Studies on the Host Selectivity of the Staphylococcal Innate Immune Evasion Protein, SPIN

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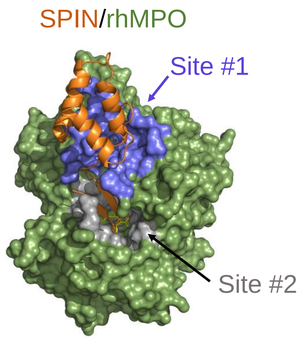
Introduction

- *Staphylococcus aureus* is a Gram-positive bacterium that is a model system for innate immune evasion as it secretes several proteins that inhibit key enzymes of neutrophils.[1,2]
- The Staphylococcal Peroxidase Inhibitor (SPIN) is a small, secreted protein from *S. aureus* that inhibits human myeloperoxidase (MPO) activity within the neutrophil phagosome.[1,2,3]
- *S. aureus* SPIN acts as a molecular plug and blocks solute exchange with the MPO active site, preventing production of cytotoxic hypochlorous acid (HOCl).[2]
- *S. aureus* is primarily a human pathogen, and its SPIN protein cannot bind MPO from non-human mammalian species (i.e. canine, feline, equine, bovine, rodent).[2]
- There is limited understanding as to whether SPIN proteins from Staphylococci that primarily infect other mammals might bind and inhibit MPO from their preferred hosts.

Objectives

1. Evaluate the interactions between SPIN homologs from Staphylococcal species known to infect dogs and recombinant canine MPO.
2. Examine whether these SPIN homologs can bind and inhibit human MPO.



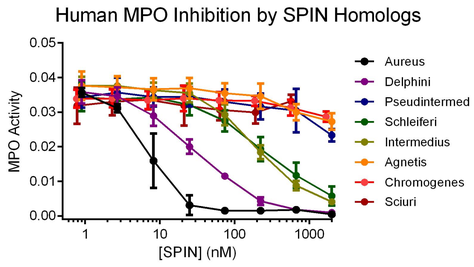
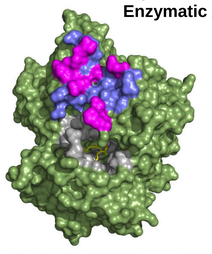


Figure 2. Subset of SPIN Homologs Inhibit Enzymatic Activity of Native Human MPO

Figure 1. Structural Basis for Inhibition of Human MPO by SPIN-*aureus*
Site #1: Primary binding interface between SPIN and MPO; Site #2: β-hairpin on SPIN is responsible for blocking the MPO active site.



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Homo      RNQINALTSFVDASMVYGSEDPLARNLRNMSNQLGLLAVNQRFQDNGRALLPFDNLEDDP  360
Canis     RNQINALTSFVDASMVYGSEDPLATRLRNLTNQLGLLAVNTRFSDNGRALLPFDNLEDDP  359
Bos       RNQINALTSFVDASMVYGSEDPLAMRLRNLTNQLGLLAVNTRFQDNGRALLPFDTLEHDP  360
Sus       RNQINALTSFVDASMVYGSEDPLAMKLRNLTNQLGLLDVNNRFRDNGRALLPFDNMEDDP  360
Loxodonta RNQINALTSFVDASMVYGSEDPLARDLRNLTNQLGLLRVNDRFRDNGRALLPFDKLEDDP  360
Mus       RNQINALTSFVDASGVYGSEDPLARKLRNLTNQLGLLAINTRFQDNGRALMPFDSLEDDP  360
          ************** *****:***  ***::****** :* ** ******:***.:::.**

Homo      CLLTNRSARIECFLAGDTRSSEMPELTSMHTLLLREHNRLATELKSLNPRWDGERLYQEA  420
Canis     CLLTNRSAGIECFLAGDTRSSEMPELASMHTLFLREHNRLATELRRLNPRWDGERLYQEA  419
Bos       CRLTNRSANIECFLAGDSRASEMPELTSMHTLFVREHNRLAKELKRLNAHWNGERLYQEA  420
Sus       CLLTNRSANIECFLAGDSRASEMPGLTSLHTLLLREHNRLATQLKRLNPRWDGERLYQEA  420
Loxodonta CLLTNRSVRIECFLAGDTRSSEMPELTSMHTLLVREHNRLATELKRLNPRWDGERLYQEA  420
Mus       CLLTNRSARIECFLAGDMRSSEMPELTSMHTLFVREHNRLATQLKRLNPRWNGEKLYQEA  420
          * *****. ******** *:**** *:*:***::*******.:*: ** :*:**:*****
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Figure 3. Sequence Features of MPO Homologs within the SPIN-MPO Binding Interface

Materials and Methods

- SPIN proteins from *Staphylococcus pseudintermedius* and *Macrococcus canis* were produced in *Escherichia coli* and purified by nickel-affinity and gel filtration chromatographies.
- Purified SPIN-*pseudintermedius* and SPIN-*canis* were characterized by electrophoresis and mass spectrometry.
- Transient-transfection of HEK293(t) cells was performed to produce recombinant canine and human MPO.
- Conditioned culture medium was harvested four days post-transfection.
- Immunoblot was used to assess production of recombinant MPO using anti-His mAb, anti-human MPO, and anti-myc mAb as primary antibodies.

