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A transwell assay method to evaluate *Borrelia burgdorferi* sensu stricto migratory chemoattraction toward tick saliva proteins

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Abstract

We developed a transwell assay to quantify migration of the Lyme disease agent, *Borrelia burgdorferi* sensu stricto (s.s.), toward *Ixodes scapularis* salivary gland proteins. The assay was designed to assess *B. burgdorferi* s.s. migration upward against gravity through a transwell polycarbonate membrane overlaid with 6% gelatin. Borreliae that channeled into the upper transwell chamber in response to test proteins were enumerated by flow cytometry. The transwell assay measured chemoattractant activity for *B. burgdorferi* s.s. from salivary gland extract (SGE) harvested from nymphal ticks during bloodmeal engorgement on mice 42 h post-attachment and saliva collected from adult ticks. Additionally, SGE protein fractions separated by size exclusion chromatography demonstrated various levels of chemoattractant activity in the transwell assay. Sialostatin L, and Salp-like proteins 9 and 11 were identified by mass spectrometry in SGE fractions that exhibited elevated activity. Recombinant forms of these proteins were tested in the transwell assay and showed positive chemoattractant properties compared to controls and another tick protein, S15A. These results were reproducible providing evidence that the transwell assay is a useful method for continuing investigations to find tick saliva components instrumental in driving *B. burgdorferi* s.s. chemotaxis.

Keywords

Borrelia burgdorferi; Lyme disease; Transwell; Tick saliva; Chemoattractant

1. Introduction

Human infection by the tick-borne spirochete, *Borrelia burgdorferi* sensu lato (s.l.), results in Lyme borreliosis, a disease that often initially presents with a skin lesion termed erythema migrans accompanied by viral-like symptoms e.g. malaise, fatigue, headache, arthralgias, myalgias, fever and regional lymphadenopathy (Steere et al., 2016; Wormser, 2006; Wormser et al., 2006). The infection can progress to more severe manifestations (arthritis, carditis, neurologic) if not accurately diagnosed and treated properly with antibiotics (Steere et al., 2016; Wormser, 2006).

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B. burgdorferi s.l. exists in an enzootic life cycle of obligate symbiosis consisting of reservoir hosts, primarily rodents and birds, and *Ixodes* spp. ticks that serve as vectors to transmit the pathogen to other hosts including humans. In vertebrate hosts, *B. burgdorferi* s.l. disseminates to tissues and organs but does not produce sustained, high density spirochetemia thereby rendering culture isolation of the organism difficult. *B. burgdorferi* s.l. is highly motile by virtue of a structure of bundled periplasmic flagella and chemotaxis proteins that are recognized as virulence factors essential for infection (Li et al., 2002; Moon et al., 2016; Motaleb et al., 2015, 2011; Sultan et al., 2013). Motility and chemotaxis are important factors enabling *B. burgdorferi* s.l. to i) move from tick midgut to salivary glands in preparation for deposition into the host, ii) disseminate within the host to distal organs and tissues, and iii) migrate from within the host to a newly feeding tick.

The processes by which borreliae navigate from disseminated tissues to the tick feeding site via the bloodstream, thereby continuing the enzootic cycle, are unknown. Assays that observe motility to chemotactic signals include swimming plate, computer assisted cell motion tracking, capillary tube, and U-tube (Bakker et al., 2007; Motaleb et al., 2007; Shi et al., 1998; Shih et al., 2002; Zhang and Li, 2018). These studies found various substances, e.g. N-acetyl glucosamine, glucosamine, chitobiose, proteins in rabbit serum, tick salivary gland extract (SGE), and saliva possess attractant properties for *B. burgdorferi*. However, few specific individual or combined proteins from saliva have been identified. Identifying chemical attractants secreted by ticks that facilitate *B. burgdorferi* migration will further our understanding how the organism traverses and survives cycling between hosts and tick vectors and could be a basis for novel diagnostic assays for active infection. We describe an in vitro transwell assay to measure borrelial migration to chemical substances as a method to identify putative chemoattractants derived from tick saliva.

2. Materials and methods

2.1. Strain

B. burgdorferi strain B31-A3 (Elias et al., 2002) was propagated to mid-logarithmic stage (1×10^6 cells/ml) in BSK-II media at 34 °C in capped tubes providing microaerophilic conditions. A full complement of plasmids (except for cp9, which is missing in the B31-A3 strain) was demonstrated based on a multiplex PCR (Bunikis et al., 2011).

2.2. Transwell assay

HTS Transwell®–96 Well Permeable Supports plate (Corning, Corning, NY) with an integral tray insert containing a 0.3 μm pore size polycarbonate membrane in each well were overlaid (50 μl/well) with 6% porcine gelatin (Sigma Aldrich, Billerica, MA) heated to 50 °C. The plate was placed at 4 °C for 5 min to let the gelatin solidify before introducing other liquids. *B. burgdorferi* were spun, washed in 1X phosphate buffered saline (PBS), resuspended in PBS or Hanks Balanced Salt solution (HBSS) and 2×10^5 in 200 μl was added to the bottom of each control or sample well. Transwell inserts were positioned into the lower compartment of the well in contact with the PBS cell suspension. Test protein or chemical (100 μl) was added to the upper compartment of the transwell insert. All test recombinant proteins were added at equivalent concentrations (450 μg/ml)

in 1X PBS. Saliva, SGE, and rabbit serum (PelFreeze, Rogers, AR) were tested undiluted. Transwell plates were sealed and incubated at 34 ° C in 5% CO_2 for 18–20 h. The transwell experimental setup is shown (Fig. 1A).

