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Abstract
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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). 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 RPNI418), 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.

References


Conflict of interest

The authors declare no conflict of interest.