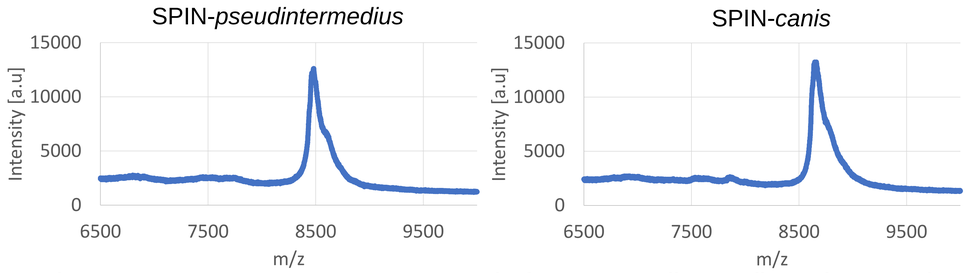

Figure 4. MALDI-TOF Mass Spectrometry Graphs for SPIN-*pseudintermedius* and SPIN-*canis*

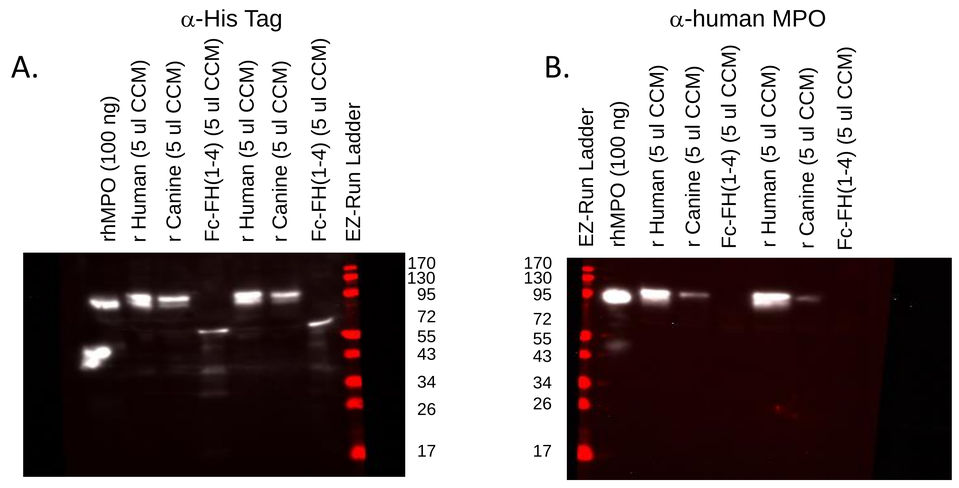

Figure 5. Immunoblot for Expression of Recombinant Canine and Human MPO.

(A) 1:1000 dilution of α-His mouse mAb; Control was 100 ng recombinant human MPO-his

(B)1:1000 dilution of α-human MPO; Control was 100 ng recombinant human MPO-his

(C) 1:2000 α-His mouse mAb; Controls were 100 ng and 30 ng recombinant human MPO-his

(D) 1:1000 α-myc mouse mAb; Conrols were 100 ng and 30 ng recombinant human MPO-his

Summary of Results

- SPIN-*pseudintermedius* and SPIN-*canis* proteins were successfully purified per SDS-PAGE electrophoresis and mass spectrometry
- Peak mass-to-charge (m/z) ratios for SPIN-*pseudintermedius* and SPIN-*canis* were 8481.63 Da and 8658.22 Da, respectively.
- Bands specific for both recombinant human and canine MPO were present on α-His mAb and α-c-myc immunoblots, while recombinant canine MPO showed weaker reactivity for α-human MPO.

Discussion

- Purification of SPIN-*pseudintermedius* and SPIN-*canis*, and recombinant human/canine MPO expression allow for further structure/function analyses.
- Surface plasmon resonance (SPR) can be used to measure the ability of these SPIN homologs to bind to recombinant human/canine MPO.
- Enzyme assays can be used to assess whether the SPIN homologs also inhibit human/canine MPO activity.
- Future plans include being able to compare the host-pathogen specificity of canine Staphylococcal species and canine MPO with that of published information on the selectivity of *S. aureus* and human MPO.

Conclusions

- Preparation of SPIN proteins from canine Staphylococcal species and canine MPO was successful for continuing studies.
- Further understanding of the host-pathogen interactions between SPIN and MPO will aid in defining the physical basis behind the host-selectivity of virulence proteins.

Acknowledgements

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References

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