2.3. Sample preparation for flow cytometry

Following the incubation period, sample from the upper transwell chamber (100 μl) was carefully removed to avoid contact with the gelatin layer on the transwell membrane. This procedure ensured that only spirochetes that migrated through the membrane/gelatin layer would be counted. Care was taken to ensure the pipet tip did not press against the gelatin/membrane surface as damage could occur resulting in contamination of liquid from the lower compartment. Samples were placed in microfuge tubes containing 0.5 μl of SYTO61 Red Fluorescent Nucleic Acid Stain (Invitrogen, Carlsbad, CA) and mixed gently for 10 min at room temperature in a light-blocking container. Tubes were centrifuged (16,000 × g, 2 min), the supernatant removed and the pellet resuspended in PBS plus 2 mM EDTA (100 μl) with addition of 1 mM paraformaldehyde followed by gentle mixing for 10 min at room temperature in a light-blocking container. Samples were centrifuged (16,000 × g, 2 min), supernatant removed, and pellets resuspended in 1X PBS plus 2 mM EDTA (100 μl). Samples were placed in 96 well flat bottom microtiter plate for flow cytometry with plate covered to avoid light bleaching of samples.

2.4. Flow cytometry

Samples were analyzed by flow cytometry with BD Accuri™ C6 Plus Benchtop Flow cytometer. Samples were run at a volume rate of 40–75 μl and gated to remove debris. Flow data was analyzed via the BD Accuri™ C6 Plus software package and graphed with GraphPad Prism Version 8.

2.5. Mouse and rabbit tick feeding

Animal experiments were approved and conducted in accordance with guidelines and regulations as established by the Division of Vector-Borne Diseases Institutional Animal Care and Use Committee (IACUC).

CD-1 mice (Charles River, Worcester, MA) were needle inoculated subcutaneously with 100 μl of log-phase *B. burgdorferi* B31-A3 (1×10^5 cells/ml) in BSK-II media. Two weeks post-inoculation, ear biopsies were collected and cultured to determine infection status of mice. Three weeks post-inoculation, approximately 100 larval ticks or 50 nymphal ticks were placed on individual *B. burgdorferi*-infected mice. A total of 7 mice were utilized for each larval or nymphal feeding in two separate replicates. At 6-hour intervals, 2–3 nymphs or 8–10 larvae from each mouse were removed and placed into 70% ethanol at 4 °C prior to genomic DNA isolation.

Female New Zealand White rabbits were tranquilized with acepro-mazine delivered intramuscularly or subcutaneously using a 26-gage needle at a dose of 0.75–10 mg/kg (typically 0.75–1.0 mg/kg range) and fitted with a plastic e-collar to prevent direct oro-anal coprophagy. One-inch wide cotton athletic tape was applied around the base of each ear. 20–50 adult uninfected *Ixodes scapularis* adult ticks were placed into a cloth ear bag affixed

to the tape at the base of the ear. A second piece of tape was placed around the base of the ear bag to hold it in place. The feeding period of adult ticks lasts approximately 7 days (range 4–10). Replete ticks were collected for saliva collection.

2.6. Tick salivary glands dissection and saliva collection

Nymphal ticks ($n = 20$) were placed on eight individual CD-1 mice (Charles River, Worcester, MA) and allowed to feed for 42 h. Ticks were removed with fine forceps and placed in microcentrifuge tubes on ice. For salivary gland dissection, individual ticks were placed in a drop of phosphate buffered saline (PBS) on a silane coated slide (Scientific Device Laboratory, Des Plaines, IL), and a scalpel was used to cut the basis capitulum and the contents of the tick were pushed out from the bottom through the cut using the side of fine tip forceps. The salivary glands were sorted from the midgut, washed in a clean drop of PBS, and placed into PBS containing protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN) on ice. The salivary glands in PBS + protease inhibitors were sonicated at a 50% output for a total of 1 min to separate the cells and generate the SGE. Material from 115 salivary gland pairs (230 total glands) were utilized for fractionation. Collection of *I. scapularis* saliva was performed as described previously (Patton et al., 2012) and stored at −80 °C.

2.7. DNA extraction and qPCR

B. burgdorferi quantification by qPCR from the collected larvae and nymphs was performed as previously described (Patton et al., 2011) with the following modifications: genomic DNA was prepared from pooled ticks by time point per mouse and homogenizing in lysis buffer (Qiagen, Germantown, MD) by bead beating in a Biospec Mini (Biospec, Bartlesville, OK), subsequently processed with DNeasy Blood and Tissue Kit (Qiagen), and eluted in 50 µl of elution buffer (10 mM Tris pH 8.5). For qPCR, 1 µl of genomic DNA was utilized. The *B. burgdorferi flaB* Taqman primers have been previously described (Gilmore et al., 2007). A parallel reaction was performed to amplify a portion of the tick actin gene using 1 µM 5′ *IscapactinSEN* primer (5′-CGTGAAATTGTCCGTGACATC-3′), 1 µM 3′ *IscapactinASN* primer (5′-TCTCGTTGCCGATTGTGATG-3′), 0.15 µM tick actin probe (5′FAM-CTACGTCGCCCTGGACTTCGAGC-3′BHQ), and 2X TaqMan® Universal PCR Master Mix (Applied Biosystems, Grand Island, NY) in a total volume of 20 µl. Amplification conditions were 1 cycle at 95 °C for 10 min and 40 cycles of 95 °C for 30 s and 60 °C for 1 min, with data collection after each cycle. Amplification of each DNA sample was performed in triplicate. To calculate copies of tick actin, a standard curve was generated by amplifying the portion of the tick actin gene which had been cloned into pCR2.1 with the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA).

2.8. Fractionation of salivary gland extract

Tick salivary gland extract was filtered using 0.22 µm Ultrafree MC GV spin column (EMD Millipore), followed by a wash with 100 µl 30% HPLC grade acetonitrile. The filtered solution and the 30% acetonitrile wash were combined and fractionated by FPLC using AKTA Purifier (GE Healthcare, Pittsburgh, PA), size exclusion column Superdex 200 10/300 GL (GE Healthcare, USA), buffer of 10 mM KH_2PO_4, 150 mM NaCl, pH 7, 0.5 ml loop, flow rate of 0.5 ml/min at 25 °C and 0.5 ml fractions collected.

