Novel *Ixodes Scapularis* Gut Protein facilitates Tick Engorgement in mammalian hosts

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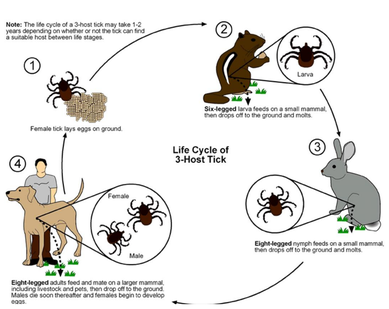
Background:

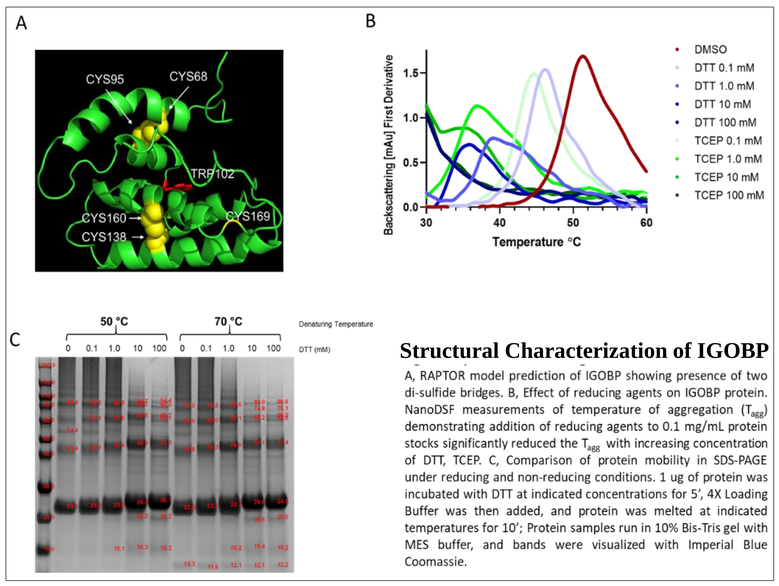
Ixodes scapularis ticks are obligate blood-feeders that target vertebrate hosts such as small rodents with humans being an accidental host. In the American north-east, *I. scapularis* (also known as blacklegged ticks) is the main carrier of *Borrelia burgdorferi*, the primary causative agent of Lyme disease.[1] There is currently no effective means of addressing tick infestation and disease spread *by I. scapularis*.[2] Tick gut and salivary proteins hold significance due to their involvement in tick feeding, tick development, host immune system regulation and transfer of tick-borne pathogens. [3-6]



A mass-spectrometric analysis of fed tick gut tissue from our laboratory identified ~5000 proteins, only 5% of which was unique to ticks. While gene silencing by RNAi of a few of the above selected genes did not have any observable effects in tick physiology, the absence of one particular gene impaired tick feeding efficiency and molting. This novel gene encodes a secretory protein with limited structural homology with other known odorant binding proteins. Named herein as Ixodes gut odorant binding protein (IGOBP). As a protein of unknown function and structure prevalent in the tick gut and saliva, IGOBP may be involved in one or more critical functions such as tick attachment to hosts, tick feeding, tick development, odorant sensing and pathogen transmission. Thus, understanding the role of IGOBP in tick biology will significantly aid in developing novel tick vaccines as well as drug targets to block host seeking, attachment and feeding as a preventive measure for tick infestations and pathogen transmission.

