ABCD_AI516 and ABCD_AI523 antibodies recognize the O-antigen of the K. pneumoniae KpGe strain by western blot

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Abstract

Recombinant antibodies ABCD_AI516 and ABCD_AI523 detect by western blot the O-antigen of the *Klebsiella pneumoniae* KpGe strain.

Introduction

Klebsiella pneumoniae is an opportunistic pathogen of concern due to the emergence of multidrug-resistant strains causing hospital-acquired pneumonia (Rice, 2008). The KpGe strain used is this study is a non-pathogenic and non-capsulated variant of *K. pneumoniae* (Lima *et al.,* 2018). The outer membrane of *K. pneumoniae* contains lipopolysaccharides (LPS) that are composed of lipid A, an oligosaccharide core region and the O-antigen (March *et al.,* 2013). Mutants unable to synthesize O-antigens, such as the *wbbM–* strain, which lacks the WbbM glycosyltransferase, fail to cause virulent infections across diverse hosts. (Guan *et al*., 2001; Benghezal *et al*., 2006). Antibodies ABCD_AI516 and ABCD_AI523 have been shown by flow cytometry to recognize the O-antigen at the surface of live KpGe cells but not on *wbbM*⁻ mutant (Crespo-Yañez and Ayadi, 2022). Here, we assess the ability of these two antibodies to detect the O-antigen of the KpGe strain by western blot.

Materials & Methods

Antibodies: ABCD_AI516 (AI516) and ABCD_AI523 (AI523), antibodies (ABCD nomenclature, http://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv portion fused to a mouse IgG Fc. The synthesized scFv sequences (GeneArt) correspond to the sequences of the variable regions of clones 9H9-H7 and G3-97 respectively, joined by a peptide linker $(GGGGS)_3$. 9H9-H7 and G3-97 antibodies both target galactan II of the O-antigen in *K. pneumoniae* O1/O2 serotype (Szijártó *et al*., 2017). HEK293 suspension cells growing in HEK TF medium (Sartorius #861-0001), supplemented with 0.1% Pluronic F68 (Sigma #P1300), were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (30 µg/mL for AI516 and 50 µg/mL for AI523) were collected after 4 days.

Antigen: *K. pneumoniae* KpGe WT and *wbbM*⁻ strains were cultivated overnight at 37 °C in 3 mL of LB medium (Froquet *et al*., 2009). For the *wbbM*⁻ strain, ampicillin was added to the LB medium at a final concentration of 100 µg/mL. LPS from these strains were purified using an LPS Extraction Kit according to manufacturer's instructions (iNtRON Biotechnology #17141). Antibodies were also tested against total KpGe WT lysate. Commercial LPS from *E. coli* were used as control (Lipopolysaccharide from Escherichia coli Serotype O55:B5, Sigma #L-2637, 1 mg/mL).

Protocol: Samples of 10 µL each of either pure or diluted (1/3, 1/9, 1/27, 1/81) purified LPS and 10 µL of KpGe WT culture were mixed with 10 µL of non-reducing 2x 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). Samples were then loaded (15 µL) and migrated (200 V, 30 min) on a 4-15% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #4561083) in Tris-Glycine-SDS buffer (Canvax #BR0063) and transferred to a nitrocellulose membrane using a dry transfer system for 7 min (iBlot gel transfer device, Invitrogen #IB23001). The membranes were blocked overnight at 4°C in PBS containing 0.1% (v/v) Tween20 and 6% (w/v) milk and washed three times for 5 min in PBS + 0.1% (v/v) Tween20. After the washing, the membranes were incubated with AI516 or AI523 (1 µg/mL in PBS-Tween) for 2 h, then washed three times for 5 min. The membranes were then incubated 1 h with horseradish peroxidase-coupled goat anti-mouse IgG (Biorad #170-6516, dilution 1:3000) and washed three times for 5 min in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences) using a PXi-4 gel imaging systems (Syngene).

Results & Discussion

Antibodies AI516 and AI523 specifically recognize the O-antigen of the *K. pneumoniae* KpGe WT strain (Kp) in both total lysate (Kp lysate) and purified LPS samples. The signal intensity decreased with dilution of purified KpGe LPS, indicating a dose-dependent response. No signal was detected with purified LPS from the *KpGe wbbM–* strain (Kp *wbbM–*), confirming the specificity of AI516 and AI523 for the *K. pneumoniae* KpGe O-antigen in western blot analysis (Fig. 1). Furthermore, no signal was observed

with LPS from *E. coli* O55:B5 (Ec), indicating that the tested antibodies do not recognize the O-antigen of this strain. Samples were also tested under reducing conditions and the results were indistinguishable with those observed in non-reducing conditions (data not shown).

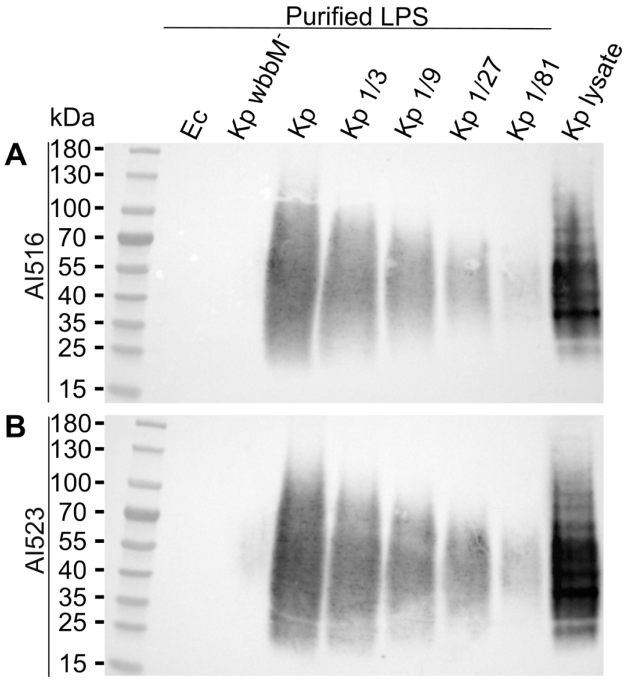

Fig. 1. AI516 and AI523 recognize the O-antigen from KpGe WT strain (Kp) by western blot in non-reducing conditions. They do not recognize purified LPS from *E. coli* O55.B5 (Ec) and purified LPS from KpGe *wbbM*- strain (Kp wbbM-).

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Conflict of interest

The authors declare no conflict of interest.