2.9. Recombinant proteins

Ribosomal S15A, Sialostatin L, Salp 9- and 11-like genes were amplified by PCR using primers listed in Table 1 from *I. scapularis* genomic DNA. Amplicons were cloned into pETite C–His-vector (Lucigen, Middleton, WI) and transformed into *E. coli* BL21 (DE3) according to the manufacturer's instructions. Recombinant proteins were expressed and purified using a QiaExpress Ni-NTA FastStart kit (Qiagen, Valencia, CA) as described previously (Brandt et al., 2019)

3. Results

3.1. Transwell assay

The assay was designed for borrelial migration to proceed upward from the bottom well to the upper transwell insert cup containing the test reagent (Fig. 1A). We initially found that borrelial movement through the transwell membrane proceeded within short timeframes that did not enable accurate counting by darkfield microscopy. We added a layer of 6% gelatin on the membrane for borreliae to gradually pass thereby allowing counting to be performed hours instead of minutes later which ensured a more precise enumeration.

Enumeration of live borreliae was initially performed by microscopy but proved onerous when counting multiple replicate wells of timepoints in real time. We turned to flow cytometry of fixed cells which had advantages over microscopy: i) borreliae were paraformaldehyde-fixed and stained enabling counts to be performed for multiple timepoints in a single run; and ii) quantification could be performed more rapidly by software. Enumeration of borreliae between the two methods was compared and found to be equivalent (Fig. 1B). The R^2=0.95 indicates a good linear trend with a low proportion of variance. The small discrepancy between counts between microscopy and flow cytometry may be due to sample loss while staining, washing, and fixing the cells during flow cytometry sample prep. Pellets prepared for flow cytometry were small and flaky, therefore, to ensure minimal sample loss, gentle removal of supernatant after each step was critical.

We found that BSK-II culture media was the best positive control for borrelial attraction (Fig. 2). Other studies have used BSK-II, N-acetyl glucosamine, chitobiose, rabbit serum, and glucosamine, as positive controls when assaying borrelial motility (Bakker et al., 2007; Motaleb et al., 2007, 2005; Shi et al., 1998). N-acetyl glucosamine and rabbit serum are components of BSK media, and we found that their chemoattractant ability was diminished compared to BSK, but N-acetyl glucosamine activity was significantly higher than controls (Fig. 2). Therefore, in our assays, *B. burgdorferi* were suspended in PBS in bottom wells and BSK was used in upper chambers as a positive control. We observed that *B. burgdorferi* were stable, viable, and motile in PBS for 24 h, therefore we performed the migration assays with both *B. burgdorferi* and the test proteins suspended in PBS.

3.2. Evaluation of I. scapularis fractionated salivary gland extract and saliva by transwell assay

The small size of nymphs and larvae, together with low yield, logistically precluded our collection of saliva. Therefore, intact salivary glands which provided ample test material,

were dissected for whole extract preparation that included saliva. Salivary glands were harvested from nymphs after attachment for 42 h on mice. This timepoint was chosen to coincide with the stage at which borrelial acquisition began to increase with the premise that putative chemoattractants would be prominent in saliva. Borrelial acquisition was determined by feeding uninfected larvae and nymphs on *B. burgdorferi*-infected mice and calculating the spirochete burden by PCR in ticks removed every 6 h during the feed (Fig. 3A). Primary acquisition of *B. burgdorferi* was observed approximately 42–48 h post-attachment with the highest densities measured from 54 to 66 h post-attachment for both tick stages (Fig. 3B). The physical limitation of dissecting larvae for salivary glands was a factor, therefore we confirmed that both stages demonstrated similar time course acquisition of borreliae and collected salivary glands from nymphs. Saliva was collected from replete adults 5–6 days post-attachment on rabbits.

Unfractionated SGE and saliva were tested for *B. burgdorferi* chemoattractant activity in the transwell assay. Both SGE and saliva demonstrated statistically significant positive borrelial migratory activity compared to the PBS control, with saliva producing levels close to that of the BSK positive control and significantly better than SGE (Fig. 4).

SGE proteins were subjected to size exclusion chromatography and collected in multiple fractions, each tested for borrelial migration activity by the transwell assay. Positive and negative chemoattractant activity were demonstrated by the SGE fractions when compared to the PBS control (Fig. 5). Each fraction contained limited volume, therefore could only be tested once in the transwell assay.

Mass spectrometry analysis from designated SGE fractions 17, 26, and 38 that demonstrated chemoattractant activity allowed us to select individual proteins for preliminary testing in the transwell assay. From fraction 17, we chose Sialostatin L, and Salp-like proteins 9 and 11 for recombinant protein expression and observed chemoattractant activity with these proteins using the transwell assay. The *I. scapularis* protein S15A, a ribosomal binding protein, was negative (Fig. 6).

We performed the transwell borrelial migration assay in a dose response experiment with a dilution series of each recombinant Sialostatin L, Salp-like 9, and Salp-like 11 proteins. The results revealed a decline in borrelial migration with a concomitant decrease in the protein concentration for all three proteins (Fig. 7). These results represented additional confirmation for the consistency of the transwell assay and proof of principle to measure borrelial migration to chemotactic test proteins.

4. Discussion

Development of the transwell method to measure borrelial migration to tick saliva components is an important step to understand the mechanism driving tick acquisition of *B. burgdorferi* s.l. from the infected host. The acquisition of *B. burgdorferi* s.l. by ticks feeding on infected individuals is the principle behind xenodiagnosis, a sensitive approach to determine the infection status of a host suspected of harboring an organism. Xenodiagnosis has been used as a method to detect *B burgdorferi* in animals (Bockenstedt et al., 2002;

Donahue et al., 1987; Embers et al., 2012; Hodzic et al., 2008) and has been modeled as a potential tool to determine latent or active *B. burgdorferi* infections in humans (Marques et al., 2014). The mechanisms responsible for signaling borreliae from disseminated tissues to an attached tick are largely unknown although undoubtedly involves the chemotactic and motility machinery of the spirochete responding to substances from tick saliva (Motaleb et al., 2015, 2011; Sultan et al., 2013; Sze et al., 2012).

