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Abstract

α-Gal Syndrome is a tick-borne delayed hypersensitivity reaction that occurs 3-6 hours after red meat consumption. It is induced by the oligosaccharide galactose-α-1,3-galactose (α-gal) found in the Lone Star tick and all mammalian-derived foods, such as beef, pork, and goat. α-gal specific IgE (sIgE) antibodies are produced after α-gal transmission from the tick bite and activated after subsequent red meat consumption. Previously in our lab, an immune-affinity approach utilized an α-Gal antibody to isolate and identify covalently linked tick saliva antigens to α-gal followed by Liquid Chromatography-tandem Mass Spectrometry (LS-MS/MS) analysis. The identified antigen included tick salivary Galectin, a carbohydrate-binding protein that is involved in many physiological functions such as inflammation and immune responses. The goal of this study is to characterize the Lone Star tick Galectin protein and its involvement in α-Gal Syndrome. Our qRT-PCR results revealed decreased gene expression of Galectin in different stages of the bloodmeal. We utilized an RNA interference (RNAi) approach to silence Galectin gene expression and assessed the functional consequences of gene depletion on tick behavior, phenotype (attachment rate, feeding duration, engorgement rate), and tick molting. The silencing of Galectin did not have a significant impact on α-gal. However, Galectin knockdown (KD) did cause a significant downregulation in galactose metabolism related genes including Galactose 1-phosphate uridyltransferase (GALT), Galactokinase (GALK), and α-D-galactosidase (AGS). Galectin-silenced ticks showed impaired oviposition as well increase microbial load in the salivary glands. We are currently conducting experiments to further characterize the role of Galectin in microbial maintenance, galactose metabolism and reproductive development to determine possible links between Galectin and α-gal.

Background and Significance

Food allergies affect an estimated 32 million Americans[5]. Red Meat Allergy, also known as α-Gal Syndrome (AGS), is an emerging food allergy caused by the bite of the Lone Star Tick, *Amblyomma americanum*, which is predominantly found in the southeast and northeast regions of the United States. Symptoms of Red Meat Allergy include sneezing, hives, coughing, shortness of breath, and anaphylaxis. AGS is a hypersensitivity to the oligosaccharide α-gal which is found on mammalian glycolipids and glycoproteins[6]. Galectin is a β-galactoside-binding lectin that functions in several intra- and extracellular processes where they bind to glycosylated proteins and lipids. Galectins are classified according to their domain organization (Figure 1). Galectin is found in *A. americanum* and is proposed to play a role in many physiological processes including tick metabolism (Figure 3). Galectin is believed to be involved in capturing glycoproteins, which are then cleaved by glycoside hydrolases and glycosyltransferases, resulting in the α-gal glycan. Therefore, Galectin is thought to play a role in tick carbohydrate metabolism (Figure 4), and transport.

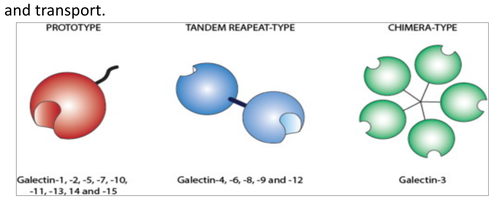

Figure 1. Galectins are grouped according to their structures.

Hypothesis

We hypothesized that the silencing of Galectin will impair the tick's ability to produce and present the α-gal antigen by hampering the tick's carbohydrate metabolism and microbial homeostasis.

Methods

Tick rearing: Ticks were reared at the University of Southern Mississippi according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) [1]

dsRNA synthesis, tick injection, tick tissue isolation: Target GRP and GFP was amplified from tick salivary glands and GFP plasmid using T7 flanked primers and purified; double- stranded RNA then in vitro synthesized using an RNA synthesis kit as described previously [3] . Ticks were injected with dsRNA. Ticks were fed on sheep, and their tissues were dissected for down-stream molecular assays using a method previously described[2,4] .

RNA isolation and cDNA synthesis, Quantitative real time (qRT-PCR), SDS-Page: RNA was extracted using Illustra RNA spin Mini Kit (GE Healthcare, Life Sciences) and concentration was measured by nanodrop spectrometer. cDNA was synthesized using Iscript cDNA synthesis kit (Bio Rad) according to manufacturer instructions. qRT-PCR was performed following established methods using actin as the housekeeping gene [4]. Western blotting was performed using a primary anti-gal antibody (1:10) and α-IgM secondary antibody (1:1000).

Data analysis: All data analysis are expressed as mean ±SEM unless otherwise stated. Statistical significance between the two experiments groups or respective controls were determined by students t-test. Comparative difference among multiple groups were determined via ANOVA with statically significant p value threshold, p<0.05 using Graphpad prism 6.05(Graph Pad Prism, la Jolla, USA). Transcriptional expression levels were determined using Bio-Rad software (Bio-Rad CFX manager); all expression values were considered statically significant if the P-Value was <0.05 when compared with control.

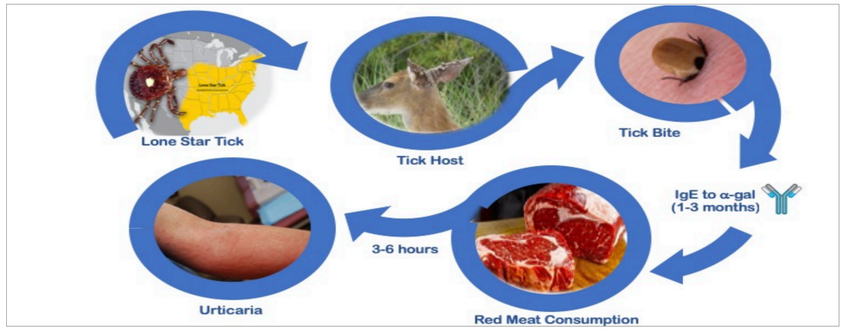

Figure 2. Schematic of α-Gal Syndrome process.