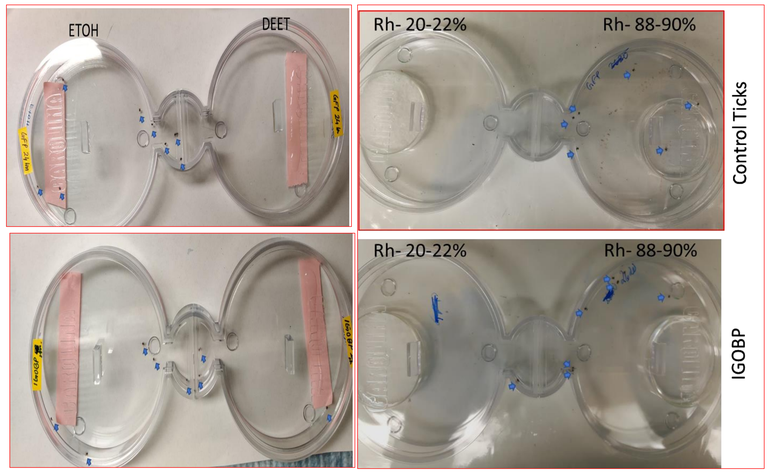
Results:

Our research focuses on understanding the structural and functional characterizations of unknown tick protein IGOBP. IGOBP is structurally similar with insect Odorant Binding Protein (OBP) and the protein sequence is conserved among other tick species.



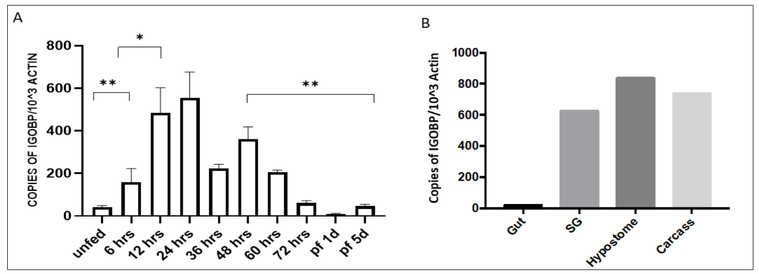
Structural Characterization of IGOBP

A, RAPTOR model prediction of IGOBP showing presence of two di-sulfide bridges. B, Effect of reducing agents on IGOBP protein. NanoDSF measurements of temperature of aggregation (T_{agg}) demonstrating addition of reducing agents to 0.1 mg/mL protein stocks significantly reduced the T_{agg} with increasing concentration of DTT, TCEP. C, Comparison of protein mobility in SDS-PAGE under reducing and non-reducing conditions. 1 ug of protein was incubated with DTT at indicated concentrations for 5', 4X Loading Buffer was then added, and protein was melted at indicated temperatures for 10'; Protein samples run in 10% Bis-Tris gel with MES buffer, and bands were visualized with Imperial Blue Coomassie.



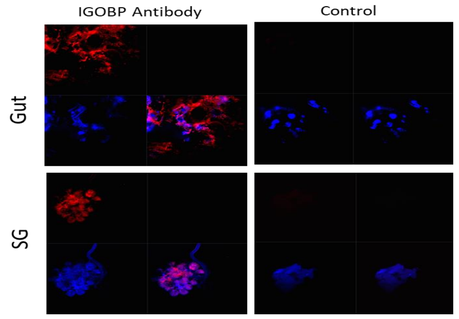
IGOBP is not responsible for humidity and repellent (DEET) sensing.

To test the attraction of ticks to water, we performed choice chamber experiments. One side of choice chamber was prepared with dH2O and other side was with $CaCl_2$ (desiccant). Before adding the ticks, choice chambers were allowed to equilibrate in the room temperature at 25°C and humidity (Rh) was measured after two hours. Ticks were microinjected with IGOBP or, GFP dsRNA (12 ticks/ group) and each group of ticks were placed in the middle of a choice chamber separately. Using similar setup we tested ticks' response to DEET in presence and absence of IGOBP. Ticks from both groups moved to humid chambers and were repelled from DEET (indicated by arrows).



