Tick saliva regulates migration, phagocytosis, and gene expression in the macrophage-like cell line, IC-21

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ABSTRACT

We studied the effects of tick saliva on cell migration, cell signaling, phagocytosis, and gene expression in the murine macrophage cell line, IC-21. Saliva increased both basal- and platelet-derived growth factor (PDGF)-stimulated migration in IC-21 cells. However, saliva did not affect PDGF-stimulated extracellular signal-regulated kinase (ERK) activity. Zymosan-mediated interleukin-1 receptor associated kinase (IRAK) activity increased when cells were pretreated with saliva. Saliva suppressed phagocytosis of zymosan particles by IC-21 cells. An RT2 Profiler™ PCR Array revealed that saliva regulates gene expression in a manner consistent with an immune response skewed toward a Th2 reaction, which may contribute to the tick’s ability to modulate immune function.

1. Introduction

The American dog tick, Dermacentor variabilis (Say), is a hard tick that feeds on the host for up to 14 days depending on environmental conditions. Damage to the host’s epidermis and dermis caused by insertion of the tick’s mouthparts should initiate the host’s wound healing processes and immune responses, including inflammation, phagocytosis of foreign bodies, and cell migration into the site of injury. Additionally, a lengthy feeding period requires evasion of host immune responses as well as prevention of wound healing at the feeding lesion. Successful feeding of this duration requires suppression of the host’s wound healing, inflammatory, and immune responses resulting in the ticks’ evolution of countermeasures to the host’s defense systems (Anderson and Valenzuela, 2008; Brossard and Wikel, 2008; Steen et al., 2006). These countermeasures include numerous anti-hemostatic, anti-inflammatory, or immuno-modulatory compounds that have been identified in tick saliva or salivary gland extract (Barriga, 1999; Champagne, 2004; Francischetti et al., 2009; Sonenshine, 1991; Ribeiro and Francischetti, 2003; Ribeiro et al., 1985; Sauer et al., 1995; Wikel, 1996). Furthermore, it is postulated that tick-induced immunosuppression helps blood meal acquisition and may be a crucial factor in the establishment and transmission of the Lyme disease organism Borrelia burgdorferi (Wikel, 1999). Ixodid ticks are pool feeders that must establish and maintain a feeding lesion to complete feeding (Sonenshine, 1991). Feeding in ixodid ticks consists of alternating cycles of bloodmeal ingestion and saliva secretion with periods where neither occurs (Sweatman and Gregson, 1970). Numerous pathogens are transmitted to the host through the tick’s saliva, and D. variabilis is known to transmit pathogens that cause Rocky Mountain Spotted fever, Tularemia, and to cause tick paralysis (Mans et al., 2008; Parola et al., 2005; Telford and Goethert, 2008). In order to maintain the feeding lesion, it is important for ticks to control the activities of immune and reparative cells that are involved in the wound healing response. We have previously demonstrated that saliva and salivary gland extract (SGx) from D. variabilis delays injury repair, suppresses migration, and reduces PDGF-stimulated ERK activity, a pro-migration signaling pathway, in fibroblasts (Kramer et al., 2008). Based on these observations, we wanted to see if saliva would affect the immune-related functions of macrophages, which play important roles in the wound healing response.

Macrophages perform many tasks within the wound site, including phagocytosis of bacteria and other foreign objects,
secretion of chemotactic factors, growth factors, and cytokines, and debriding and remodeling. Control of this cell type by saliva components could provide the tick with a means of delaying wound healing, regulating inflammation, and successfully transmitting pathogens. Macrophages arrive at the wound site several hours after neutrophils and remain through several stages of the healing process. In addition, mononuclear cells (including macrophages) are the second most abundant cells after neutrophils at tertiary infestations on New Zealand White rabbits and their numbers increase as feeding progresses (Gill and Walker, 1985). Thus, it is possible that the tick promotes maintenance of the feeding lesion and efficient feeding by regulating macrophage activities at the injury site.

