Inhibition of Leukotoxin Activity Through Receptor-Based Peptides

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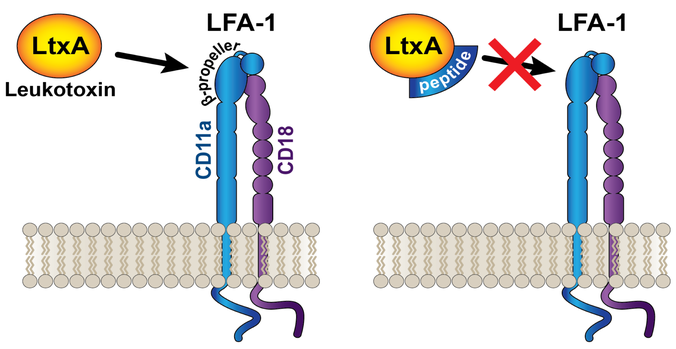
Background

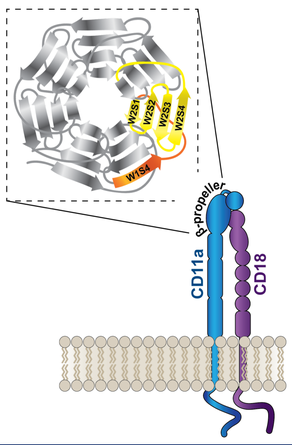
- *Aggregatibacter actinomycetemcomitans (A.a.)* is a Gram-negative bacterium that colonizes the human oral cavity.
- It is a causative agent for localized aggressive periodontitis (LAP), a form of periodontal disease that occurs in adolescents, but it is also linked to infective endocarditis.
- *A.a.* 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.



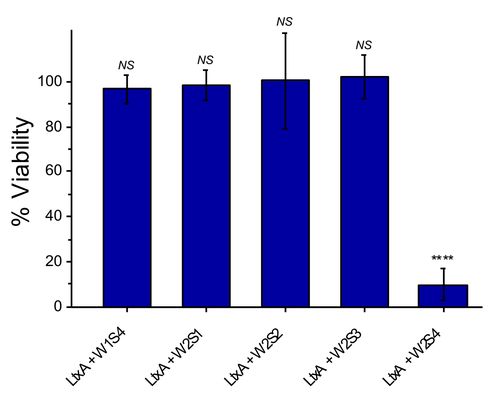
Methods

- 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



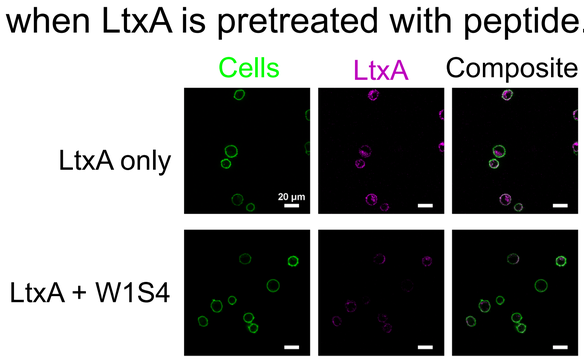
Peptide Inhibition of LtxA

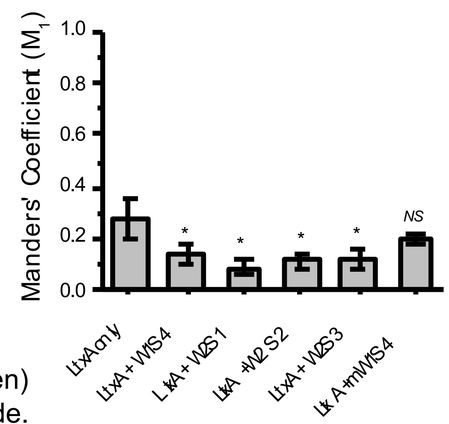
- **W1S4, W2S1, W2S2**, and **W2S3** fully inhibited the cytotoxicity of LtxA to the THP-1 cells.
- **W2S4** did not inhibit LtxA cytotoxicity.



Peptides Inhibit LtxA-LFA-1 Interaction

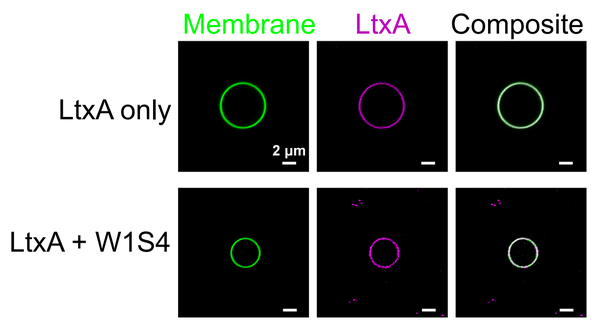
- Confocal images reveal a decreased co-occurrence with LtxA and LFA-1 when LtxA is pretreated with peptide.

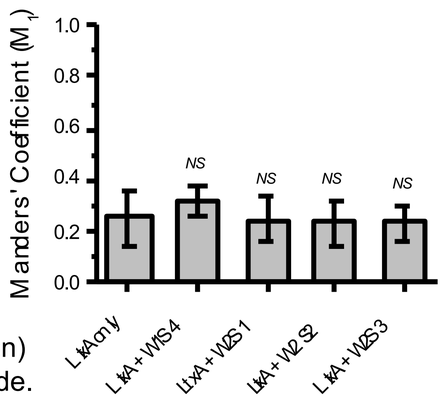




Mean Manders' coefficient M_1 of labeled LFA-1 (green) and labeled LtxA (magenta) preincubated with peptide.
NS, $P > 0.05$; * $P \leq 0.01$

- As a control, we analyzed the binding between peptide-treated LtxA and LFA-1 free GUV. Peptides do not inhibit the LtxA-cholesterol interaction.

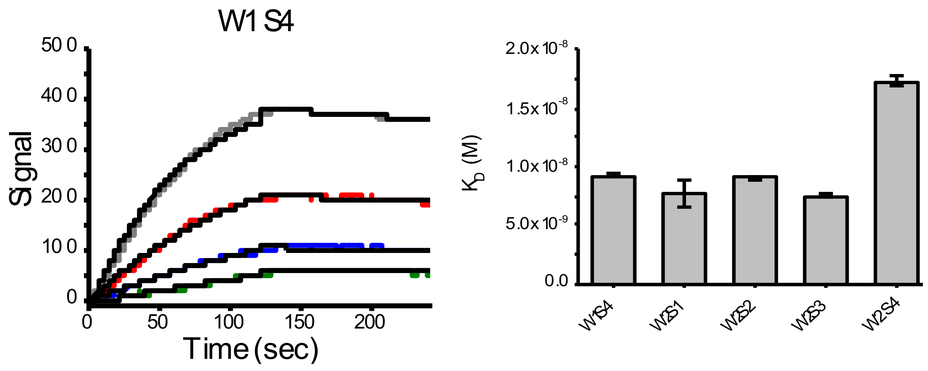




Mean Manders' coefficient M_1 of labeled GUV (green) and labeled LtxA (magenta) preincubated with peptide.
NS, $P > 0.05$

LtxA-Peptide Affinity

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



SPR sensorgrams 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.

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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