AI842, AI843, AI844 and AI177 antibodies do not recognize a FLAG-tagged protein expressed in D. discoideum by western blot

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Abstract

The recombinant antibodies AI842, AI843, AI844 and AI177 do not detect by western blot an N-terminal FLAG-tagged RavZ protein expressed in *Dictyostelium discoideum*.

Introduction

RavZ is an effector of the bacterium *Legionella pneumophila*, used to establish an infection in its host cell (Choy *et al.*, 2012). We expressed this protein fused to a FLAG-tag in *D. discoideum* and tested several recombinant antibodies directed against the tag. Here, we describe that four recombinant antibodies (AI842, AI843, AI844 and AI177) were not able to detect the full-length protein by western blot.

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 $(GGGGS)_3$. 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 RavZ coding sequence was amplified by PCR from *L. pneumophila* genomic DNA, cloned into pGEM-T and verified by sequencing. The fragment was then cut by enzymatic digestion with BglII and SpeI and inserted into pDM320 containing the FLAG tag sequence for N-terminal tagging (Veltman *et al.*, 2009). AX2(Ka) *D. discoideum* cells were then transfected with the pDM320-FLAG-RavZ, and selected with G418. *D. discoideum* cells were collected, washed in Sorensen-120 mM Sorbitol, counted and resuspended as to have $5x10^7$ cells/ml in Laemmli Buffer (125 mM Tris pH 6.8, 4% (w/v) SDS, 20% glycerol, 0.01% (w/v) bromophenol blue, 10 mM DTT). 10 µL of each sample was migrated (50 V stacking and 150 V running, 1h30) in a 10% homemade acrylamide gel and transferred to a nitrocellulose membrane (Amersham, Protran GE10600002) in 25 mM Tris, 192 mM glycine, 20% MeOH, 0.01% SDS at 4 °C, 30 V, 16 h. After checking transfer by Ponceau Red staining, the four membranes used for the recombinant antibodies were blocked during 1 hour in PBS containing 5% (w/v) BSA (bovine serum albumin fraction V, pH 7.0 (SERVA Electrophoresis GmbH 11930)) and the other membrane was blocked during 1 hour in PBS containing 5% (w/v) milk (GE Healthcare RPN418), 0.2% (w/v) Tween20. The membranes were then incubated with each of the four recombinant antibodies (dilution 1:2 in PBS and 3% (w/v) BSA) or anti-FLAG M2 antibody (Sigma, F-3165, dilution 1:1000 in PBS with Tween and 3% (w/v) milk), overnight at 4 °C, then washed three times for 10 minutes in PBS with or without Tween. The membranes probed with the recombinant anti-FLAG antibodies were then incubated during 1 h with goat anti-human IgG (Biorad #172-1050; in PBS and 3% (w/v) BSA) coupled to horseradish peroxidase and anti-FLAG M2 with goat anti-mouse IgG coupled to horseradish peroxidase (Brunschwig, dilution 1:10'000 in PBS-Tween (w/v) and 3% (w/v) milk) and washed three times for 10 minutes in PBS with or without Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences RPN2232) using a Fusion Fx device (Vilbert Lourmat).

Results

Antibodies directed against the tag were tested on lysates from wild-type AX2(Ka) *D. discoideum* cells as a negative control and cells expressing FLAG-RavZ. The commercial anti-FLAG M2 did recognize the tagged RavZ protein (Fig. 1). Antibodies AI842, AI843, AI844 and AI177 did not recognize the tagged protein in *D. discoideum* cells (Fig. 1). The M2 antibody also recognizes nonspecifically a protein of ~65kDa (Fig. 1, marked with an asterisk).

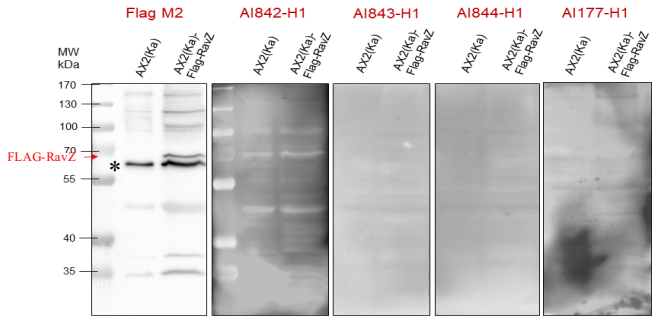

Fig. 1. Western blot with lysates extracted from AX2(Ka) cells as negative control, and AX2(Ka) cells overexpressing the FLAG-tagged *L. pneumophila* effector RavZ (66 kDa). Membranes were incubated with the indicated antibodies. The commercial anti-FLAG M2 antibody was used as a positive control.

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Conflict of interest

The authors declare no conflict of interest.