To address the possibility that ticks regulate macrophage function, we examined the effects of tick saliva on IC-21 cells, a continuous monoclonal murine macrophage-like cell line, chosen for its similarity to macrophages. IC-21 cells are morphologically indistinguishable from macrophages for the first few days after subculturing and differ only slightly after that (Mauel and Defendi, 1971). Like macrophages, IC-21 cells are phagocytic and cytolytic (Walker and Gandour, 1980) and express IgG2a, IgG2b, complement receptor C3, and platelet-activating factor receptors (Walker, 1976). These cells display macrophage-specific antigens, undergo apoptosis when exposed to rabbit anti-macrophage serum, and can be activated by lipopolysaccharide via TLR4 (Mauel and Defendi, 1971). Our results suggest that D. variabilis saliva may alter macrophage activity within the feeding lesion resulting in a modified host immune response.

2. Materials and methods

2.1. Materials

Adult D. variabilis males and females were purchased from the Tick Rearing Facility at Oklahoma State University, and kept in 96% humidity with a saturated K2SO4 solution at room temperature. Specific pathogen free New Zealand White rabbits were purchased from Myrtle’s Rabbitry (Thompson's Station, TN). Dr. Richard A. Smith (University of Tennessee at Memphis) generously provided the IC-21 cells. RPMI-1640 was from HyClone (Logan, UT). Fetal bovine serum and Alexa Fluor 488-conjugated zymosan provided the IC-21 cells. RPMI-1640 was from HyClone (Logan, UT). Fetal bovine serum and Alexa Fluor 488-conjugated zymosan

2.2. Methods

2.2.1. Cell culture

IC-21 cells were grown in 75 cm² flasks at 37 °C in 5% CO2 and given fresh growth medium three times per week (RPMI-1640 with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine). Upon reaching confluence, cells were subcultured by incubating with Ca²⁺/Mg²⁺-free Hank's Balanced Salt Solution (HBSS) for ~15 min to disrupt cell-to-cell contact followed by forcefully pipetting up and down to release the cells from the surface of the flask. IC-21 cells used in phagocytosis assays were plated at a density of ~80,000 cells/well in eight well chambers. Phagocytosis assays were performed 3 days after plating and cell counts were done immediately preceding addition of zymosan particles in order to determine the number of zymosan particles to be added to each experimental well. IC-21 cells used for ELISA were plated at a density of 200,000 cells/100 μl in 96 well dishes and were used 1–3 days after plating.

2.2.2. Collection of tick saliva

Female New Zealand White rabbits weighing 9–10 lb and approximately 6 months of age were used as hosts for adult male and female D. variabilis as previously described (Patrick and Hair, 1975) under a protocol approved by the University of Memphis Institutional Animal Care and Use Committee. On approximately day 6 of feeding, partially engorged female D. variabilis were removed from the host, weighed to ensure that they met our definition of partially engorged (~90–350 mg), and immobilized on double-sided scotch tape attached to a clean glass slide. Ticks were injected with 10 μl of tick saline/MOPS (TS/MOPS) (10 mM dophamine/thesophyllylino in MOPS buffer in tick saline, pH 7.0, with 3% DMSO) into the hemolymph using care to avoid damaging the salivary glands (Needham and Sauer, 1979). Ticks that produced saliva (an average of ~4 μl/tick) were injected a total of three times, with the follow-up injections coming 5 min after the previously induced salivary production ceased. Saliva was collected using non-heparinized soda lime glass pipettes and transferred into a microcentrifuge tube on ice. A Bio-Rad protein assay was performed on the collected saliva, which was subsequently aliquoted and stored at −20 °C until used.

