AI842, AI843, AI844 and AI177 antibodies do not recognize a FLAG-tagged protein by immunofluorescence in D. discoideum *cells*

Manon Mottet and Thierry Soldati

Department of Biochemistry, Faculty of Science, University of Geneva, Geneva, Switzerland.

Abstract

The recombinant antibodies AI842, AI843, AI844 and AI177 failed to detect by immunofluorescence a C-terminally FLAG-tagged LmpA fusion protein expressed in *Dictyostelium discoideum*.

Introduction

The Lysosomal Membrane glycoprotein (LmpA; DDB_G0267406; UniProt #Q9XYS8) is the homologue of the mammalian Lysosomal Membrane Protein (LIMP-2) and belongs to the CD36-SCARB family. LmpA regulates phagocytosis and phagolysosome biogenesis (Sattler *et al.*, 2018). To confirm the expression and the mainly postlysosomal location of the tagged protein, we tested several recombinant antibodies directed against the FLAG-tag. Here, we report that four recombinant antibodies (AI842, AI843, AI844 and AI177) were not able to detect the full-length tagged protein by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AI177, ABCD_AI842, ABCD_AI843, and ABCD_AI844 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2019) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a human IgG1 Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of the clones 2H8, EEh14.3, EEh13.6, and EEf15.4 (Sasaki *et al.*, 2012, and Entzminger *et al.*, 2017) joined by a peptide linker $(GGGS)_3$. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (30 mg/L for AI177) were collected after 4 days; AI842, AI843, and AI844 have a low production yield in this system (<5 mg/L).

Protocol: The *lmpA* coding DNA sequence (CDS) was synthesized with a C-terminal FLAG-tag (Life Technologies). The Tet-ON LmpA-FLAG construct was generated by inserting the synthetic CDS into the pDM310 vector (Veltman *et al.*, 2009) between the *Bgl*II and *Spe*I sites. *D. discoideum* Ax2(Ka) cells were transformed, and cells carrying the FLAG construct were selected with G418 without doxycycline for constitutive expression. Ax2(Ka) LmpA-FLAG cells were seeded to adhere on 12 mm coverslips grade 0 (Assistant, ref. 01105209) at a density of $1x10^6$ cells/ml. The coverslips were then fixed using the ultracold methanol fixation (Hagedorn *et al.*, 2006). Unspecific binding was then blocked by incubating twice 15 minutes in PBS + 0.2% (w/v) gelatin (Sigma) (PBG). The coverslips were then incubated with each of the four antibodies (dilution 1:2 in PBG) or anti-FLAG M2 antibody (Sigma, F-3165, dilution 1:500 in PBG) for 1 hour, then washed 4 times with PBS and once with PBG. The coverslips stained with the recombinant antibodies were then incubated during 1 hour with goat anti-human IgG coupled to CF640 (Sigma, SAB4600160, 1:500 in PBG) and anti-FLAG M2 with goat anti-mouse IgG coupled to Alexa594 (Thermofisher, A-11005, 1:500 in PBG). Concomitant to the secondary antibody incubation, nuclei were stained with DAPI (Thermofisher, D1306) at a final concentration of 1 µg/ml. Coverslips were washed 4 times in PBS and finally briefly rinsed with distilled water before mounting in Prolong (Thermofisher, P36934). Coverslips were left to dry overnight and the samples were analysed with a confocal LSM800 microscope (Zeiss).

Results

Antibodies directed against the FLAG-tag were tested on Ax2(Ka) *D. discoideum* cells expressing the postlysosomal transmembrane protein LmpA-FLAG. The commercial anti-FLAG M2 mouse monoclonal antibody successfully recognized LmpA-FLAG located in postlysosomes (Fig. 1, bottom left panel). However, there was no difference in signal for the antibodies AI842, AI843, AI844 and AI177 (Fig. 1). Note that the brightness of the images was pushed to make the background, unspecific staining, visible. We conclude that none of the recombinant antibodies did recognize the vacuolar FLAG-tagged protein (Fig. 1). For antibodies AI842, AI843 and AI844, the failure to generate a signal may be caused by their very low production yield.

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Conflict of interest

The authors declare no conflict of interest.

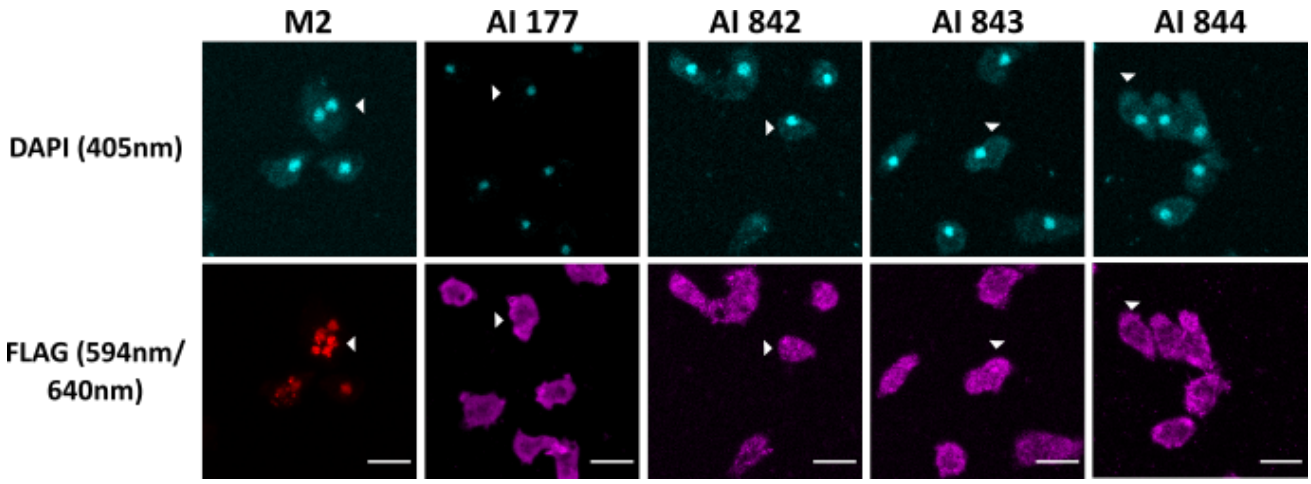

Fig. 1. Immunofluorescence microscopy of cells expressing a FLAG-tagged protein. The commercial M2 anti-FLAG (red) was used as a positive control. Negative controls of the secondary antibodies did not reveal any signals. AI177, AI842, AI843 and AI844 recombinant antibodies (pink). In cells expressing LmpA-FLAG, the protein is accumulated in a postlysosomal compartment. The arrowheads point to the cells expressing the FLAG-tagged proteins. Representative confocal images of Ax2(Ka) cells stained with DAPI (blue) and anti-FLAG. Scale bar: 10µm