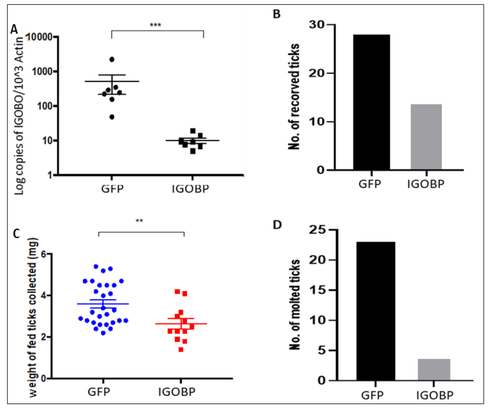
Temporal and Spatial Expression of IGOBP

A, Ticks were collected at different time points after tick placement on mice and relative expression of IGOBP was measured in individual ticks. IGOBP expression was highest at 6-12 hours of feeding. B, IGOBP expression was measured in different tick organs. Salivary glands, Hypostomes and carcasses express IGOBP, while guts has very low expression



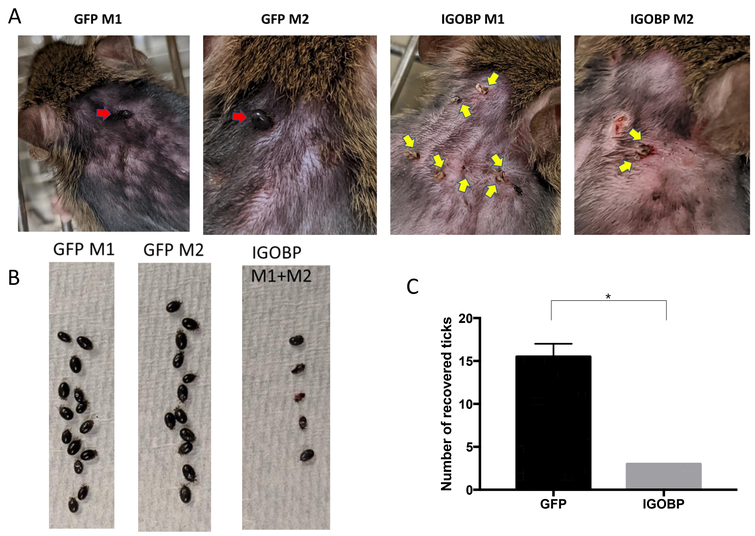
Localization of IGOBP by confocal Microscopy

Confocal immunofluorescence staining of tick tissue sections (gut and salivary glands) was performed using IGOBP antibodies (labeled *red*). The nuclei in tick tissues were labeled with DAPI (*blue*)



Silencing of IGOBP impairs tick feeding

A, Knockdown of IGOBP transcripts induced by RNA interference. B, IGOBP knockdown impairs tick feeding as reduced number of ticks were recovered from IGOBP knockdown group. C, Weights of recovered ticks were measured, which is significantly lower in IGOBP group compare to control group. D, Molting efficiency was reduced in IGOBP group.



IGOBP is not responsible for tick attachment but has role in feeding process.

Ticks are feeding in control group (GFP) at day 4 (A) or were recovered as shown in Figure B. Ticks from IGOBP group were not feeding well and appears dead and only few ticks were recovered.

Discussion:

We expect to understand the molecular mechanism underlying IGOBP silencing mediated phenotypic effects of ticks from our studies. Furthermore, presence of IGOBP in saliva indicates possible interaction with host immune molecules. Therefore, elucidating the molecular mechanism of IGOBP will improve our understanding of the biology at the tick-host interface. Lastly, identification of IGOBP interacting partners will be essential in determining the cellular pathways involved in tick feeding process and this might lead to identification of several antigens for vaccine development.

Acknowledgments:

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References:

Trends Parasitol **34**, 295–309 (2018) [1] ; *Expert Review of Vaccines* **14**, 1367–1376 (2015)[2]; *Parasit Vectors* **8**, (2015) [3]; *Trends in Parasitology* **29**, 276–285 (2013) [4]; *Front Cell Infect Microbiol* **4**, 116 (2014) [5]; *Front Cell Infect Microbiol* **3**, (2013) [6]