RB110 and RB157 antibodies recognize by ELISA specific phosphorylation patterns on CCR5 C-terminal peptides

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Abstract

Recombinant antibodies RB110 and RB157 can be used in ELISA assays to specifically detect human CCR5 phosphorylated at serines 336/337 and at serine 349, respectively.

Introduction

CCR5 (C-C chemokine receptor type 5, UniProt #P51681) is a chemokine receptor belonging to the G protein-coupled receptor (GPCR) superfamily (Mueller and Strange, 2004). Typically, upon agonist activation, GPCRs are phosphorylated at serine and/or threonine residues at their intracellular loops and/or tail (Tobin *et al.*, 2008). CCR5 features four serine residues (at positions 336, 337, 342 and 349) previously shown to be phosphorylated upon stimulation by its native ligands (Oppermann *et al.*, 1999, Pollok-Kopp *et al.*, 2003). Here we describe two recombinant antibodies (RB110 and RB157) that specifically detect peptides corresponding to the CCR5 C-terminal tail phosphorylated at positions 336/337 (RB110) and 349 (RB157) in ELISA assays.

Materials & Methods

Antibodies: ABCD_RB110 and ABCD_RB157 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2020) 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 IgG2a Fc (MRB110 and MRB157). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~20-100 mg/L) were collected after 5 days.

Antigen: The antibodies were originally raised against a synthetic biotinylated peptide corresponding to the CCR5 C-terminal region (residues 325-356; `SIFQQEAPERASSVYTRSTGEQEISVGL`) incorporating phosphoserine at positions 336, 337, 342 and 349 (underlined). Peptides were synthesized using Fmoc chemistry on a MultiPep RSi synthesizer (Intavis). A set of sixteen different synthetic biotinylated CCR5(325-356) peptides with either phosphoserine or serine at positions 336, 337, 342 and 349 were also synthesized and used to investigate antibody specificity for different phosphorylation patterns.

Protocol: Maxisorb ELISA plates (Nunc) were coated with streptavidin 5 µg/ml (50 µl/well) for 15 h at 4°C. Wells were washed three times with Wash Buffer (25 mM Tris, 150 mM NaCl, pH 7.2, supplemented with 0.1% BSA and 0.05% Tween-20) and blocked for 1 h at room temperature (RT) with PBS supplemented with 3% BSA. Biotinylated peptides (0.5 µg/ml, 50 µl) dissolved in Wash Buffer were added to wells and incubated for 2 h at RT on a benchtop shaker. Following three washes, wells were incubated for 30 min at RT with recombinant antibody supernatants (100 µl) diluted in Wash Buffer (1:50 for MRB110; 1:500 for MRB157). Wells were then washed three times and incubated with HRP-conjugated anti-mouse antibody (DAKO, #P0447, 1 µg/ml) for 30 min at RT on a benchtop shaker. Following three washes, wells were revealed with 100 µl 3,3',5,5'-Tetramethylbenzidine solution (Pierce). Reactions were stopped with 2 M H_2SO_4 and absorbance (OD) was measured at 450 nm on a VMax ELISA Absorbance Microplate Reader (Molecular Devices).

Results

ELISA experiments (Fig. 1) show that MRB110 only detects CCR5 peptides incorporating phosphoserines at both positions 336 and 337 (pS1 and pS2), and MRB157 only detects CCR5 peptides incorporating phosphoserines at position 249 (pS4).

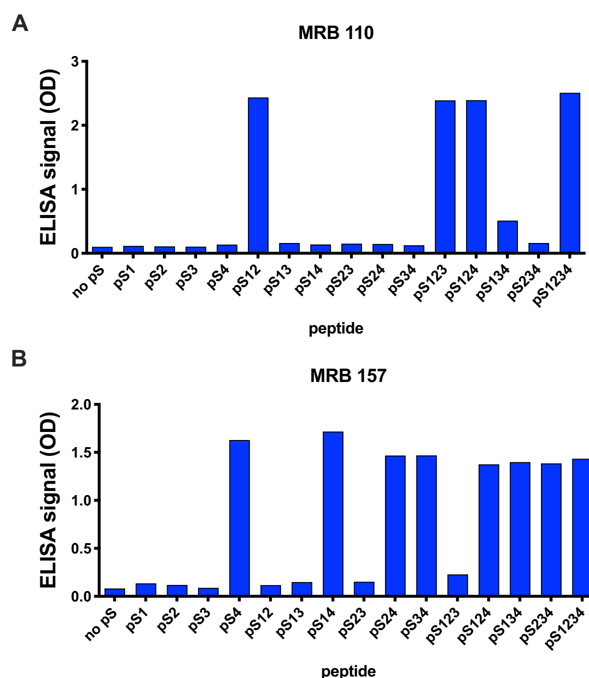

Fig. 1. ELISA binding signals for MRB antibodies to a panel of CCR5(325-352) phosphopeptides. pS1, pS12, etc. indicate the sites in the peptide at which phosphoserine was incorporated: pS1=pS336, pS2=pS337, pS3=pS342, pS4=pS349. **A)** MRB110. **B)** MRB157.

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Conflict of interest

The authors declare no conflict of interest.

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