Evaluation of new variants of CIGB55 in two Interleukin-15-dependent cell lines

RODRIGUEZ-ALVAREZ Y[1], GARAY-PEREZ H[2], CABRALES-RICO A[2], REYES-ACOSTA O[2] and SANTOS-SAVIO A[1]

1. Pharmaceutical Division, Center for Genetic Engineering and Biotechnology, CP 10600, Havana, Cuba.
2. Chemistry and Physics Division, Center for Genetic Engineering and Biotechnology, CP 10600, Havana, Cuba.

Introduction

Interleukin-15 (IL-15) is a 14-15 kDa glycoprotein that belongs to the four-helix bundle cytokine family and was first characterized by its ability to substitute IL-2. Although, IL-15 shares with IL-2, β and γ subunits receptor but has a unique private α subunit (IL-15Rα) that is responsible for high-affinity binding [1]. IL-15 is essential to the development, function and survival of natural killer (NK) cells, NK T cells and memory $CD8^+$ T cells [2,3]. However, deregulated IL-15 expression was demonstrated in patients with autoimmune inflammatory diseases, including rheumatoid arthritis, inflammatory bowel diseases, celiac diseases, psoriasis and sarcoidosis [4,5]. Therefore, various strategies have been developed to target IL-15, its receptor or the molecules involved in IL-15-induced signaling for the treatment of such autoimmune diseases. In this sense, several agents that inhibit IL-15 activity have been developed, including soluble IL-15Rα, mutant IL-15 molecules, and specific antibodies for IL-15 or IL-2/15Rβ. Our group identified a peptide (CIGB55 or P8) that binds to IL-15Rα and blocks the activity of IL-15 [6]. Because of the high-affinity interaction between IL-15 and IL-15Ra, it was important to optimize binding of the CIGB55 peptide to IL-15Rα, in order to reduce its 50% inhibitory dose (IC50 = 130 μM). In this work we evaluated, in CTLL-2 and Kit 225 cell proliferation assay, the effects of new variants of CIGB55 with D-aminoacids or a complex with four peptide molecules called MAP CIGB55.

Methods

Peptide Synthesis

The peptides were synthesized on solid phase using the Fmoc/tBu chemistry on Fmoc-AM-MBHA resin. Removal of the Fmoc group was carried out with 20% of piperidine in DMF, and the Fmoc-amino acids were coupled with DIC/HOBt activation. Cleavage from the resin and removal of side chain-protecting groups were accomplished by treatment with TFA/H2O/EDT/TIS (95/2.5/2.5/1) for 2 h, and the peptides were then precipitated with cold ether, dissolved in 40% acetonitrile/water, and lyophilized. Peptides were purified by RP-HPLC and identified by mass spectrometry. Peptide P44 and CIGB55 were dimerized by oxidation of the Cys residue with 20% DMSO in water. Briefly, the peptide was dissolved in water at 4 mg/ml, pH was adjusted to 6 with 25% ammonium hydroxide, and then DMSO was added to a final concentration of 20%. Completion of the oxidation reaction was monitored by RP-HPLC and ESI-MS. Finally, the dimer was purified by RP-HPLC.

Effect of Peptides on Proliferation of CTLL2 and Kit 225 Cell Lines

For evaluation of the antagonist effects of the peptides, serial dilutions of the peptide under examination were performed in 96-well plates (Costar, Corning Inc.,Corning, NY USA) in a volume of 25 μl of RPMI medium (Gibco, Invitrogen Corp., Carlsbad, CA,USA) supplemented with 10% fetal bovine serum (Gibco). Then, previously washed CTLL-2 cells were added in amounts of 5000 cells/well and further incubated for 30 min at 5% CO2 and 37°C. Afterwards, 300 pg of IL-15 was added to each well, and the plate was incubated for 72 h in the same conditions. In Kit 225 we used 3,8 ng of IL-15 and 10000 cell/well. Proliferation was measured by MTT mitochondrial staining.

Results

New Variants of CIGB55 Peptide

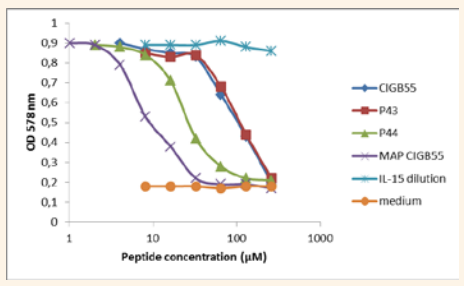
^{36}KVTAMKCFLL^{45} **CIGB55 Peptide**

P43 y P45 peptides: an aminoacid residue of CIGB55 sequence was changed by D-aminoacid.
P44 peptide: Two aminoacid residues of CIGB sequence were changed by D-aminoacid.
MAP CIGB55: a complex with four peptide molecules.

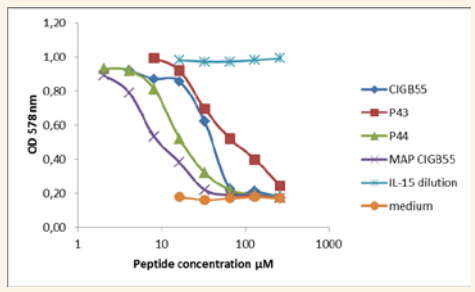
Peptide	IC50 in CTLL-2	IC50 in KiT 225
P43	130 μM	65 μM
P44	32 μM	24 μM
MAP P8	4 μM	4,8 μM

Solubility: All of the peptides, except for the P45, were soluble in aqueous solution or culture medium at a concentration of 1mg/mL.
Antagonist effect: P44 and MAP CIGB55 peptides are more active than CIGB55 peptide in CTLL-2 and Kit 225 proliferation assays (A,B). P44 dimer peptide (IC50 26 μM) is more active than the P44 monomer and the CIGB55 dimer (IC50 29 μM) in the CTLL-2 cell line (C).

Evaluation in CTLL-2 (A, C) and Kit 225 (B) cell proliferation assay



A



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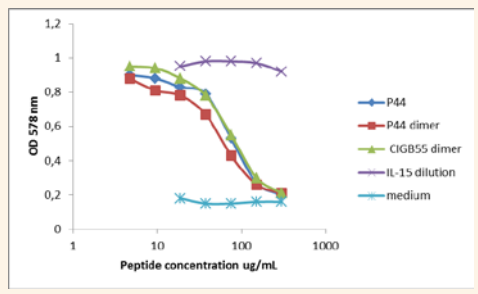

C

Conclusions

The P44 and MAP CIGB55 peptides are two new variants of CIGB55 more active in the CTLL-2 and Kit 225 cell lines.

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