2.2.3. Cell migration assay

IC-21 cells grown to confluence in 75 cm² flasks were incubated 15 min with Ca²⁺/Mg²⁺-free HBSS and removed from the surface by pipetting up and down. Cells were centrifuged then resuspended in RPMI-1640 (without supplements) to a concentration of 100,000 cells/ml. The bottom portion of a blind well chemotaxis chamber was loaded with serum-containing RPMI-1640 or PDGF as described in the figure legends (see Fig. 1). An 8 μm polycarbonate track-etch filter was placed over the filled well and the top portion of the chamber was screwed into place forming an upper well. Cells were pretreated with saliva for 30 min then 100 μl were loaded into the upper well. The IC-21 cells were incubated uncovered for 4 h in the blind well chemotaxis chamber at 37 °C in a 5% CO2 incubator. The solution in the upper well was removed and a sterile cotton swab was gently rubbed across the surface of the filter to remove excess liquid as well as any cells on the top surface of the filter. The filter was removed with forceps and placed on a polycarbonate track-etch filter. The filter was placed on a polycarbonate track-etch filter. The filter was placed on a polycarbonate track-etch filter.

Fig. 1. Saliva increases migration of IC-21 cells. Saliva increases both basal and PDGF-stimulated cell migration. Cells were pretreated with 0, 2, or 4 μl/ml saliva for 30 min then placed in the upper chamber of a blind well chemotaxis chamber with PDGF (100 ng/ml) in the bottom chamber. Data are means ± SEM, n = 3. *p < 0.01, **p < 0.001 compared to basal migration.
upside-down on a clean glass slide then fixed with 75% methanol. The filter was then stained with Harris’ hematoxylin, and a coverslip was mounted using fluoromount. IC-21 cells that had migrated through the filter were counted using the 40× objective of a light microscope. Data were reported as number of cells migrating/4 h.

2.2.4. Phosphoantibody cell-based ELISA (PACE)
ERK and IRAK activity were measured using the phosphoantibody cell-based ELISA described in Kramer et al. (2008). Briefly, IC-21 cells were treated as described in figure legends and treatments were stopped by adding ice-cold PBS to each well. Cells were washed, fixed with 4% formaldehyde, and incubated overnight with primary antibody. The primary antibody was added to the wells after another wash cycle and the plates were incubated overnight at 4 °C. The primary antibody dilutions were as follows: phospho-ERK (Thr202/Tyr204), 1:4000; phospho-IRAK (Ser376), 1:250. The plates were then washed and incubated for 1 h with an HRP-conjugated secondary antibody. The Pierce 1-Step Ultra TMB ELISA substrate was used to develop the assay and the absorbance was read at 450 nm using a Bio-Tek ELx808 Ultra Microplate Reader. Data were normalized to time-matched controls.

2.2.5. Phagocytosis assay
IC-21 cells plated in eight chambered slides were treated as described in figure legends. Cells were pretreated with increasing volumes of saliva then incubated for 2 h with zymosan particles (~10 particles/cell). Cells were washed three times with PBS, stained for 10 min with trypan blue to quench fluorescence of extracellular zymosan A BioParticles, and fixed for 20 min with 10% formalin. The plastic chamber top was removed from the slides and coverslips were applied using fluoromount. Ingested Alexa Fluor 488-conjugated zymosan A BioParticles (100 cells/treatment) were counted using a fluorescent microscope. For each saliva concentration, the phagocytic index was calculated by multiplying the average percentage of phagocytes by the average number of particles ingested per phagocytic cell, and the data are presented as such.

2.2.6. PCR array
IC-21 cell treatments correspond with the experimental conditions used in the ELISA experiments (+/+− saliva, +/- TLR ligand). RNA was isolated from IC-21 cells using the Pure Link RNA Purification System (Invitrogen). RNA concentration and purity were quantified using a Nanodrop Spectrophotometer. cDNA was synthesized using the RT2 First Strand Kit (SABiosciences). A RT2 Profiler™ Innate and Adaptive Immune Response PCR Array was used (SABiosciences). Data were analyzed with the SABiosciences Web-Based PCR Array Data Analysis, where p values were calculated based on a Student’s t-test of the replicate 2−ΔΔCT values for each gene in the control group and treatment groups, and p values less than 0.05 were considered significant. The SABiosciences Web-Based PCR Array Data Analysis protocol recommends choosing a housekeeping gene as a control based on the following criteria: it should have Ct values that differ by less than one cycle across the compared samples. GAPDH was used as the control housekeeping gene, because its Ct values differed by only 0.5 across the compared samples.