Transwells are commonly used in biomedical research to study cell migration, invasion, and motility (Justus et al., 2014). Previous studies have shown *B. burgdorferi* migratory responses to tick SGE and saliva, however little is known regarding chemoattractant activity for specific individual or combinations of saliva proteins (Shih et al., 2002). Currently, one *I. scapularis* protein, Salp 12, has been described as a chemoattractant (Murfin et al., 2019). A study using two-photon intravital microscopy indicated the process of borrelial acquisition by the tick from an infected mammal does not appear to be a passive mechanism whereby the vasculature surrounding the tick bite site harbors the spirochete (Bockenstedt et al., 2014).

When designing the transwell assay, resuspending *B. burgdorferi* in BSK would have conceivably resulted in diminished migration to a test protein since nutrients and the environment preferred by the spirochetes were available. Previous investigations suspended *B. burgdorferi* in “motility medium” which was an osmotic buffer like PBS or HBSS (Shih et al., 2002; Zhang and Li, 2018). Bakker et al., described a flow cytometry method to enumerate borreliae and we modified and incorporated that methodology in our assay as opposed to direct counting by microscopy which we also found cumbersome (Bakker et al., 2007).

We considered timing for production of potential chemoattractant proteins when collecting salivary glands from feeding nymphs. We postulated that optimal production of attractant proteins might be highest during the beginning of borrelial acquisition from the infected host. Previous work determined the time between larval tick attachment and acquisition being as short as 8 h (Piesman, 1991). A separate study also demonstrated 8 h as the earliest time point of acquisition with 48 h being the time point of highest acquisition efficiency (Shih et al., 1995). Although components of larval salivary gland extracts would have been of interest, it was unworkable to dissect salivary glands from this tick stage. We determined the acquisition time courses for both larvae and nymphs were similar and opted to harvest nymphal salivary glands at 42 h post-attachment.

We note that time course considerations for potential salivary chemoattractant proteins being secreted and delivered during tick feeding may or may not be relevant. Production of saliva components may be constitutive with the likelihood that ticks do not sense whether a host is infected or uninfected and it would not be in a tick’s survival interest to attract borrelia into their bloodmeal. Conversely, recent studies have described differential gene expression and protein production in saliva as a function of time post-attachment, the tick’s infection status, and between adults and nymphs (Kim et al., 2021; Ribeiro et al., 2006). The array of proteins and peptides produced and presented in *I. scapularis* saliva has been documented

and will contribute to future work to decipher putative chemoattractant functions (Kim et al., 2021; McNally et al., 2012; Ribeiro et al., 2006; Valenzuela et al., 2002).

We observed borrelial migration when using both SGE and saliva as test agents. We separated our sample of SGE proteins into size fractions as another test for our transwell assay. The fractionated proteins provided material for only one test run and was meant as a preliminary trial to assess the efficacy of the transwell assay. Accordingly, our results showed positive attractant activity for recombinant proteins Sialostatin L, a salivary cystatin, that displays anti-inflammatory and immunosuppressive activity (Kotsyfakis et al., 2006), and the functionally undefined Salp-like proteins 9 and 11. PBS or HBSS alone were used as negative controls, therefore negative control proteins were necessary to validate the assay. The lack of chemotactic activity observed by multiple SGE fractions and the recombinant *I. scapularis* protein S15A served the purpose. Recombinant proteins were generated in *E. coli*, but future considerations should be given to produce candidate proteins in eukaryotic expression systems to assess potential augmented functional properties from post-translational modifications.

Additional verification for the transwell assay was demonstrated by the dose response experiment for the recombinant proteins. The concentration of individual proteins in tick saliva is unknown but likely lower than that reflected in our in vitro experiments with recombinant proteins and is a limitation to be addressed. Experiments addressing identification of saliva proteins with chemoattractant activity, in vivo and in vitro protein concentration correlations, and validation by replicate trials for statistical significance is the focus of our continuing work.

In conclusion, we have developed a method to assay borrelial migration to identify and characterize chemoattractants in tick saliva. Identification of chemoattractant saliva proteins may have utility in development of xenodiagnostic tests and anti-tick vaccines. The transwell assay will allow for ongoing work to address questions relating to i) chemotactic properties between cultured and host-adapted spirochetes, ii) identification of *B. burgdorferi* s.l. receptors to chemoattractants, and iii) comparisons between the transwell assay and other methods for measuring borrelial migration.

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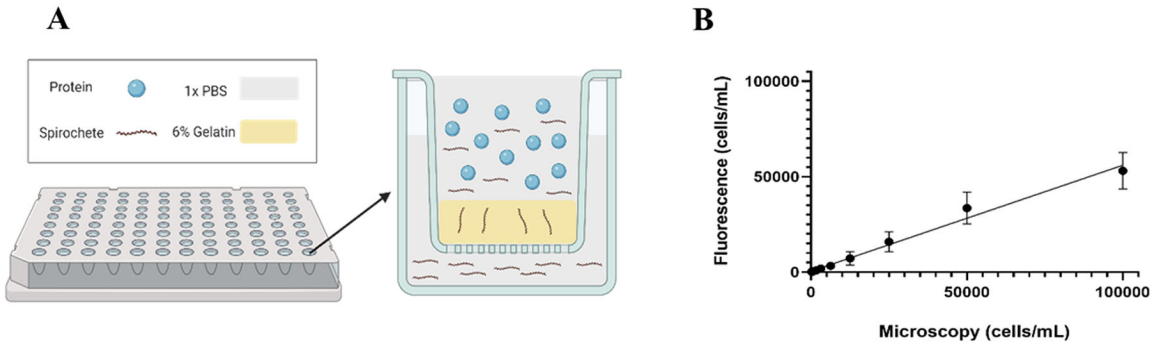

Fig. 1.

