The AJ514 antibody recognizes the common antigen 1 from Dictyostelium discoideum by Western blot

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Abstract

The AJ514 antibody, derived from the 221-342-5 hybridoma, detects by Western blot the common antigen 1 from *Dictyostelium discoideum*.

Introduction

The common antigen 1 (CA1) is a mannose-6-SO_4-carbohydrate epitope shared by lysosomal enzymes of *Dictyostelium discoideum*, recognized by the 221-342-5 monoclonal antibody (Knecht *et al*., 1984; Neuhaus *et al*., 1998). This lysosomal carbohydrate epitope is absent in a sulfation-defective knock-out of *D. discoideum* (*kil1* KO cells) (Benghezal *et al*., 2006). Here we describe the ability of the AJ514 antibody, a single chain fragment (scFv) derived from the 221-342-5 hybridoma, to detect the common antigen 1 by Western blot.

Materials & Methods

Antibodies: ABCD_AJ514 antibody (ABCD nomenclature, web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) 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 joined by a peptide linker $(GGGGS)_3$. The sequencing of the 221-342-5 hybridoma was performed by the Geneva Antibody Facility. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (~50 mg/L) were collected after 4 days.

Antigen: $5x10^6$ *D. discoideum* (strain DH1) WT or *kil1* KO cells, cultivated in HL5 medium, were used to detect the common antigen 1.

Protocol: Cells were pelleted and resuspended in 200 µL of sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6,8, 10 mM EDTA, 0,1% (w/v) bromophenol blue, 4% (w/v) SDS; 6% (v/v) β-mercaptoethanol was added for reducing condition). 20 µL of each sample was migrated (200 V, 30 min) in a 4-20% acrylamide gel (Genscript, SurePAGE Bis-Tris, #M00655), and transferred to a nitrocellulose membrane using a dry transfer system for 7 minutes (iBlot gel transfer device, Invitrogen #IB23001). The membranes were blocked during 1 hour in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk, and washed three times for 15 minutes in PBS + 0.1% (v/v) Tween20. The membranes were then incubated during 2 hours with either the 221-342-5 monoclonal or the AJ514 scFv antibody (diluted 1:10 or 1:300 in PBS-Tween, respectively). The membranes were then washed three times and incubated for 1h with the horseradish peroxidase-coupled goat anti-mouse IgG (Biorad #170-6516, dilution 1:3000) or anti-rabbit IgG (Sigma #A8275, dilution 1:3000) and washed twice for 15 minutes and once for 5 minutes in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences) using a PXi-4 gel imaging systems (Syngene).

Results

Similarly to the original 221-342-5 hybridoma, the AJ514 antibody recognizes lysosomal enzymes of different molecular sizes exhibiting sulfated carbohydrates (Knecht *et al*., 1984). The signal was not detected in the sulfation-defective cells (Benghezal *et al*., 2006) (Fig. 1). The staining with both antibodies appears almost indistinguishable (Fig. 1).

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Conflict of interest

The authors declare no conflict of interest.

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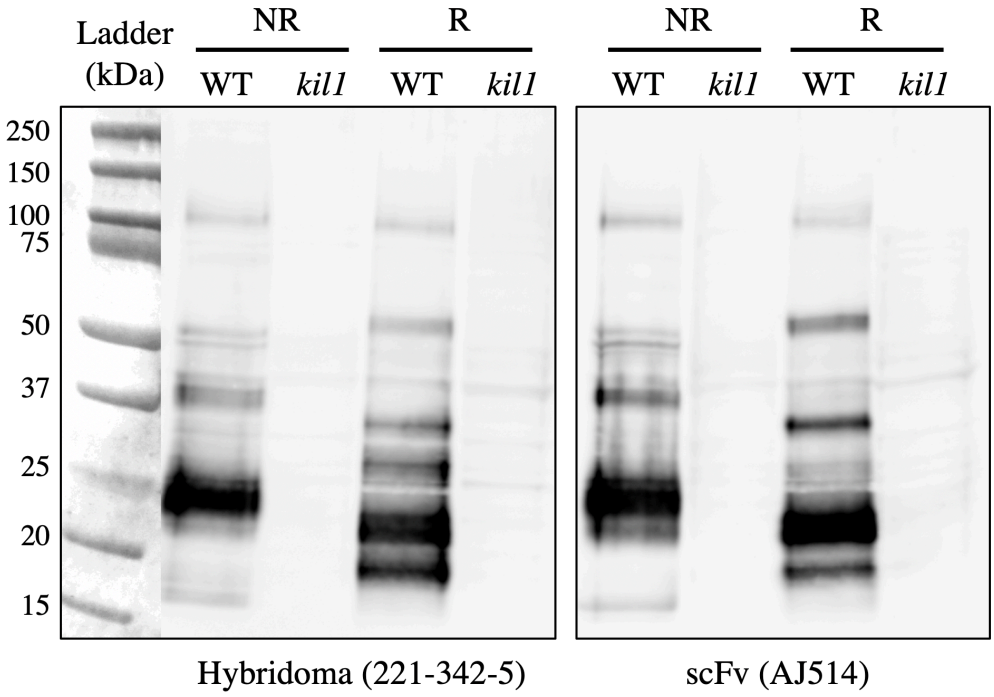

Fig. 1. The 221-342-5 hybridoma and the AJ514 scFv antibodies recognize the common antigen 1 in *Dictyostelium* WT cells but not in the sulfation-defective *kil1* KO cells (NR: non-reducing conditions; R: reducing conditions).