2.2.7. Statistical analyses
Data are presented as means ± SEM of 3–6 experiments assayed in triplicate and performed over several passages of IC-21 cells. Statistical significance was determined by one-way ANOVA and Dunnett’s post-test for multiple comparisons using GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego CA, <www.graphpad.com>). Differences in means were considered significant at p < 0.05.

3. Results
All saliva used in this study was from a single pooled batch (resulting from feedings of ~300 ticks fed on six different rabbits) with a protein concentration of 0.27 mg/ml. This protein concentration is consistent with previously published studies using multiple batches of saliva (Kramer et al., 2008). To test the impact of tick saliva on the IC-21 cell line, we used blind well chemotaxis chambers. Cells were pretreated for 30 min with increasing concentrations of saliva (0, 2, or 4 µl/ml) then loaded into the upper chamber of a blind well where the lower chamber contained RPMI with or without PDGF (100 ng/ml). Cells were all treated with the same volume of liquid, only the concentration of saliva within the treatment volume changed. Fig. 1 shows that saliva dose-dependently increased basal cell migration (341 ± 17 cells/h) to 573 ± 14 cells/h and 866 ± 32 cells/h, with 2 or 4 µl/ml saliva, respectively. PDGF increased the total number of cells migrating (771 ± 19 cells/h), and saliva enhanced the effect of PDGF (1006 ± 29 cells/h and 1223 ± 64 cells/h, with 2 or 4 µl/ml saliva, respectively). The magnitude of the increases in migration due to saliva pretreatment were similar in basal and PDGF-stimulated migration; these additive effects suggest that a component of saliva regulates migration through a pathway or receptor that is different than that of PDGF.

The ERK signaling pathway regulates growth factor-mediated cell migration (Pintucci et al., 2002). Therefore, we examined the effect of saliva on PDGF-stimulated ERK activity. Saliva alone did not significantly affect ERK activity (fold over basal) as compared to untreated cells (Fig. 2, white bars). PDGF-stimulation of ERK activation was only significantly different from the untreated control (p < 0.05) control when cells were exposed to 0, 0.1 and 0.25 µl/ml of saliva. The effect of saliva on PDGF-stimulated ERK activity was analyzed by comparing the PDGF plus saliva treatments to cells treated with PDGF alone (Fig. 2, black bars). A 30-min pretreatment with low volumes of saliva (0.1 and 0.25 µl/ml) did not significantly change PDGF-stimulated ERK activation; however, there was a significant decrease in PDGF-stimulated ERK activation when cells were pretreated with 2 µl/ml saliva (Fig. 2).

One of the essential roles of macrophages within the wound site is cytokine secretion caused by Toll-like receptor (TLR) activation. Therefore, we studied the effect of tick saliva on IRAK-1 in IC-21

![Fig. 2. PDGF-stimulated ERK activation is decreased by 2 µl/ml saliva. Saliva does not affect PDGF-stimulated ERK activity in IC-21 cells at low volumes; however, saliva does decrease PDGF-stimulated ERK activity in IC-21 cells by 2 µl/ml. Cells were pretreated with increasing volumes from 0–2 µl/ml saliva for 30 min then incubated for 15 min with 1 ng/ml PDGF. For each experiment (n), the treatments were performed in triplicate and each experiment was performed using a different cell passage. Data are presented as fold over basal and are means ± SEM, n = 3. Two microliter per milliliter saliva decreases PDGF-stimulated ERK activity in cells treated with 1 ng/ml PDGF. *p < 0.05 compared to untreated control, #p < 0.01 compared to PDGF-treated cells without saliva.](https://www.graphpad.com)
cells. IRAK-1 is a key component of the TLR pathways. As reviewed by Gottipati et al. (2008), IRAK-1 has regulatory functions ranging from activation of NFkB and MAPK pathways to increasing IFNγ and IL-10 gene expression.