Transwell assay method. (A) Illustration of a 96- well transwell plate with each well consisting of the lower chamber containing a PBS suspension of *B. burgdorferi* and the upper transwell insert chamber with a polycarbonate membrane overlaid with 6% gelatin containing the test attractant placed in contact with the lower chamber cell suspension. (B) Cross comparison between cells counted by microscopy (x-axis) and cells counted by flow cytometry via SYTO61 nucleic acid stain (y-axis). R-value of 0.95 and a slope of $Y = 0.555X + 490.8$.

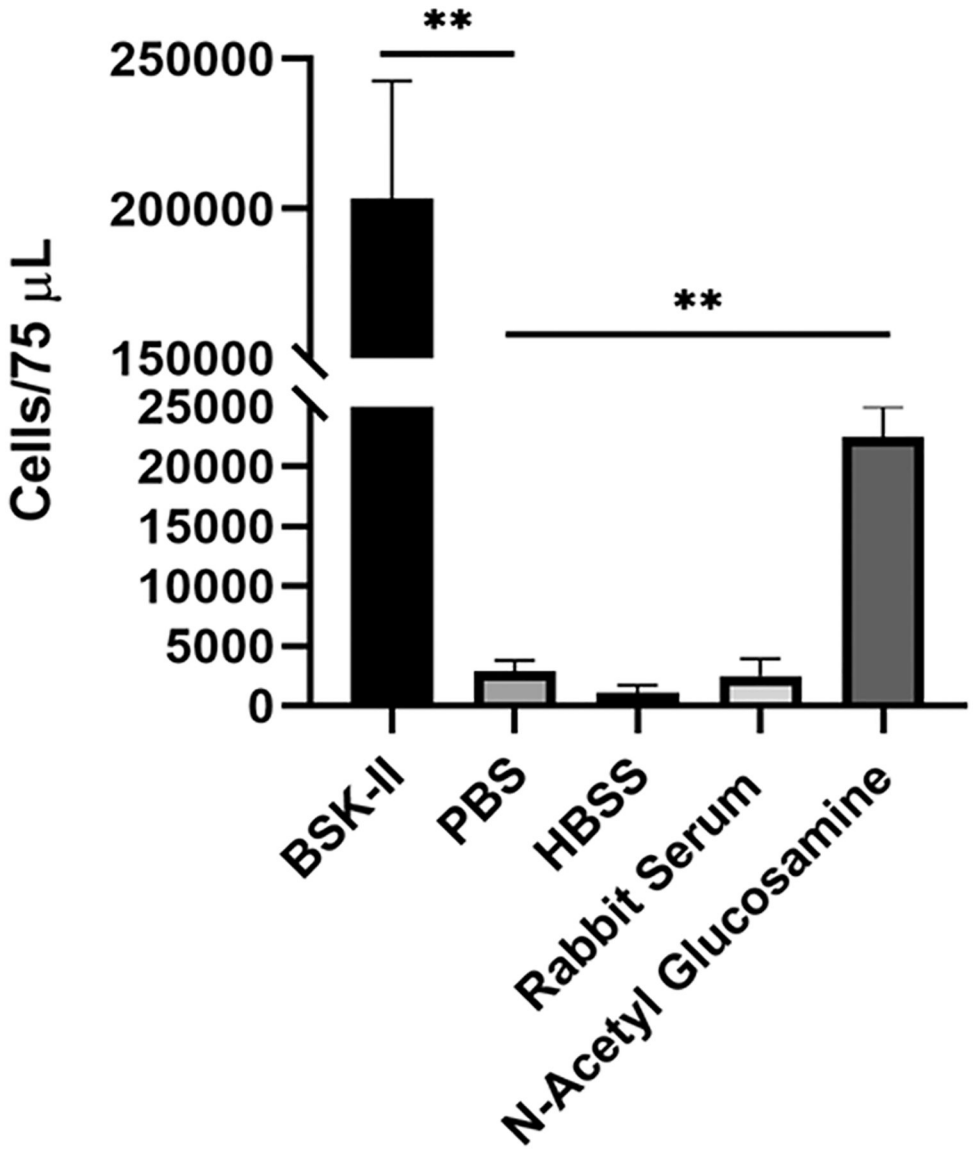

Fig. 2.

Comparison of known chemoattractants and controls to evaluate consistency of the transwell assay method. *B. burgdorferi* were enumerated by flow cytometry (total cells in 75 µl upper chamber sample). Statistical comparisons were made to PBS negative control. Unpaired *t*-test compared: BSK-II vs. PBS (Welch's corrected, $t = 1.627$, $p < 0.001$), N-acetyl glucosamine (Welch's corrected, $t = 7.382$, $p < 0.0092$). Other comparisons were not significant. Error bars represent standard error of means (SEM). Biological and technical replicates were performed in triplicate.

