Design of Fab-based chimeric antibodies against Bothrops asper toxins

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Design of Fab-based chimeric antibodies against *Bothrops asper* toxins

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Addressing the problem of immunogenic antivenoms

Snakebite is one of the world’s most neglected tropical diseases, with an estimated 5 million bites per year, resulting in about 125,000 deaths.1 The only current treatment for snakebite envenoming is antiserum derived from the blood of immunized mammals (typically horses).2,3 These antiserums are expensive to produce and carry a high risk of causing hyper-allergic reactions in human recipients due to their heterologous origin.4,5 Here we report the discovery of chimeric scFvBs against *Bothrops asper* toxins.

### Composition of *B. asper* venom

<table>
<thead>
<tr>
<th>Component</th>
<th>Composition</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVMPs</td>
<td>Snake Venom MetalloProtease</td>
<td>43.3%</td>
</tr>
<tr>
<td>PLA2</td>
<td>Phospholipases A2</td>
<td>4.8%</td>
</tr>
<tr>
<td>CRISP</td>
<td>Cysteine-Rich Secretory Proteins</td>
<td>4.4%</td>
</tr>
<tr>
<td>LAD</td>
<td>L-amino Acid Oxidases</td>
<td>4.6%</td>
</tr>
<tr>
<td>SP</td>
<td>Serine Proteases</td>
<td>4.6%</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic Cell Fragments</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

Results presented here were obtained prior to the project.6

### Construction of scFvBaP1 from a monoclonal antibody

Hybridoma cells secreting the monoclonal antibody against BaP1 (MABA1) were made, and from these a recombinant single chain variable fragment (scFv) was constructed.

### Results from the recombinant scFvBaP1

The scFvBaP1 was cloned into the pMST3 expression vector in frame with a SUMO (Smith-Ulrich-Maison-Dubreuil) sequence. When subjected to SDS-PAGE, the sequenced SUMO-scFvBaP1 showed a band at 40 kDa as was predicted and SUMO protein around 13 kDa.7

*In vivo* tests showed that scFvBaP1 specifically recognizes BaP1 and whole *B. asper* venom, and to a large extent neutralizes the main toxic effects of BaP1.

Results presented here were obtained prior to the project.7

### Transformation of antibody formats into E. coli

Recombinant FabBaP1 and scFabBaP1 will be made from scFvBaP1. These are going to be expressed in *Escherichia coli* using a pUHE23-2 expression vector. The final protein model shown here is of the recombinant scFab.8,9

### Binding strength testing of chimeric antibodies by ELISA

Binding strengths of the antibodies to BaP1 will be determined by ELISA. This will allow us to compare the different antibody formats in regards to how likely they are to neutralize BaP1.

### Next steps: Determining the best antibody format

If good chimeric antibodies are obtained in good yield, the next steps will be to determine their Kd and test the antibodies in a preclinical model. This will allow an *in vivo* comparison between the different formats.

### References


### Contact information

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### Acknowledgements

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*Bothrops asper* venom composition:

- SVMPs: Snake Venom MetalloProtease (43.3%)
- PLA2: Phospholipases A2 (4.8%)
- CRISP: Cysteine-Rich Secretory Proteins (4.4%)
- LAD: L-amino Acid Oxidases (4.6%)
- SP: Serine Proteases (4.6%)
- DC: Dendritic Cell Fragments (0.1%)

Results presented here were obtained prior to the project.

**Construction of scFvBaP1 from a monoclonal antibody**

1. B cells are harvested.
2. Hybridoma cells are seeded.
3. Myeloma cells are seeded.
4. scFvBaP1 is made.

**Results from the recombinant scFvBaP1**

The scFvBaP1 was cloned into the pMST3 expression vector in frame with a SUMO (Smith-Ulrich-Maison-Dubreuil) sequence. When subjected to SDS-PAGE, the sequenced SUMO-scFvBaP1 showed a band at 40 kDa as was predicted and SUMO protein around 13 kDa.

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