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-15Ra) 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-15Ra, 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-15Ra 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-15Ra, 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 and ESi-MS. 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, Invtrogen 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. It was found that in CTLL-2 we used 3,8 ng of IL-15 and 10000 cell/well. Proliferation was measured by MTT mitochondrial staining.

**New Variants of CIGB55 Peptide**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IC50 in CTLL-2 (μM)</th>
<th>IC50 in Kit 225 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P43</td>
<td>150 μM</td>
<td>65 μM</td>
</tr>
<tr>
<td>P44</td>
<td>12 μM</td>
<td>24 μM</td>
</tr>
<tr>
<td>MAP P46</td>
<td>4 μM</td>
<td>4,8 μM</td>
</tr>
</tbody>
</table>

**Results**

**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).

**Conclusions**

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

**References**