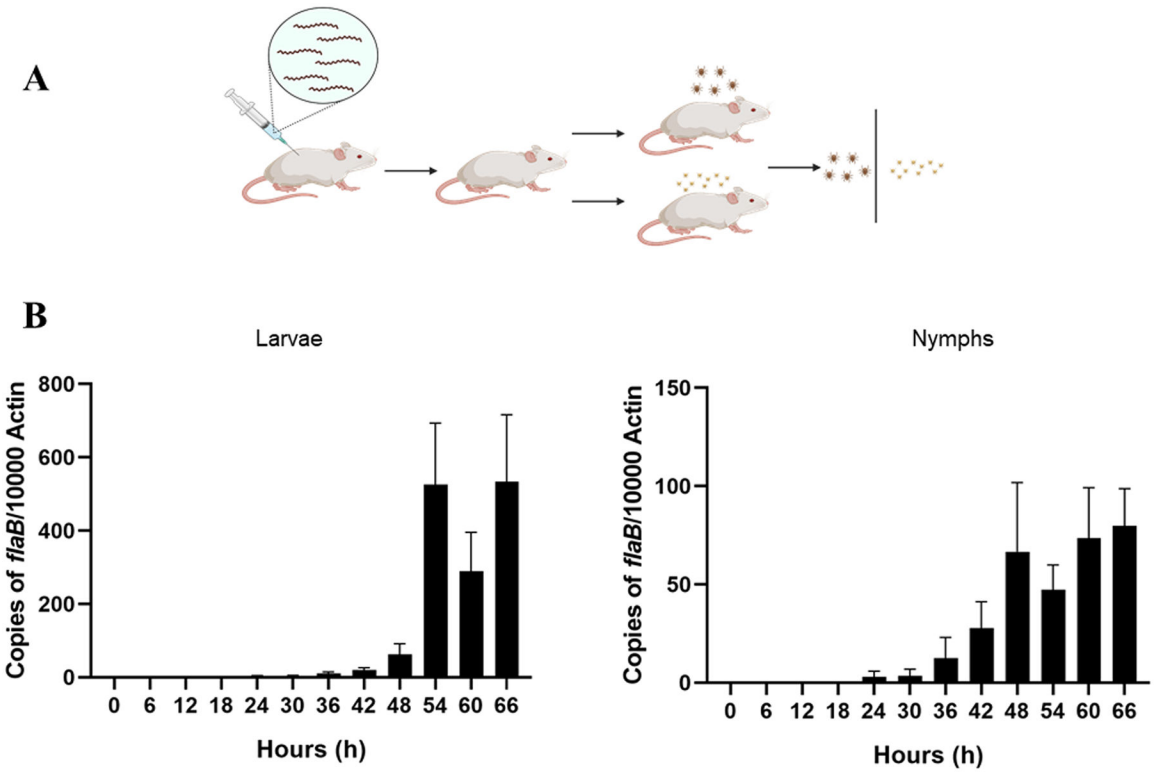

Fig. 3.

Comparison of larval and nymphal acquisition of *B. burgdorferi*. (A) Experimental setup whereby mice were infected with *B. burgdorferi* by needle injection and larvae and nymphs allowed to feed. Tick cohorts were collected every 6 hrs post-attachment and spirochete burden was assessed by qPCR. (B) qPCR of *B. burgdorferi* per time post-attachment as measured by copies of *flaB* over 10,000 copies tick actin. Left panel = larvae; right panel =nymphs. Error bars represent standard error of means (SEM).

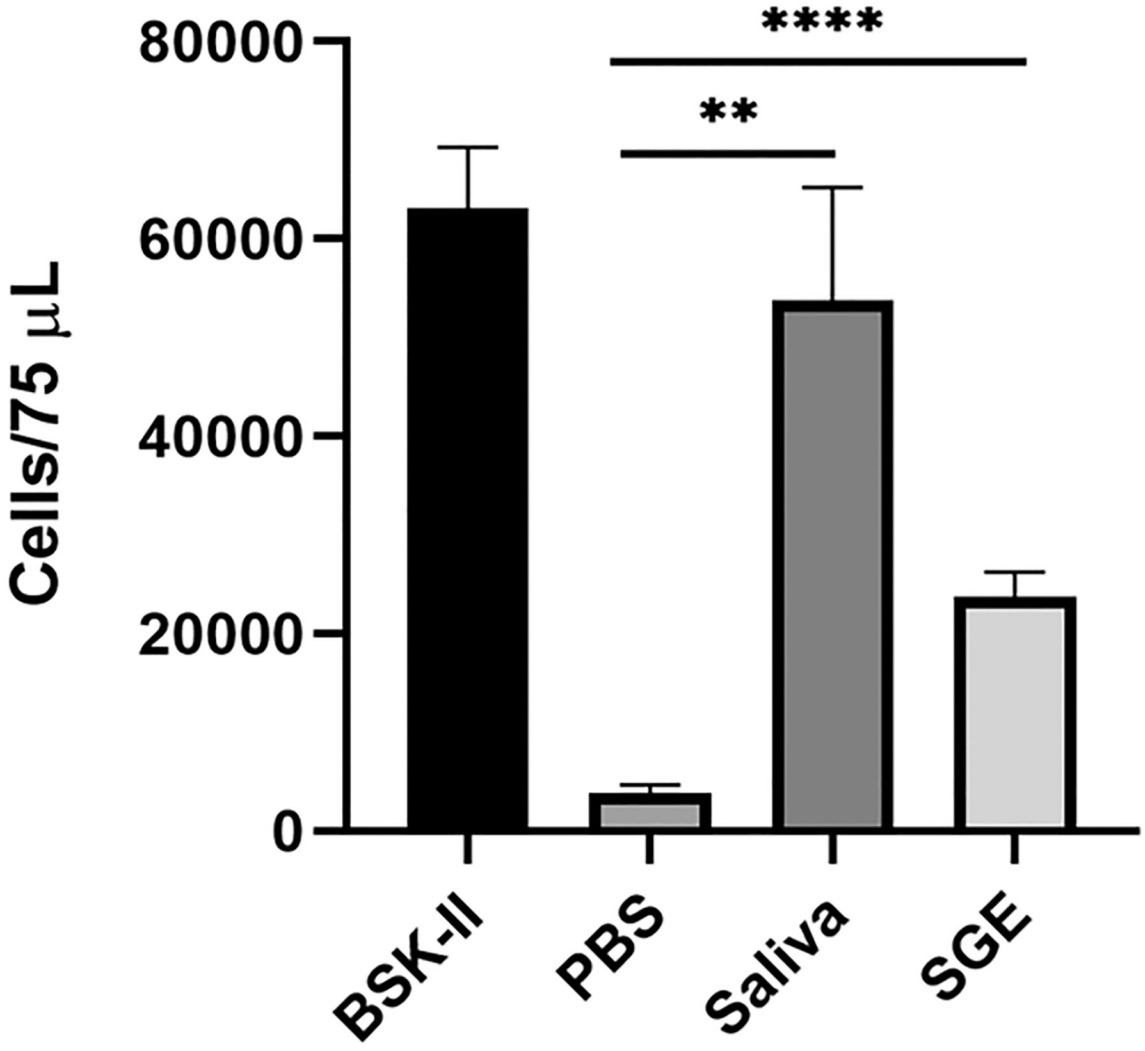

Fig. 4.

