The AW519 antibody specifically detects αvβ6 integrin by flow cytometry

Jérémy Kessler, Michael Bachmann, Bernhard Wehrle-Haller

Cell Physiology and Metabolism Dpt., Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The recombinant antibody AW519 detects specifically the β6 (ITGB6) subunit of αVβ6 integrin on MDA-MB-168 cells and on NIH-3T3 cells when transfected with human β6 integrin as demonstrated with flow cytometry.

Introduction

Integrin β6 pairs exclusively with the subunit integrin αV, making cell surface detection of β6 synonymous with detection of αVβ6 integrin. This integrin is expressed by many epithelial cells and tumors of epithelial origin. A major function of αVβ6 integrin is to activate the cytokine Transforming Growth Factor beta 1 and beta 3 (TGF-β1 and -β3) (Bachmann *et al.*, 2019). Here we demonstrate that the recombinant single chain antibody AW519 is able to detect αVβ6 integrin on the surface of different cell lines when analyzed by flow cytometry.

Materials & Methods

Antibodies: The ABCD_AW519 antibody (ABCD nomenclature, https://web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as a mini-antibody with the antigen-binding domain fused to a mouse IgG2A Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions of the clone 6.4B4 (Weinreb *et al.*, 2004). 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. The supernatant (10 mg/L) was collected after 4 days.

Antigen: The human breast cancer cell line, MDA-MB-168, was used to detect endogenously expressed αVβ6. In parallel, mouse NIH-3T3 cells were analyzed with or without transfection of a human GFP-coupled β6 integrin construct (the αV subunit is endogenously present).

Protocol: Multiparametric flow cytometry was used to characterize AW519 antibody binding to αVβ6 integrin. After trypsin-mediated detachment, antibody staining was carried out at 4 °C.

Cells were incubated for 30 min at 4 °C with different concentration of AW519 (10, 1, 0.2 and 0.05 µg/mL). Antibody dilutions were prepared in DMEM + 1% bovine serum albumin without FBS.

After one washing step, cells were incubated for 30 min at 4 °C with the secondary antibody (goat anti-mouse Ig PE coupled; SouthernBiotech 1010-09, 1:1000 dilution), followed by one washing step. Finally, samples were assessed with an Accuri Flow Cytometer.

Data were analyzed using the Flowjo v10 software (Becton Dickinson Ashland, Oregon). After compensation for bleed through, signals were gated for cells (FFS/SSC) and for single cells (FFS-A/FFS-H). For NIH-3T3 cells, background GFP signal of non-transfected cells was used to identify GFP-positive cells in the β6-GFP transfected condition. PE signal was acquired for single MDA-MB-168, non-transfected single NIH-3T3, and single, NIH-3T3 β6-GFP expressing cells (gated for GFP-signal).

Results

The AW519 antibody detected αVβ6 integrin by flow cytometry at the cell surface. This detection was observed for endogenously expressed αVβ6 integrin in MDA-MB-168 (Figs. 1A, 1B and 1E) and for recombinantly expressed β6-GFP in transfected NIH-3T3 (Figs. 1C, 1D and 1E).

Only background staining was detected in non-transfected NIH-3T3 cells (Figs. 1C and 1E), confirming the specificity of AW519 for β6-integrin since NIH-3T3 fibroblasts do not express β6-integrin.

References

Bachmann M, Kukkurainen S, Hytönen VP, Wehrle-Haller B. Cell adhesion by integrins. Physiol Rev. 2019; 99:1655-99. PMID: 31313981.

Weinreb PH, Simon KJ, Rayhorn P, *et al*. Function-blocking integrin alphavbeta6 monoclonal antibodies: distinct ligand-mimetic and nonligand-mimetic classes. J Biol Chem. 2004; 279(17):17875-87. PMID: 14960589.

Conflict of interest

The authors declare no conflict of interest.