Zymosan A prepared from the cell wall of Saccharomyces cerevisiae was used as a ligand for TLRs 2 and 6. Zymosan increased IRAK-1 activity in IC-21 cells in a time-dependent fashion, with the largest increase occurring after 30 min (a 1.5 fold increase over the time-matched control). Interestingly, saliva increases zymosan-stimulated IRAK activity, which indicates activation of signaling through the TLR-2 pathway (Fig. 3). Cells were pretreated with 0, 0.5, 1, or 2 μl/ml saliva for 30 min then incubated for 5, 15, 30, or 60 min with zymosan (100 μg). The increases in zymosan-induced IRAK activity by saliva were both time- and dose-dependent, with the maximum response occurring at 15 min with 2 μl/ml saliva (a 3 fold increase over the time-matched control). The effects of saliva and zymosan on IRAK activity were additive; saliva alone also increases IRAK activity (data not shown), which may indicate that saliva can affect more than one Toll-like receptor pathway.

Another essential role for macrophages within a wound is phagocytosis of bacteria and apoptotic neutrophils. In order to assess the effect of saliva on the phagocytic ability of IC-21 cells, the cells were pretreated with 0, 0.5, 1, or 2 μl/ml saliva for 30 min then incubated for 2 h with fluorescent zymosan particles (~10 particles/cell). Ingestion of zymosan particles by IC-21 cells was suppressed by saliva (1 and 2 μl/ml saliva, but not 0.5 μl/ml saliva), indicating that a dose-dependent response could possibly be seen using a broad range of lower concentrations of saliva. ~82% of untreated IC-21 cells ingested at least one particle as compared to ~78%, ~66%, and ~65% for IC-21 cells treated with 0.5, 1, and 2 μl/ml saliva, respectively (Fig. 4a). The average number of particles ingested by each phagocytic cell also decreased (~6, ~3, ~3, and ~2 particles for 0, 0.5, 1, or 2 μl/ml saliva, respectively) (Fig. 4b). Fig. 4c shows the decrease in the phagocytic index of IC-21 cells, which is calculated by multiplying the % of phagocytic cells (Fig. 4a) by the mean # of ingested particles per cell (Fig. 4b).

To extend on our observations that tick saliva controlled immune function, we measured changes in the expression of 84 genes related to innate and adaptive immune responses using a qRT-PCR array (Table 1). Twelve of the 84 tested genes showed significant differential expression in response to treatment with saliva and/or zymosan (Fig. 5). Up- or down-regulation based on PCR data analysis was determined by comparing saliva, zymosan, or saliva + zymosan-treated cells to untreated control cells. CCL2 gene expression is down-regulated in cells treated with saliva. CCL2 gene expression is up-regulated in zymosan-treated cells, but this up-regulation is diminished in cells treated with both zymosan and saliva. TLR4 gene expression was down-regulated in cells treated with saliva. TLR4 gene expression was also down-regulated in zymosan and zymosan-treated cells, but not to the same extent. The remaining genes were up-regulated to varying degrees in response to tick saliva. CD14, which encodes a protein involved in innate immunity, showed the greatest change in expression.

4. Discussion

We studied the effects of saliva from partially engorged adult female D. variabilis on migration, signaling, phagocytic activity, and gene expression of macrophages, cells that play a crucial role
Fig. 5. Effects of saliva on the expression of genes involved in innate and adaptive immune responses. RNA was isolated from untreated cells as well as cells exposed to saliva (30 min pretreatment), zymosan (2 h), or saliva (30 min pretreatment) + zymosan (2 h). Real-time PCR was performed using cDNA synthesised from the isolated RNA and results are presented as fold up- or down-regulation compared to untreated cells.

