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Student-Faculty Summer Undergraduate Research Fellowship Research Synopsis: Complement Resistance in *Bartonella* Species

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Student-Faculty Summer Undergraduate Research Fellowship Research Synopsis

Benjamin J. Mason

Complement Resistance in *Bartonella* Species

Abstract

Of the 31 validated species in the genus *Bartonella*, 11 are agents of serious infectious diseases of humans, among them Carrión's disease, trench fever, cat-scratch disease, and bacillary angiomatosis. *Bartonella* are extremely widespread, having been found in virtually every type of mammal surveyed. As facultative intracellular parasites employing hemotrophy (infection of red blood cells), the key to their success comes in their ability to survive within the bloodstream of their host or reservoir. It has been demonstrated that bartonellae are resistant to the effects of complement proteins in serum, the primary agents of the innate immune system. Although this effect has been repeatedly observed, the molecular basis of *Bartonella*'s resistance remains undetermined. The **overall objective** of this research is to examine the genetic and molecular components of complement resistance in bartonellae using *Bartonella bacilliformis* (*Bb*) as a model species. In order to address our hypothesis that complement resistance has a genetic basis and likely encodes a surface-exposed protein or component of the bacterium, we had three original experimental aims. In **aim 1**, we planned to use the *Himar1* transposon to generate a signature-tagged mutagenesis (STM) library of *Bb*. In **aim 2**, we were to screen the library to identify the specific gene(s) involved with serum complement resistance. In **aim 3**, we were going to analyze the gene(s) that confer this resistance by automated DNA sequencing

and genetic manipulation. The results of this study would allow us to analyze complement resistance in *Bartonella* and increase our understanding of complement resistance in other pathogenic bacteria.

Initial Findings

Samples of 4-day-old Bb culture were washed three times with 0.9M saline and sent to Dr. Richard P. Marconi of Virginia Commonwealth University Medical Center. Concentrations were determined with a BCA assay against bovine serum albumin (BSA). Using a far-Western blot with human factor H as a probe, Dr. Marconi identified a distinct factor H-binding protein (Fhbp) of Bb of ~90kDa molecular weight. A sample of Bb culture media (HIBB) was also analyzed and used as a control. On replicate analyses of first, second, and third wash supernatants, a protein band of similar molecular weight was observed. The intensity of this band appeared to decrease with each subsequent wash, suggesting a weak association with the bacterial cell wall.

Refocusing Experimental Protocol

Since far-Western blots identified a potential protein candidate as the Fhbp of Bb, a more streamlined approach was undertaken to directly characterize it. Five new experimental aims were designed as follows:

Aim 1: Screen genetic and proteomic databases of Bb to identify potential genes that could encode the factor H-binding protein (Fhbp) based on molecular weight.

Aim 2: Use TOPO cloning kits to isolate the corresponding genes of candidates identified in Aim 1.

Aim 3: Analyze complement resistance in Bb.

Aim 4: Confirm cellular location of Fhbp by immunofluorescence assay (IFA).

Aim 5: Analyze the factor H-binding protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Aim 1

Based on the 90kDa weight of the Fhbp identified by far-Western blots, the Bb KC583 genome from the NCBI databases was searched for genes either identified or hypothesized to produce peptides within ± 10 kDa of this mass. Of the initial list of over 30 proteins identified, five were highlighted based on protein type or genetic homology to virulence factors in other species.

Aim 2

Cloning of the short list of genes began with the first gene as the first target. Primers were prepared and optimized based on genomic data and used in polymerase chain reaction (PCR) procedures. PCR success was verified using agarose gel electrophoresis. Cloning attempts using a TOPO 2.1 cloning kit were unsuccessful, potentially due to the relatively large size of the DNA target and associated long coding sequence. A TOPO XL cloning kit was then used in successive attempts, but successfully transformed colonies verified by blue-white screening were found to possess only vector plasmid DNA. Inability to successfully clone this gene could have stemmed from a problem with the vector strain or cytotoxicity due to leaky expression from the *lacZ* promoter on the TOPO constructs.

In a paper on the Fhbp of *Moraxella catarrhalis*, researchers noted that there is often interspecific homology among Fhbps due to the structure of factor H (20 homologous short consensus repeats of 60 residues with 3-8 residue spacers). Using the sequence of the Fhbp of

Rickettsia conorii in a BLAST search against the KC583 genome identified a gene from our short list of five candidate Fhbps with significant sequence homology. New primers have been designed to amplify this gene, and we are cloning the target for future research.

Aim 3

Complement assays were carried out on *E. coli* HB101 and Bb (strain KC583). *E. coli* showed statistically significant differences in survival that were directly correlated to concentration of serum, and at 50% serum showed significant death relative to *E. coli* treated with heat-killed serum. Bb resistance to complement was demonstrated using both guinea pig serum and pooled human complement. There was no statistically significant difference between Bb survival following treatment with normal human serum or heat-killed serum for either complement assay. To determine the role of factor H in the survival of Bb, another complement assay was carried out using factor H-depleted serum. Due to issues of contamination, these assays were unsuccessful. In order to eliminate the effects of contamination, the assay will be repeated using selective plates and a kanamycin-resistant strain of Bb.

Aim 4

An immunofluorescence assay was carried out on cells from a four-day culture of Bb. Harvested cells were incubated in pooled human serum and washed with phosphate-buffered saline (PBS). The cells were then treated with either goat anti-human factor H antigen, normal goat serum, or PBS. To visualize antibody binding, AlexaFluor conjugated IgG was added. The cells were then washed and examined under UV microscopy. After initially inconclusive results,

a blocking step using donkey serum was added. Results clearly showed factor H-binding to the surface of Bb.

Aim 5

Four-day-old cultures of Bb were harvested into HIB and washed with PBS. The cells were then incubated in normal human serum and washed again. The cells were then washed in PBS and treated with goat serum and goat anti-factor H antiserum. The isolated protein content was then immunoprecipitated using protein A sepharose (PAS). The concentration of protein was extremely low, and so there was low resolution on SDS-PAGE analysis of the immunoprecipitate. Even after further concentration of the precipitate using a Slider cassette, gel readouts were still inconclusive. This aim will now be done using recombinant Bb Fhbp protein, prepared as described in revised aim 2, for analysis.