RB137 and RB138 antibodies recognize human cathelicidin LL37 by ELISA

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Abstract

LL37 is a cationic antimicrobial peptide (AMP), which can undergo post-translational modifications (PTM), such as citrullination and carbamylation. We demonstrate here that recombinant antibodies RB137 and RB138 are specific for native LL37 and do not recognize modified LL37 (citrullinated and carbamylated). They thus represent tools to assess the presence of native, unmodified LL37 in body fluids by ELISA.

Introduction

Human cathelicidin antimicrobial peptide (CAP18 or FALL39, UniProt #P49913) is encoded by the human gene CAMP. The peptide LL37, which corresponds to the COOH-terminal part of the molecule (residues 134-170), represents the native form, which exerts antimicrobial and immune-modulatory activity (Zanetti, 2005; Hancock *et al.*, 2016). Several native LL37 functions are altered by post-translational modifications (PTM), in particular if citrullination and/or carbamylation occur in response to inflammatory processes (Kilsgård *et al.*, 2012; Koro *et al.*, 2016). Here we analyzed the ability of two recombinant antibodies (RB137 and RB138) to detect specifically the native form of the LL37 peptide by ELISA.

Materials & Methods

Antibodies: ABCD_RB137 and ABCD_RB138 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv portion fused to a mouse IgG2a Fc (MRB137 and MRB138). HEK293 adherent cells (growing in DMEM, Gibco #11960044 supplied with 8% FBS) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~1-5 mg/l) were collected after 5 days.

Antigen: The antibodies were raised against an N-biotinylated synthetic native LL37 peptide (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES). This peptide was also used as antigen for ELISA detection. As a negative control, an N-biotinylated scrambled LL37 sequence was used, as well as an N-biotinylated citrullinated LL37 (cit-LL37), where the 5 arginines were replaced by 5 citrullines (LLGDFF-**Cit**-KSKEKIGKEFK-**Cit**-IVQ-**Cit**-IKDFL-**Cit**-NLVP-**Cit**-TES) and an N-biotinylated carbamylated LL-37 (carb-LL37) (**L***LGDFFR**K***S**K***E**K***IG-K-EF**K***RIVQRI**K***DFLRNLVPRTES, in which the asterisks describe substitutions with homocitrullines) (Koro *et al*., 2016).

Protocol: The whole procedure was carried out at room temperature. Biotinylated peptides at saturating concentration (10 pmol/well) were immobilized on streptavidin-coated ELISA plates (Pierce #15124) for 30 min. Each well was rinsed three times with 100 µl of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 µl of MRB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-mouse IgG (Bio-Rad #170-6516, dilution 1:1000, 50 µl per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 µl per well). The reaction was stopped by the addition of 25 µl of 2 M H_2SO_4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted. For comparing reactivity of the antibodies to cit-LL37 and carb-LL37, non-biotinylated native LL37, cit-LL37 and carb-LL37 were directly coated on ELISA plates (Costar) and the procedure carried out as above.

Results

Antibodies RB137 and RB138 bound in a concentration-dependent manner to the native LL37 peptide (against which they were raised), but not to the negative control scrambled peptide (Fig. 1), nor to cit-LL37 or carb-LL37 (Fig. 2). RB137 has since been shown to recognize native LL37 in human tissue by immunofluorescence and immunohistochemistry (Lande *et al.*, 2020).

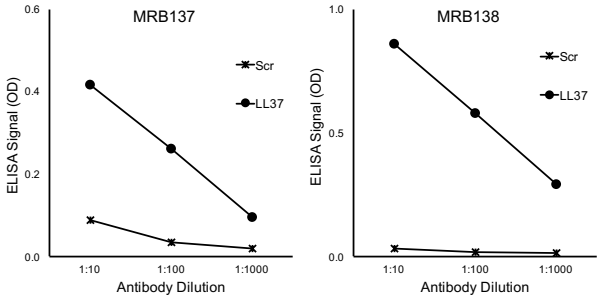

Fig. 1. Specific binding of RB antibodies to the target native LL37 peptide (LL37), as detected by ELISA. 'Scr' indicates binding to scrambled LL37.

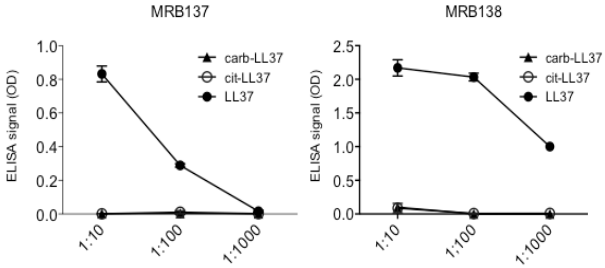

Fig. 2. Specific binding of RB antibodies to the target native LL37 peptide (LL37), as detected by ELISA. 'cit-LL37' indicates binding to citrullinated LL37 and 'carb-LL37' to carbamylated LL37.

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Conflict of interest

The authors declare no conflict of interest.

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