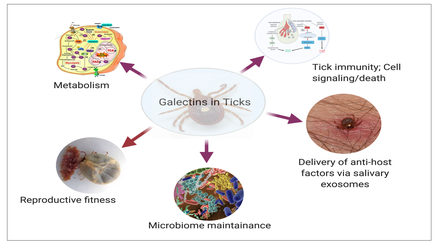

Figure 3. Schematic of proposed roles of Galectin in *Amblyomma americanum* physiology.

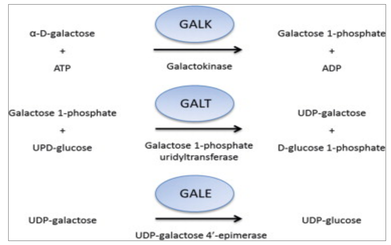

Figure 4. The primary enzymes involved in galactose metabolism pathway.

Results

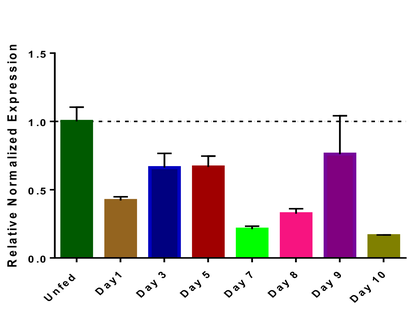

Figure 5. Temporal gene expression of Galectin in *Amblyomma americanum* salivary glands in unfed and fed stages. Gene expression was normalized with unfed ticks (dashed line), Actin as housekeeping gene.

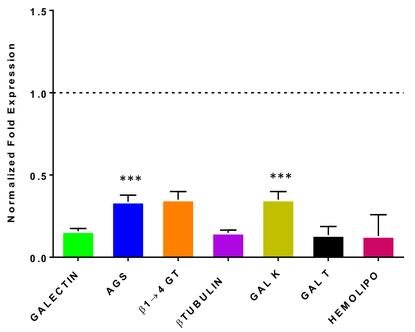

Figure 6. Transcriptional gene expression of 5-day Galectin KD in *Amblyomma americanum* salivary gland tissue. Transcriptional expression was normalized to GFP using Actin as the housekeeping gene.

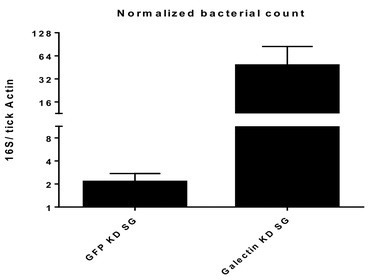

Figure 7. Total microbial load in Galectin KD and GFP control in 5-day *Amblyomma americanum* salivary glands.

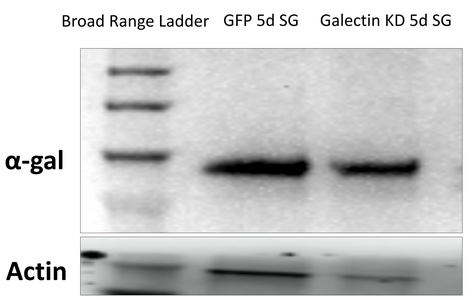

Figure 8. Identification of α-gal and actin in Galectin KD and GFP control in 5-day *Amblyomma americanum* salivary glands. Western blot using monoclonal actin antibody.

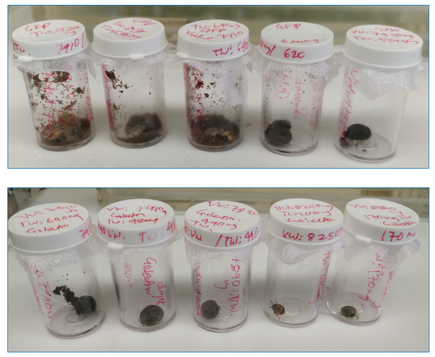

Figure 9. Oviposition of *A. americanum* ticks injected with dsGFP (irrelevant control; top) and dsGalectin (bottom).

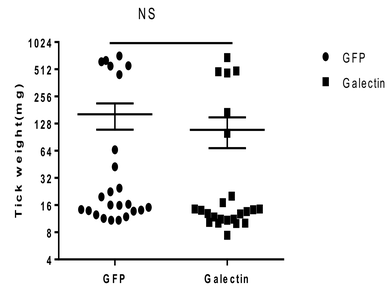

Figure 10. Mean weight of 5-10 day GFP (irrelevant control) and Galectin KD engorged tick. Mann-Whitney U test determined that there was not a statistically significant weight loss or gain in Galectin KD ticks.

Summary & Future Work

- No significant reduction of α-gal was observed in Galectin knocked down (KD) ticks compared to GFP ticks.
- Galectin KD ticks showed impaired oviposition compared to the GFP ticks suggesting its role in reproductive fitness.
- The temporal gene expression of Galectin downregulates during slow and feeding phases.
- Galectin KD significantly downregulated galactose metabolism associated genes namely α-galactosidase (AGS) and Galactosidase (GALK).
- Galectin KD ticks did not have a significant weight loss or gain after a bloodmeal compared to the GFP control ticks.
- The microbial load was significantly increased in Galectin knocked down tick salivary glands which suggests its role in microbiome homeostasis.

Future Work

- Basophil Activation Assay (BAT) to test its role in α-gal, production, presentation and basophil sensitization.
- N-Glycan analysis in Galectin KD ticks.
- Microbiome analysis of Galectin KD ticks.
- Testing the developmental anomalies associated with ovarian development in Galectin KD ticks via microscopy.

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Acknowledgements

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