AE098, AE456, AE510, AE596, AE597 and AE598 antibodies recognize the HIV-1 envelope protein by immunofluorescence

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Abstract

The recombinant antibodies AE098, AE456, AE510, AE596, AE597 and AE598 detect by immunofluorescence the HIV-1 envelope protein in paraformaldehyde-fixed cells. AE603 and AE724 antibodies do not.

Introduction

The HIV-1 envelope protein is first inserted in the endoplasmic reticulum, then transported through the Golgi apparatus to the cell surface and endosomes. In the Golgi apparatus, it is proteolytically cleaved into the surface protein gp120 and the transmembrane protein gp41. Here we describe the ability of six recombinant antibodies to successfully detect the HIV-1 envelope protein by immunofluorescence in gp160-transfected Hela cells.

Materials & Methods

Antibodies: ABCD_AE098, ABCD_AE456, ABCD_AE510, ABCD_AE596, ABCD_AE597, ABCD_AE598, ABCD_AE603 and ABCD_AE724 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) target the gp41 subunit of the HIV-1 envelope protein (Table 1). They were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies; Blanc *et al*., 2014) as mini-antibodies with the antigen-binding scFv fused to a mouse Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequence of the variable regions joined by a peptide linker $(GGGS)_3$ (see Table 1 for clone names and references). HEK293T cells (growing in DMEM GlutaMAX™ (Gibco, #31966) supplemented with 8% Fetal Bovine Serum (Gibco, #10270)) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~5 mg/L) were collected after 5 days.

Table 1: Clone number, recognized epitope and reference for the anti-gp41 antibodies used in this study.

Ab	Clone	Epitope	Reference
AE098	3D6	SGKLICTTAVPWNAS	Felgenhauer 1990
AE456	K14	----------	van der Donk 1994
AE510	No.86	YLKDQQLLGIWGCSGLI CTTAVPWNASWSNKSL	Moran 1993
AE596	DaB2B3	----------	David 1995
AE597	NG3B7	----------	David 1995
AE598	DZ33	----------	David 1995
AE603	M25	----------	Watkins 1996
AE724	2F5	ELDKWA	Kunert 1998

Antigen: HeLa cells (growing in DMEM GlutaMAX™ supplemented with 10% Fetal Bovine Serum) cultured on a glass coverslip (Menzel-Gläser, 22x22 mm) were transfected 3 days before the experiment with the VB9 vector coding for the full-length HIV-1 envelope glycoprotein gp160 (UniProt #P03375).

Protocol: The whole procedure was carried out at room temperature. Transfected HeLa cells were rinsed once with PBS, fixed with PBS + 4% paraformaldehyde (w/v) (Applichem, #A3013) for 30 min, and blocked with PBS + 40 mM ammonium chloride (NH_4Cl) (Applichem, #A3661) for 5 min. Cells were then permeabilized in PBS + 0.2% saponin (w/v) (Sigma, #S7900) for 10 min, washed once (5 min) with PBS + 0.2% (w/v) BSA (PBS-BSA), and incubated for 30 min with the antibody-containing supernatants (non-diluted). After 3 washes (5 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-mouse IgG conjugated to AlexaFluor-488 (1:300, Molecular Probes, #A11029). After 3 washes (5 min) with PBS-BSA, cells were mounted on slides (Menzel-Gläser, 76x26 mm) with Möwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka, #33480). Pictures were taken using a Zeiss LSM700 confocal microscope, with a 63x Neofluar oil immersion objective.

Results

Antibodies AE098, AE456, AE510, AE596, AE597 and AE598 detected specifically a signal in transfected cells reminiscent of an ER network (Fig. 1). The precise labelling pattern of each specific antibody was not specifically studied here. No signal was detected in non-transfected cells (data not shown) or when the primary antibody was omitted (Fig. 1). Antibodies AE603 and AE724 yielded no signal above background levels (Fig. 1).

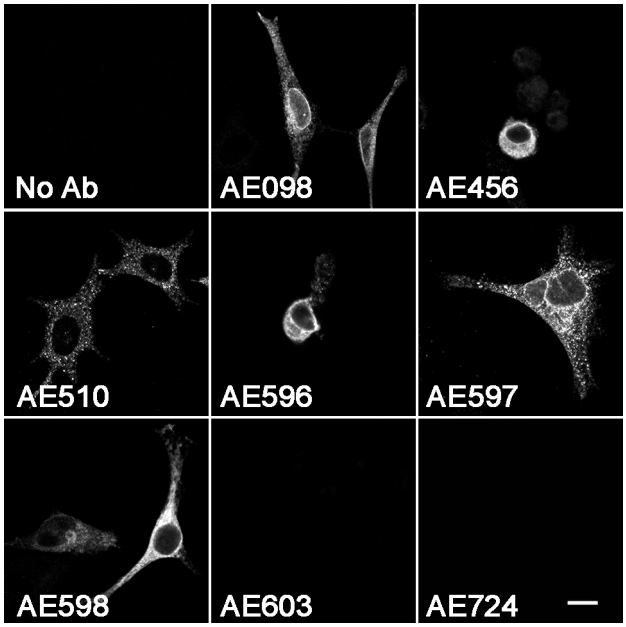

Fig. 1. AE098, AE456, AE510, AE596, AE597 and AE598 antibodies successfully labelled HeLa cells expressing the HIV-1 gp160 protein. No labelling was seen with AE603 and AE724, or when the primary antibody was omitted (No Ab). Scale bar: 10 μm.

References

Blanc C, Zufferey M, Cosson P. Use of in vivo biotinylated GST fusion proteins to select recombinant antibodies. ALTEX. 2014;31(1):37-42. PMID:24100547

David D, Goossens D, Desgranges C, Thèze J, Zouali M. Molecular characterization of human monoclonal antibodies specific for several HIV proteins: analysis of the VH3 family expression. Immunol Lett. 1995 47(1-2):107-12. PMID:8537086

Felgenhauer M, Kohl J, Rüker F. Nucleotide sequences of the cDNAs encoding the V-regions of H- and L-chains of a human monoclonal antibody specific to HIV-1-gp41. Nucleic Acids Res. 1990 18(16):4927. PMID:1697678

Kunert R, Rüker F, Katinger H. Molecular characterization of five neutralizing anti-HIV type 1 antibodies: identification of nonconventional D segments in the human monoclonal antibodies 2G12 and 2F5. AIDS Res Hum Retroviruses. 1998 14(13):1115-28. PMID:9737583

Moran MJ, Andris JS, Matsumato Y, Capra JD, Hersh EM. Variable region genes of anti-HIV human monoclonal antibodies: non-restricted use of the V gene repertoire and extensive somatic mutation. Mol Immunol. 1993 30(16):1543-51. PMID:8232339

van der Donk EM, Schutten M, Osterhaus AD, van der Heijden RW. Molecular characterization of variable heavy and light chain regions of five HIV type 1-specific human monoclonal antibodies. AIDS Res Hum Retroviruses. 1994 10(12):1639-49. PMID:7888223

Watkins BA, Davis AE, Fiorentini S, di Marzo Veronese F, Reitz MS Jr. Evidence for distinct contributions of heavy and light chains to restriction of antibody recognition of the HIV-1 principal neutralization determinant. J Immunol. 1996 156(4):1676-83. PMID:8568275

Conflict of interest

The authors declare no conflict of interest.