Table 1

<table>
<thead>
<tr>
<th>Function/group</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute phase response to bacteria</td>
<td>Casp1, Casp4, Nfkbia, Tgfb1, Tnf, Tnfs1a</td>
</tr>
<tr>
<td>Chemokine receptor</td>
<td>Cxcr4</td>
</tr>
<tr>
<td>Complement activation</td>
<td>C8a, C8s</td>
</tr>
<tr>
<td>Defense response to bacteria</td>
<td>Ccl2, Clec7a, Defb4, Irf1, Lbb1, Ly6, Lyz2, Ncf4, Nlrc4, Pglyrp3</td>
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<tr>
<td>Detection of bacteria</td>
<td>Cdi1d1, Pglyrp1, Pglyrp2, Pglyrp3</td>
</tr>
<tr>
<td>IL-18 pathway</td>
<td>Il18bp, Mapk11, Mapk18</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Adora2a, Ccl2, Ccr3, Cldn4, Cypb, Hc, Ly96, Mif, Nos2, Ptg2, Ptprf, Stabl</td>
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<tr>
<td>Innate immunity</td>
<td>Colec12, Dimb1, Stpl</td>
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<tr>
<td>Interferons/receptors</td>
<td>Ifn1, Ifn2, Ifn4</td>
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<tr>
<td>Interleukins/receptors</td>
<td>Il10, Il12b2, Il1a, Il1b, Il1f10, Il1f5, Il1f6, Il1f8, Il1f9, Il1r1, Il1r2, Il1rap, Il1rapl2, Il1r2, Il1r3, Ile6</td>
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<tr>
<td>NFκB signaling</td>
<td>Chuk, Traf6</td>
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<td>Septic shock</td>
<td>Hmox1, Nfkbiaa, Serpina1a, Serpina1, Tmem1</td>
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<tr>
<td>TLR pathway</td>
<td>Irak1, Irak2, Mdy88, Tlr1, Tlr2, Tlr3, Tlr4, Tlr6, Tlr8, Tlr9, Tollip</td>
</tr>
</tbody>
</table>

in normal wound healing. As described in the Methods, saliva production was induced by injecting a dopamine-containing solution into the tick’s haemocoel. While the amount of dopamine that crosses the salivary gland membrane is unknown, the half-life of dopamine is very short making it unlikely that it would produce the effects seen. This is particularly true for those that were time-dependant, such as a maximum increase in zymosan-induced IRAK activity at 15 min (as opposed to 5 min). In addition, catecholamines rapidly oxidize in solution and are not likely to persist in the saliva once it is collected. Finally, we have shown that the effects of saliva were qualitatively similar to those of salivary gland extract where dopamine/theophylline were not used in the collection process (Kramer et al., 2008).

We previously reported that SCG from adult female D. variabilis suppressed fibroblast migration (Kramer et al., 2008), and Skallová et al. (2008) have reported similar results with dendritic cells, which are antigen-presenting cells. Conversely, our results indicate that macrophage migration is increased when the cells are pretreated with saliva. These data are consistent with in vivo studies showing that macrophage numbers increase at the feeding lesion throughout the course of tick feeding (Szabó and Bechara, 1999). We speculate that the tick saliva has evolved in such a way that the presence of macrophages within the wound site can be used to the ultimate advantage of the tick rather than the host. Tick saliva seems to promote a regulatory macrophage population with anti-inflammatory activity characterized by the production of the immunosuppressive cytokine IL-10 (reviewed by Mosser and Edwards, 2008), and our data show that saliva up-regulates IL-10 expression.

Dillon et al. (2004, 2006) showed that zymosan modulates the immune response by inducing the secretion of at least one immunomodulatory protein, TGF-β, by macrophages while another TLR2 ligand (Pam-3-cys) stimulates a Th2 response in dendritic cells. TGF-β inhibits activation of phagocytes such as macrophages and dendritic cells and also displays antiinflammatory effects against epithelial cells. A Th1 response activates macrophages, specifically stimulating phagocytosis, and produces pro-inflammatory cytokines such as IL-12. A Th2 response suppresses the Th1 response by negative feedback through cytokines such as IL-4 and IL-10 and inhibits phagocytic activity of both macrophages and dendritic cells. Thus, we speculate that the increase in IL-10 expression associated with saliva treatment could mediate the inhibition of macrophage phagocytosis observed in our studies.

