Inhibition of Leukotoxin Activity Through Receptor-Based Peptides

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Background

- *Aggregatibacter actinomycetemcomitans* (A.a.) is a Gram-negative bacterium that colonizes the human oral cavity.
- It is a causative agent for localized aggressive periodontitis (LAP), a form of periodontal disease that occurs in adolescents, but it is also linked to infective endocarditis.
- A.a. secretes leukotoxin (LtxA), a “key” virulence regulator of the bacterium.
- LtxA is a member of the RTX (repeats-in-toxin) family, and it specifically kills white blood cells, thus inhibiting the immune response to the infection.
- In its initial interaction with the host white blood cell membrane, the toxin must bind to an integrin receptor, lymphocyte function-associated antigen-1 (LFA-1).

Our goal is to investigate the inhibition of LtxA-LFA-1 binding through the use of small peptides to block LtxA from binding to its binding site on LFA-1.

Methods

- Synthesized peptides based on the sequence of the LtxA binding site of LFA-1 and measured their ability to block LtxA activity by increasing the viability of toxin-treated cells.
  - Last β-strand on β-sheet 1
  - All four β-strands on β-sheet 2

Peptide Inhibition of LtxA

- W1S4, W2S1, W2S2, and W2S3 fully inhibited the cytotoxicity of LtxA to the THP-1 cells.
- W2S4 did not inhibit LtxA cytotoxicity.

Peptides Inhibit LtxA-LFA-1 Interaction

- Confocal images reveal a decreased co-occurrence with LtxA and LFA-1 when LtxA is pretreated with peptide.

LtxA-Peptide Affinity

- Surface Plasmon Resonance (SPR) sensor chips functionalized with each peptide to measure LtxA-peptide binding.

Summary

- Receptor-based peptides are an effective strategy to inhibit bacterial toxin activity.
- Human CD11a peptides inhibit the interaction of LtxA with LFA-1.
- Peptides and LtxA have a strong affinity to each other, revealing the mechanism to the inhibition of LtxA activity.

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[Image of Confocal images and SPR sensorgrams]

Percent Viability

<table>
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<th>Time (sec)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>200</th>
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<tbody>
<tr>
<td>LtxA only</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>LtxA + W1S4</td>
<td>100</td>
<td>100</td>
<td>100</td>
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SPR sensograms of LtxA binding to W1S4. For LtxA-W1S4 binding, \( K_D = 2.01 \times 10^{-8} \) M.

- Peptides exhibit a low dissociation constant \( K_D \) demonstrating that the peptides have a strong affinity for LtxA.