies: ABCD_AA841 and ABCD_AJ072 antibodies (https://web.expasy.org/abcd/, ABCD nomenclature) were produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as mini-antibodies (with the antigen-binding scFv fused to a rabbit IgG Fc, for AA841 and AJ072), and as a full-length double-chain mouse IgG (for AJ072). HEK293 suspension cells (growing in HEK TF medium, Xell 861-0001, supplemented with 0.1% Pluronic F68, Sigma P1300) were transiently transfected with the vector coding for the scFv-Fc or IgG. Supernatants (120 mg/L for AA841, <5 mg/L for AJ072-scFv, and 15 mg/L for AJ072-IgG) were collected after 4 days.

Antigen: HEK293T cells were cultured on glass coverslips (Menzel-Gläser, 22x22 mm) and grown in DMEM GlutaMAX™ (Gibco 31966) supplemented with 10% Fetal Bovine Serum (Gibco 10270) and 100 µg/mL of penicillin-streptomycin (Gibco 15070-063) at 37 °C and 8% CO_2.

Protocol: Fluorescent transferrin was used to label the cell surface and early endosomes as follows: cells were incubated with serum-free preheated DMEM GlutMAX™ for 10 min at 37 °C. Cells were then incubated with transferrin Alexa Fluor™ 546 conjugate at 5 µg/mL in the same medium for 30 min at 37 °C (ThermoFisher T23364).

Surface immunolabelling was performed on ice: cells were rinsed once with ice-cold PBS + 0.2% (w/v) BSA (PBS-BSA), incubated with either AJ072-IgG (5 µg/mL), AJ072-scFv (undiluted), or AA841 (5 µg/mL) for 30 min, rinsed thrice with cold PBS-BSA and incubated for 30 min with secondary goat anti-mouse (for AJ072-IgG) or anti-rabbit (for AA841 and AJ072-scFv) IgG conjugated to AlexaFluor™-488 (1:400, Thermo Fisher A11001 or A11034). Cells were then rinsed thrice with cold PBS-BSA (3 min) and once with cold PBS (3 min).

Then, immunofluorescence of permeabilized cells was performed at room temperature: 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). Cells were then permeabilized in PBS containing 0.2% (w/v) saponin (Sigma S7900) for 5 min, washed once (3 min) with PBS-BSA, and incubated for 30 min with AA841, AJ072-IgG or AJ072-scFv. After 3 washes (3 min) with PBS-BSA, cells were incubated for 30 min with secondary goat anti-mouse (for AJ072-IgG) or anti-rabbit (for AA841 and AJ072-scFv) IgG conjugated to AlexaFluor™-647 (1:400, Thermo Fisher A21235 and A21245, respectively), washed thrice with PBS-BSA and once with PBS, and mounted on slides (Menzel-Gläser, 76x26 mm) with Möwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka 33480). For the control cells, no primary antibody was used.

To check whether AJ072-IgG and AJ072-scFv stain the same structures, cells were incubated with fluorescent transferrin Alexa Fluor™ 546, and fixation, quenching and permeabilization were performed as described above. Cells were then incubated for 30 min with AJ072-IgG and AJ072-scFv, washed thrice with PBS-BSA and incubated for 30 min with secondary goat anti-mouse AlexaFluor™-647 (binding AJ072-IgG) and anti-rabbit IgG conjugated to AlexaFluor™-488 (binding AJ072-scFv), washed thrice with PBS-BSA and once with PBS, and mounted on slides as previously described.

Pictures were taken using a Zeiss LSM800 Airyscan confocal microscope (Bioimaging Core Facility, Faculty of Medicine, University of Geneva), with a Plan-APO 40x/1.4 Oil DIC (UV) VIS-IR objective.

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Results

Using a cell surface labelling protocol, AJ072-IgG successfully labelled the plasma membrane of HEK cells (Fig. 1A). Endocytosed fluorescent transferrin was used as a marker to label early endosomes (Fig. 1A). In permeabilized cells, AJ072-IgG labelled the same endosomes as fluorescent internalized transferrin (Fig. 1A). No staining was observed when the primary antibody was omitted, nor when AA841 was used (Fig. 1A).