Zymosan-induced TLR2 activation also inhibits IFN-γ-induced killing of mycobacteria by murine macrophages (Arko-Mensah et al., 2007). TLR2 activation (by hsp60) also enhances the immunosuppressive effects of regulatory T cells (Zanin-Zhorov et al., 2006). Thus, macrophages at the wound site could be regulated by components of tick saliva such that they ultimately suppress the host’s immune response to the tick as well as to any transmitted pathogens and it seems likely that the TLR2 pathway plays a role in this regulation.

Phagocytosis by macrophages is an essential component of the host’s immune response to tissue damage, and our results indicate that saliva reduced the number of phagocytic macrophages as well as the average number of phagocytosed particles per cell. Reduced phagocytic activity due to saliva has also been reported in neutrophils (Montgomery et al., 2004; Ribeiro et al., 1990). Suppression of phagocytic activity in neutrophils has been cited as a contributing factor in “saliva-activated transmission” of pathogens, and our data further support these conclusions. In addition to bacteria and apoptotic cells, red blood cells are also phagocytosed by macrophages (Mosser and Edwards, 2008). Since ticks must ingest heme through their bloodstream (Donohue et al., 2009), it would benefit the tick to reduce the phagocytic activity of macrophages.

CCL2, also known as monocyte chemotactic protein 1 (MCP-1), mediates both acute and chronic inflammatory responses (Conti et al., 1999). Neutralization of CCL2 is associated with a decrease in IFNγ and an increase IL-4 and IL-5, which polarizes the T helper cell reaction toward Th2 and also increases IL-10, an anti-inflammatory cytokine (Traynor et al., 2002). IL-6, although usually considered a pro-inflammatory cytokine, also has anti-inflammatory activity, such as stimulating production of IL-10 (Steensberg et al., 2003; Xing et al., 1998). Although it was surprising that the expression of the pro-inflammatory cytokines IL1a and IL1b were up-regulated, it is important to note that the gene for their
receptor antagonist, IL1ra, was enhanced to greater extent, thus potentially counter-acting any pro-inflammatory effects of these cytokines. There may be similar reasons for the tick to up-regulate CD14, which is also pro-inflammatory. Up-regulation of this gene has been documented in the transmission of B. burgdorferi, which suggests a role in salivary-activated transmission. In addition, CD14 induces monocyte differentiation into macrophages, and as we have shown, tick saliva increases migration of the macrophage-like IC-21 cells while decreasing phagocytic efficiency.

Of the TLR and related genes, only TLR4 was down-regulated. TLR4 recognizes LPS on Gram-negative bacteria and promotes a Th1 response through the production of IL-12 (Agrawal et al., 2003). TLRs 2 and 6, which function together to recognize yeast, were both up-regulated as were IRAK1 and MYD88, which play critical roles in most TLR signaling pathways (see review by Gottipati et al., 2008). TLR2 stimulates proliferation of regulatory T cells and promotes a Th2 response through the production of IL-10 (Re and Strominger, 2004; Sutmuller et al., 2006). It selectively controlling the host’s response. Other studies have shown that tick saliva increases migration and zymosan-stimulated IRAK activity (signaling through the TLR2 pathway) in IC-21 cells but suppresses zymosan-induced phagocytic activity. ERK signaling pathways as well as LPS-induced IRAK activity in IC-21 cells appear to be unaffected by tick saliva. In addition, our gene expression data reveals a pattern of regulation consistent with an increase in TLR2-associated immune responses. These results suggest that D. variabilis has evolved a mechanism for selectively controlling the host’s response. Other studies have revealed similarly complex effects, such as selective promotion of a Th-helper 2 cytokine profile (Ferreira and Silva, 1999; Mejri et al., 2001). Our data indicate that ticks use macrophages to orchestrate a modified host immune response and exploit the complex interactions between cytokines and TLRs to create a delicate balance of inflammatory and immune reactions, polarizing the response toward the anti-inflammatory TLR2 pathway and cytokine profile.

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