The AI516 and AI523 antibodies recognize the Klebsiella pneumoniae KpGe strain *by flow cytometry*

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Abstract

The recombinant antibodies AE516 and AI523 bind to the surface of the *Klebsiella pneumoniae* KpGe strain, as detected by flow cytometry. They do not bind to a *K. pneumoniae* strain defective in O-antigen synthesis.

Introduction

Klebsiella pneumoniae is a Gram-negative bacterium of clinical importance due to its ability to provoke infections like pneumonia, and to the emergence of multidrug resistant strains like *K. pneumoniae* 52145 (Rice, 2008). The KpGe strain is a non-pathogenic, non-capsulated strain of *K. pneumoniae* (Lima *et al*., 2018). Mutants defective for O-antigen synthesis, such as *wbbM*⁻ strains, lost the ability to mount virulent infections in various hosts (Guan *et al*., 2001; Benghezal *et al*., 2006). Here, we demonstrate by flow cytometry the ability of the recombinant antibodies AI516 and AI523 to bind to live *K. pneumoniae* KpGe strain, but not a *wbbM*⁻ mutant.

Materials & Methods

Antibodies: ABCD_AI516 and ABCD_AI523 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding portion fused to a rabbit IgG Fc. The synthesized scFv sequences (Twist Bioscience) correspond to the sequences of the variable regions of the clones 9H9-H7 and G3-97 respectively, joined by a peptide linker $(GGGGS)_3$. 9H9-H7 and G3-97 antibodies were originally raised against the O-antigen of *Klebsiella pneumoniae* O1/O2 serotype (Szijártó *et al*., 2017).

HEK293 suspension cells (growing in HEK TF medium, Xell 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 (15 mg/L for AI516 and 30 mg/L for AI523) were collected after 4 days.

Antigen: *K. pneumoniae* KpGe WT and *wbbM*⁻ were cultivated overnight at 37 °C in 3 mL of LB medium (Froquet *et al*., 2009).

Protocol: 1 mL of bacterial culture was centrifuged for 3 min at 4500 rpm. Bacteria were washed in 1 mL of SBS buffer (2 mM $Na_2HPO_4 \cdot 2H_2O$, 14.7 mM KH_2PO_4, 100 mM sorbitol, pH 6.0). All subsequent steps were performed in SBS buffer. Bacteria were resuspended in 1 mL of buffer and diluted 1:100. 200 µL of diluted bacteria were incubated for 15 min with 5 µg/mL of primary antibody at room temperature and under agitation. Bacteria were then centrifuged, washed with 1 mL of buffer, resuspended in 400 µL, then incubated with Alexa 488-coupled goat anti-rabbit IgG (Life Technologies A-11008, diluted 1:200) for 15 min. Bacteria were washed once with 1 mL of buffer and resuspended in 400 µL before analysis by flow cytometry (BD LSRFortessa Cell Analyzer, 647800E6).

Results

K. pneumoniae KpGe strain incubated with AI516 and AI523 antibodies exhibited a clear fluorescent signal compared to the negative control, where no primary antibody was used (Fig. 1, upper panels). As a specificity control, AI516 and AI523 antibodies did not measurably bind to the O-antigen deficient strain, KpGe *wbbM*⁻ (Fig. 1, lower panels).

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Conflict of interest

The authors declare no conflict of interest.

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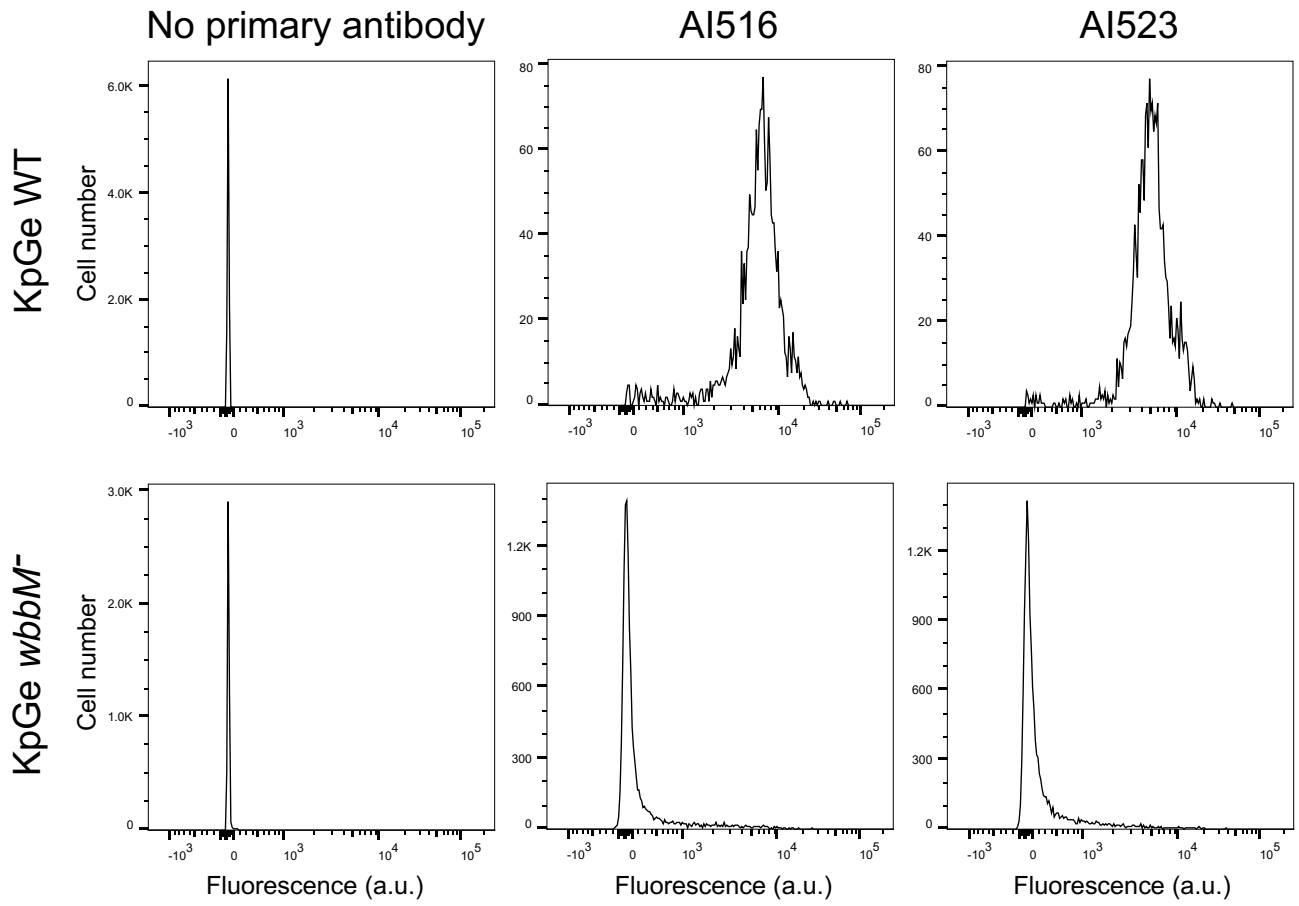

Fig. 1. Live *K. pneumoniae* KpGe are coated with AI516 and AI523 antibodies, as detected by flow cytometry. Graphs depict the number of events (Cell number axis) vs. the Alexa Fluor 488 signal (Fluorescence axis). AI516 and AI523 antibodies bound specifically to KpGe WT strain, but not to the O-antigen deficient KpGe *wbbM*⁻ strain. No labelling was seen when the primary antibody was omitted ("No primary antibody" panels).

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