Moreover, AJ072-IgG labelled the same compartments as AJ072-scFv (Figs. 1A and 1B; Bian, 2022). Because of the higher production yields of AJ072-IgG (15 mg/L, against less than 5 mg/L of AJ072-scFv), it is preferable to use AJ072-IgG for surface and early endosome labelling.

References

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Conflict of interest

The authors declare no conflict of interest.

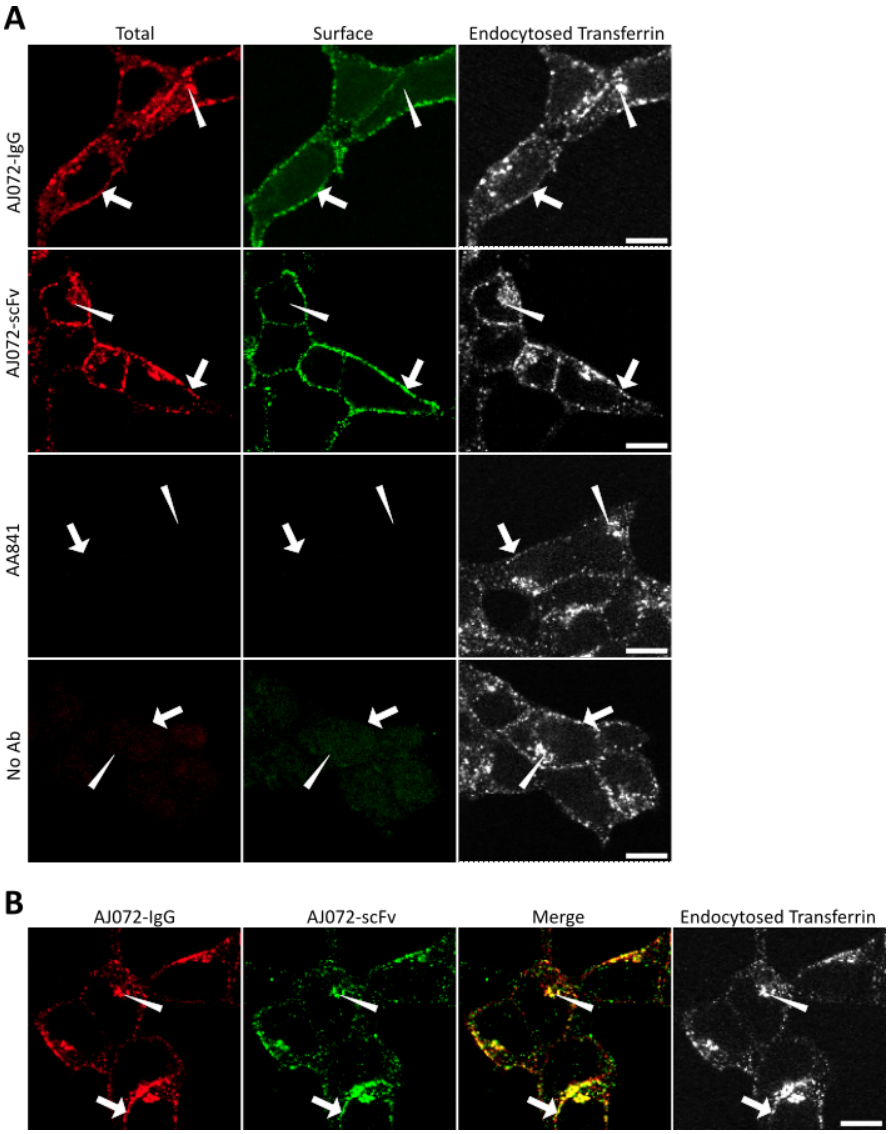

Fig. 1. (A) Early endosomes (arrowheads) were labelled by allowing endocytosis of fluorescent transferrin (white); AJ072-IgG labelled the cell surface (green, arrows) of HEK cells by surface immunofluorescence and early endosomes after cell permeabilization (red). Endosomes (arrowheads) were labelled by both the AJ072-IgG and internalized fluorescent transferrin. AJ072-scFv also labelled the cell surface and early endosomes. No labelling was observed when the primary antibody was omitted (No Ab panel), nor when the AA841 antibody was used (AA841 panel). **(B)** Endocytosed transferrin (white), AJ072-IgG (red) and AJ072-scFv (green) stained identically the cell surface (arrows) and endosomes (arrowheads). Scale bar: 10 μm