Analysis of chemoattractive properties of *I. scapularis* saliva and SGE by the transwell assay method. Cells were enumerated by flow cytometry. All statistical comparisons were made against the negative control, PBS. Error bars represent standard error of means (SEM). Unpaired *t*-test compared: PBS vs. saliva (Welch's corrected, $t = 4.345$, $p < 0.0024$) and PBS vs. SGE (Welch's corrected, $t = 7.665$, $p < 0.0001$). Biological and technical replicates were performed in triplicate.

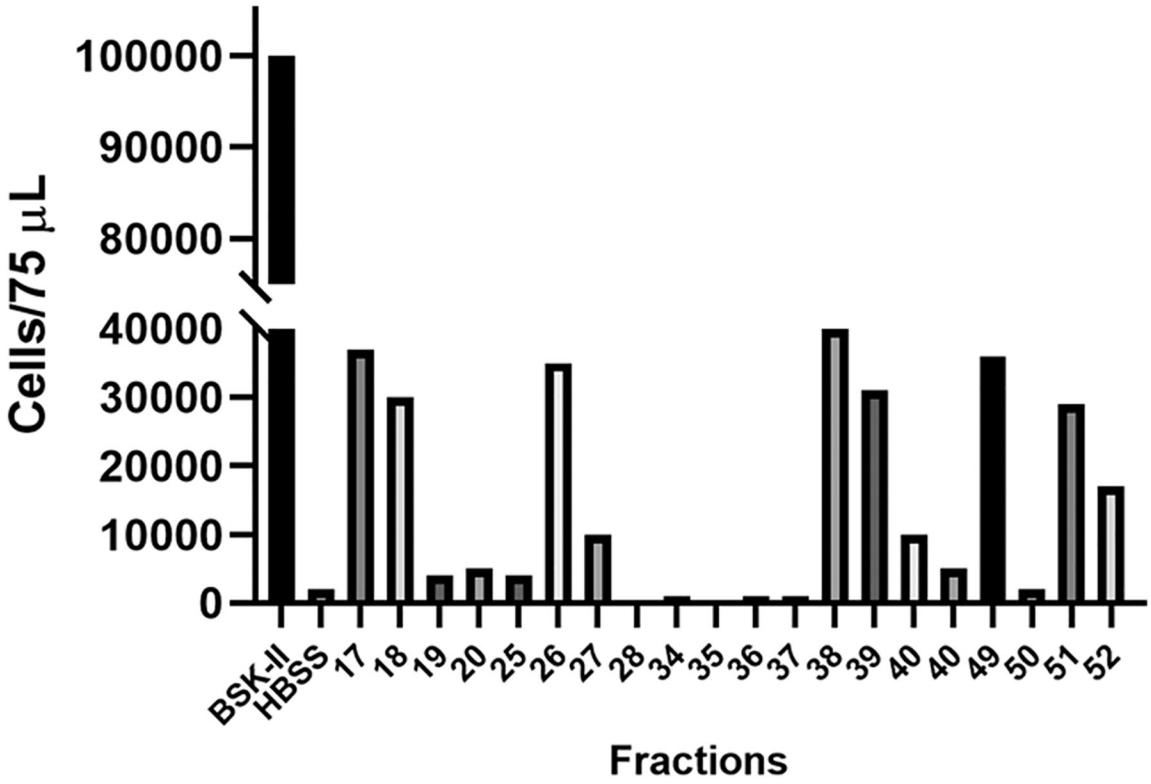

Fig. 5.

Transwell assay analysis of salivary gland extract fractions. Whole SGE was fractionated into 52 × 0.5 ml samples; fractions 17–52 were analyzed by the transwell assay. Cells were counted by flow cytometry. BSK-II was used as the positive control and HBSS was used as the negative control to compare active fractions.

