Role of the Arp Lipoprotein in the Pathogenesis of *B. burgdorferi* during Murine Infection.

**Petronella Hove**¹ and Troy Bankhead¹,²

**Paul G. Allen School of Global Animal Health**¹, Department of Veterinary Microbiology and Pathology²

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**Abstract**

Infection with the bacterial agent of Lyme disease, *Borrelia burgdorferi* (Bb), results in a severely debilitating disease with clinical manifestations including arthritis, neurological impairment and cardiac inflammation. Lyme arthritis associated with Bb infection occurs in a substantial number of untreated animal and human patients that can range in disease from mild to severe(2, 3). Important in Lyme disease pathology is the broad number of surface lipoproteins that can trigger host inflammatory responses.(4) Arp is one such surface lipoprotein that is upregulated during host infection and is encoded on the linear plasmid 28-1 (lp28-1). While the function of this gene is unknown, anti-Arp antibody has been shown to reduce severity of arthritis in immunodeficient mice suggesting a role for this lipoprotein in inflammation(5, 6). Moreover a Bb strain lacking lp28-1 displayed reduced arthritis levels during murine infection(4, 7). Despite this evidence for a potential role in joint inflammation, direct mutational analysis of the *arp* gene has not been carried out to date. In this study, we demonstrate that a targeted deletion mutant of *arp* produces an intermediate level of joint arthritis in mice compared to the wild-type control. Future work should provide details on the exact timeline for the role of Arp in inflammation during infection, as well identifying any additional lp28-1-resident having a role in Lyme arthritis. Identifying factors involved in Bb pathogenesis and colonization of host joints is important as it may provide new targets for therapeutic intervention.

**Introduction**

- A model of Lyme arthritis in C3H mice duplicates many of the features of the acute phase of human Lyme arthritis (8).
- In C3H mice, infection by wild type Bb strain induces marked edema and inflammation 10 to 14 days after inoculation of the spirochete. In mice, the arthritis occurs mainly in the tibiotarsal joints, peaks 3 to 5 weeks after infection (5).
- Low passage wild type infectious strains have been used in the laboratory to study the dynamics of *Bb*.
- Other strains that have lost certain plasmids have been correlated with loss of infectivity and persistence in mice suggesting that they carry genes involved in disease propagation (7).

In order to assess involvement of *arp* in Lyme arthritis we compared levels of arthritis caused by wild type, *arp* knockout (*arp KO*) and a strain lacking lp28-1lp28-1 minus) which results in an attenuated level of infection.

**Experimental approach**

**Targeted deletion of *arp***

**Verification of mutants**

**Assessment of Joint inflammation**

**Figure 2**. Schematic of the construction strategy for the *arp* knockout plasmid. The deletion construct was created by PCR amplification of a 1000 basepair (bp) region upstream of the *arp* locus. Resulting fragment was then cloned into a shuttle vector which carries a kanamycin resistance gene (*kan*) for selection, and a replicated telomere (*retel*) which is specifically recognized by the telomere resolvase (*Telo*). After transformation of deletion construct into a fully infectious clone of Bb, forced integration of the plasmid at the homologous target site and resolution of the *retel* by endogenous ResT resulted in the loss of a DNA fragment containing only the *arp* gene.

**Figure 3**. Southern blot confirmed successful mutants. Four transformants were recovered and screened by polymerase chain reaction (PCR) for the presence of the *kan* and the absence of *arp*. Two clones matched these criteria and were further screened by Southern blot. Both clones contained a deletion of lp28-1 of the expected size. The blot also confirmed the presence of the kan gene (closed arrow) and the absence of *arp* (open arrow) by use of respective probes on lp28-1.

**Figure 4**. Effects of Bb strain on ankle swelling in C3H mice. Four groups of C3H mice were infected subcutaneously or intraarticularly with either WT, *arp KO* or lp28-1 minus strain. Uninfected control mice were injected with sterile medium. Weekly measurements of the most severely swollen ankle were made for each animal, and the values were used to determine average differences within each treatment group at each time point.

**Conclusions**

- *arp KO* showed the same level of infectivity and persistence as wild type strain suggesting that *arp* plays no role in infectivity and persistence.
- *arp KO* exhibited an intermediate severity of joint inflammation compared to wild type strain within the study period indicating *arp* contributes significantly to the inflammatory phenotype
- Initial level of arthritis in the KO strain was not the same as the lp28-1 minus strain suggesting that another gene on lp28-1 may be involved in joint inflammation.

**References**