Adaptation of the Human-Specific S. aureus Leukocidin, LukGH, to Rabbit, by Protein Engineering

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Background and Aims

- Host defense against Staphylococcus aureus greatly depends on clearance by phagocytic cells. S. aureus expresses up to five cytolytic bicomponent cytotoxins (LukSF, HlgABC, HlgDEF, LukGH and LukIV-P) that specifically target phagocytes.
- The bicomponent cytotoxins exhibit species specificity that hinders their use in S. aureus pathogens in small animal models (mice and rabbits).
- Bicomponent cytotoxins secreted by S. aureus Cell receptors and species specificity

<table>
<thead>
<tr>
<th>Human Cell</th>
<th>Mouse Cell</th>
<th>Rat Cell</th>
<th>Rabbit Cell</th>
<th>Guinea Pig Cell</th>
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<tbody>
<tr>
<td>LukGH-P1</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>LukGH-P2</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>LukGH-WT</td>
<td>+</td>
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Characterization of Ala mutants of LukH

<table>
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<tr>
<th>Residue</th>
<th>Mutation</th>
<th>CD spectra (Amax)</th>
<th>Melting temperature (Tm) of Ala mutants measured by DSC</th>
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</thead>
<tbody>
<tr>
<td>LukGH-P1</td>
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Cytotoxicity of S. aureus strains expressing rabbit adapted LukGH variants

- Chromosomal integration of LukGH-D312K and LukGH-E263Q/D312Q into S. aureus LA5635A and TCH1516 strains did not alter expression of the toxins.
- Production of toxins by LA5635A can be indirectly linked to LukGH activity: lower for rabbit adapted LA5635A compared to the wild-type strain, implying that rabbit adapted LukGH has higher activity towards rabbit PMNs than wt LukGH.
- No difference was observed in toxicity of LA5S wild-type, rabbit adapted strains towards human PMNs.

Conclusions

- The binding epitope of CD11b-1 was determined.
- D312K and E263Q/D312Q mutants of LukH showed 10-fold improved activity towards rabbit PMNs in vitro and approx. 2-fold higher affinity for HLA-DRB1 than LukGH, while its activity towards human PMNs was not significantly changed compared to wt LukGH.
- The improved activity towards rabbit PMNs was maintained after chromosomal integration of LukGH variants in S. aureus strain LA5S, with no change in activity towards human PMNs.
- The effect of the LukGH mutations need to be explored in rabbit S. aureus infection models

References


Conflict of interest

The authors declare a potential conflict of interest as the work was performed at Arsanas Biosciences (Innsbruck, Austria), a biotechnology company developing a monoclonal antibody-based bacterial vaccine, S. aureus LA5S.