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DEFENSE OF THE DOCTORAL

DISSERTATION

"Borrelia glycosaminoglycan binding protein: a novel virulence factor for the Lyme disease causing spirochete, *Borrelia burgdorferi*"

by

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Molecular Biology, Genetics & Cancer Track

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11:00 A.M.
ICPH Auditorium

ABSTRACT

Lyme disease, caused by the spirochete, *Borrelia burgdorferi*, is the most common tick-borne illness in the United States, with the CDC estimating ~300,000 new cases/year. The bite of an infected tick deposits *B. burgdorferi* into the mammalian host initiating infection. As an extracellular pathogen, the spirochete relies on the expression of a large number of adhesins to colonize the host. We describe our studies on **B**orrelia **G**lycosaminoglycan-binding **P**rotein (Bgp) that functions both as an adhesin, facilitating adherence to heparan sulfate on the host cell surface, and as a 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase, facilitating detoxification/removal of metabolic byproducts and nutrient salvage. Given its role in two critical pathways, we hypothesized that Bgp is an important virulence factor of *B. burgdorferi*. To investigate this, *bgp* mutants in two highly infectious and clinically relevant strains, B31 and N40, were characterized. Our approach included *in vitro* adherence assays to screen for loss of function, and *in vivo* infection studies with Lyme-susceptible C3H mice. In the B31 background, wild type, *bgp*mutant, and trans-complemented strains were compared. In the absence of Bgp reduced adherence to heparin and mammalian cell lines was observed compared to wild type and complemented strains *in vitro*. The *bgp*mutant strain was also attenuated in its ability to colonize tissues in mice compared to the wild type and *bgp*comp strains, particularly at inoculum doses of $\leq 10^3$ spirochetes. In the mutant strain we show poor recovery of spirochetes from organs of infected mice, reduced seroconversion, decreased spirochete burden in tissues, and attenuated inflammatory disease. In the N40 background, bioluminescent *bgp*mutants were generated and characterized in parallel with WT-N40ΔBBE02ΔBbluc strain. A more pronounced reduction in tissue colonization was detected in two N40-*bgp*mutant strains. *In vivo* bioluminescent imagining of *bgp*mutant infected mice, in real-time, showed reduced spirochete dissemination/colonization as compared to the wild type N40 strain. These results were supported by culture-recovery and molecular tests; even at high inoculum doses of 10^4-$5x10^5$ spirochetes. Taken together, our studies in two strains show that the loss of Bgp results in an infectivity deficit, thus confirming that Bgp is an important virulence factor. The dual function of Bgp complicates straightforward understanding of how the adhesin and MTA/SAH nucleosidase activities affect virulence. A site-directed mutagenesis strategy was developed to mutate critical residues in either functional domain. Purification of recombinant BgpΔHeparinbinding or BgpΔNucleosidase proteins demonstrated loss of either heparin-binding or nucleosidase activity respectively by *in vitro* assays. Future use of these constructs for *in vitro* and *in vivo* analyses will identify the function of Bgp that is critical for full manifestations of Lyme disease pathogenesis.