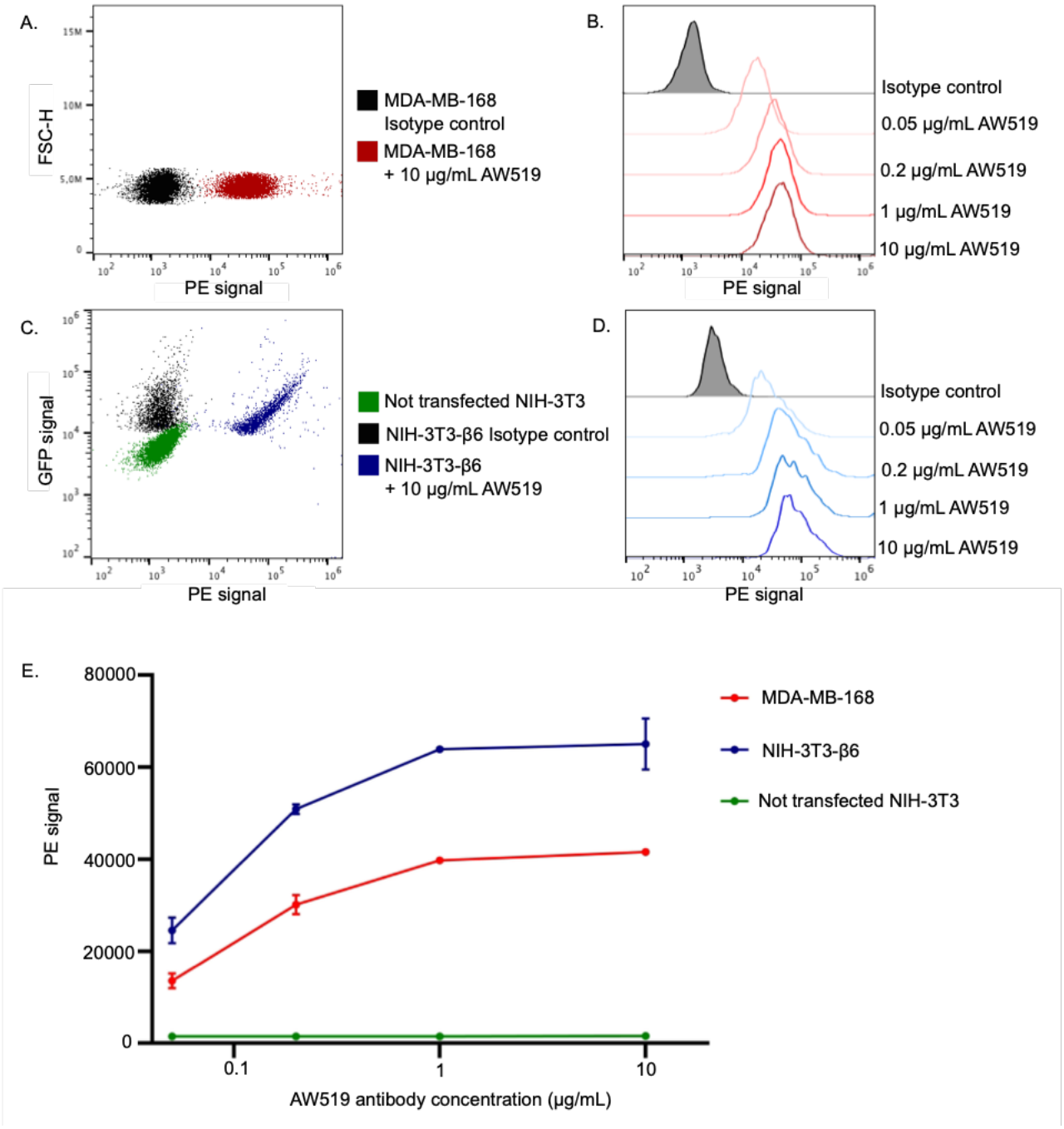

Fig. 1. AW519 binds to endogenous αVβ6 integrin and to recombinantly expressed human β6 integrin.
(A-B and E) Detection of endogenous αVβ6 integrin in MDA-MB-168 cells. **(A)** Scatter plot of FSC-H vs PE signal of AW519 (10 µg/mL) staining of MDA-MB168 cells (red) in comparison to isotype control (black). **(B)** Binding of AW519 (10, 1, 0.2 and 0.05 µg/mL; red) to MDA-MB-168 cells in comparison to isotype control (black).
(C-E) Detection of β6 subunit on β6-GFP transfected NIH-3T3 cells. **(C)** Scatter plot of GFP vs PE signal of non-transfected NIH-3T3 (green) and β6-GFP transfected NIH-3T3 (blue) in comparison to isotype control (black). **(D)** Binding of AW519 (10, 1, 0.2 and 0.05 µg/mL; red) to NIH-3T3 β6-GFP integrins in comparison to isotype control (black).
(E) Mean PE signal ± SEM for binding of AW519 to MDA-MB-168 αVβ6 integrins (red), β6-GFP transfected NIH-3T3 (blue) and non-transfected NIH-3T3 (green) at indicated concentrations.