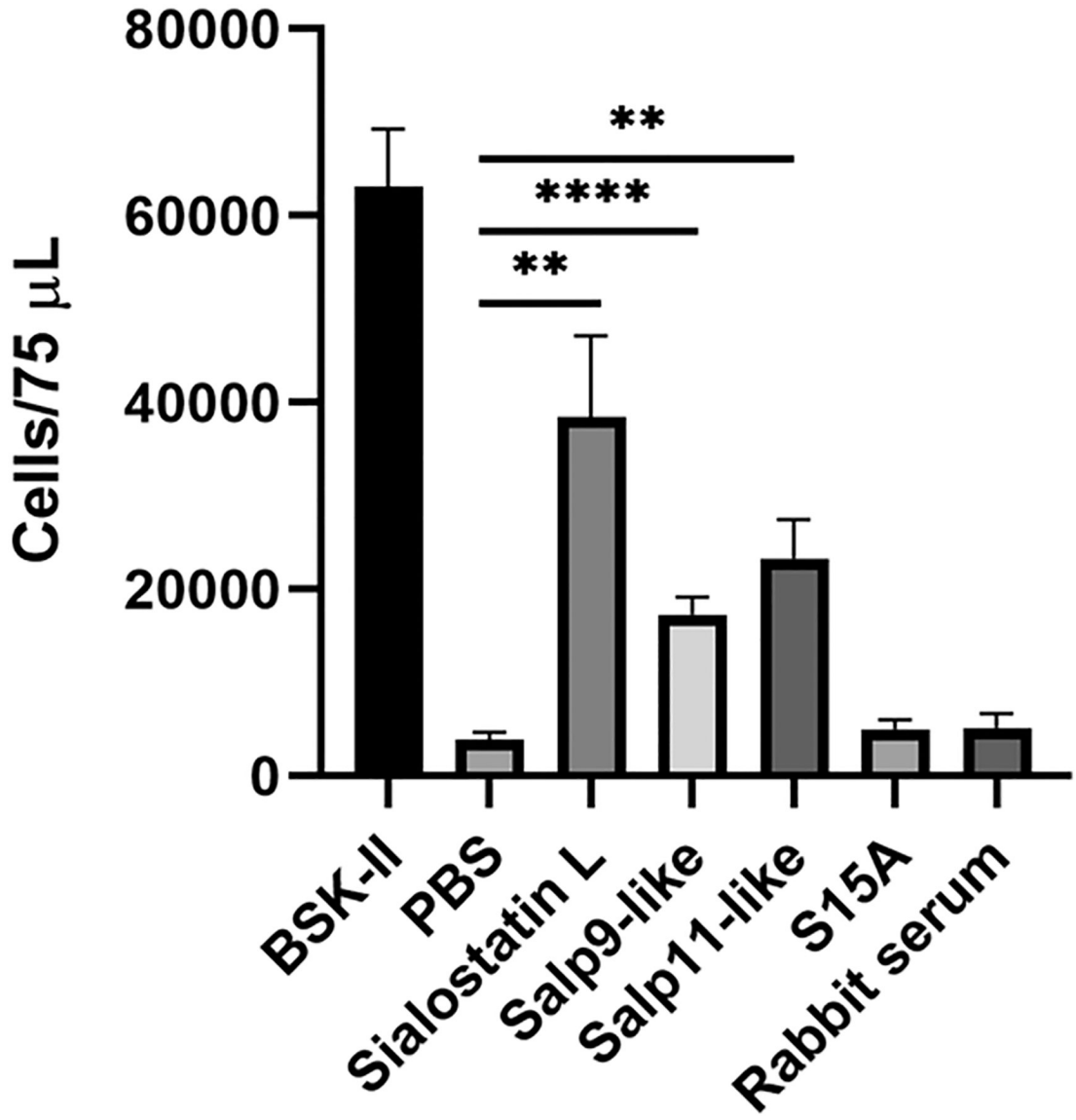

Fig. 6.

SGE recombinant proteins tested by the transwell assay. Three experimental proteins (Sialostatin L, Salp9-like and Salp11-like), one negative control protein (S15A), two negative controls (rabbit serum and PBS), and one positive control (BSK-II) were tested. Cells were counted by flow cytometry. Statistical comparisons were made against PBS, the negative control. Error bars represent standard error of means (SEM). Unpaired t-tests compared: PBS vs. Sialostatin L (Welch's corrected, $t = 3.956$, $p < 0.0053$), PBS vs. Salp9-like (Welch's corrected, $t = 6.646$, $p < 0.0001$), and PBS vs. Salp11-like (Welch's corrected, $t = 4.577$, $p < 0.0022$). Biological and technical replicates were performed in triplicate.

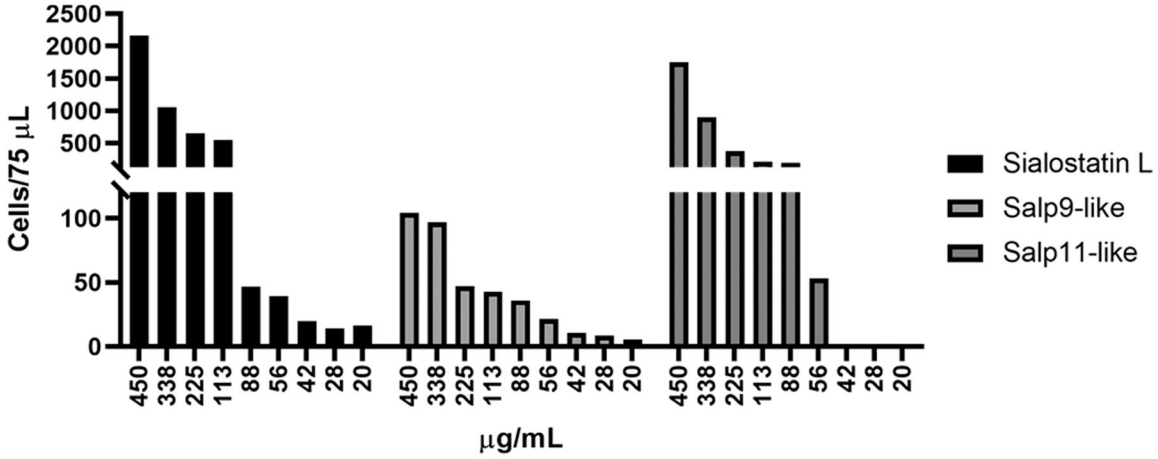

Fig. 7.

Recombinant SGE proteins exhibit dose-dependent properties. Three protein recombinants (Sialostatin L, Salp9-like, and Salp11-like) were serial diluted, assayed for chemoattractant activity, and counted by flow cytometry. Empty bar slots represent a count of zero. Proteins concentrations in µg/ml.

Table 1

PCR primers to generate recombinant proteins.

Primer name	Target Gene	5′ −3′ sequence
PetCH_SiaL_F	Sialostatin L	GAAGGAGATATACATATGACTTCTACCTTCGCTTTGGTC
PetCH_SiaL_R	Sialostatin L	GTGATGGTGGTGATGATGTGCGGCTTCACACTCGAAGGG
PetCH_Salp9-Like_F	Salp9-like protein	GAAGGAGATATACATATGGGGTTGACTGAGATCATGCTG
PetCH_Salp9-Like_R	Salp9-like protein	GTGATGGTGGTGATGATGGGCTTCTAACTCTTCTTCGGT
PetCH_Salp11-Like_F	Salp11-like protein	GAAGGAGATATACATATGGGGTTGACTGAGATCACGCTG
PetCH_Salp11-Like_R	Salp11-like protein	GTGATGGTGGTGATGATGGACATCATTGTCATTGGGCAC
Pet_S15a_CHIS_F	S15a 40S ribosomal protein	GAAGGAGATATACATATGGTGCGAATCAACGTGTTGGCA
Pet_S15a_CHIS_R	S15a 40S ribosomal protein	GTGATGGTGGTGATGATGGAAGAAGTAGCCCAGTATTTT