Effects of Tick Saliva on Musculoskeletal Inflammatory Molecules

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Significance
Controlling inflammatory processes in bone and cartilage could have a very high impact on treatment of musculoskeletal diseases. There already exists in nature organisms, such as ticks, that have evolved complex fluids for controlling host immune and inflammatory responses. Identifying and characterizing the molecules involved could lead to novel treatments in disease processes such as arthritis and osteolysis.

Introduction
Ixodid tick species such as Dermacentor variabilis take a long blood meal (up to 14 days) after attachment to its host. The tick must damage both the dermis and blood vessels to obtain a meal. Normally this would activate host immune and reparative responses. Ticks have evolved an array of countermeasures in order to fight off these responses. The anti-inflammatory and immunomodulatory components of the saliva may stop the rejection of the tick, which allows the tick to continue to take in blood. We hypothesize that establishment and maintenance of the feeding lesion requires the coordinated regulation of immune and reparative cells critical to the wound healing response and that proteins found in the tick saliva provide these functions. Past research conducted has identified some of these proteins, as well as discovering various effects of these proteins on different cytokines and other important cells used in immune responses. The purpose of this study was to examine the effects of tick saliva on molecules important in musculoskeletal inflammatory responses such as those found in osteolysis and arthritis.

Materials and Methods
The experiments were performed using tick saliva collected from the species Dermacentor variabilis (provided by Dr. Lewis Coons). Specific disease free rabbits were used as hosts. Ticks were removed from the host to obtain the saliva. The mouse macrophage cells, IC-21 (ATCC), were grown in RPMI-1640 medium supplemented with L-glutamine, gentamicin, and 5% fetal bovine serum (FBS) for growth (2% FBS for experiments). Each experiment consisted of 250,000 IC-21 cells placed in each well of a 24 well culture dish. The wells consisted of medium alone (no cells), only cells; cells plus 0.76 µg/ml lipopolysaccharide (LPS, a gram negative bacterial toxin known to elicit an inflammatory reaction) as a positive stimulator for inflammatory cytokines; cells plus tick saliva; and cells plus LPS plus tick saliva. Experiments were done overnight and medium from each well aspirated and frozen. Levels of TNFα, and IL-1alpha were measured using the Quantikine Assay kit (R&D Systems Inc.) and levels of Prostaglandin E2 (PGE2) were measured using Correlate-ELA kit (Assay Designs, Inc.). In addition, cell viability was measured after adding fresh growth medium to the cells and 20 microliters of MTS from CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega), incubated for one hour and 100microliters from each well transferred to a 96 well plate and absorbance read and recorded.

Results
The MTS assays indicated there were no differences in cell numbers between groups for any of the experiments (data not shown). LPS increased secretion of TNF-α as expected, however the addition of 8µl/ml of tick saliva had an inhibitory effect on the secretion of TNF-α (Figure 1). Figure 2 indicates that this inhibition occurs in a dose dependent manner. Results were similar for IL1-alpha, with saliva decreasing the amount secreted from 235 to 82 pg/ml. Interestingly the saliva had no effect on the amount of IL6 secreted from the cells (data not shown).

Discussion
In these experiments, tick saliva decreases the LPS induced secretion of the inflammatory cytokines, TNF-α and IL1, both of which have important functions in musculoskeletal pathologies. Interestingly the saliva had no effect on LPS induced secretion of IL6, which has been reported to be both pro and anti-inflammatory and to be both a positive and negative influence on bone formation. Tick saliva greatly increases the secretion of PGE2, which has been shown to inhibit fibroblast migration, synthesis of extracellular matrix proteins and inhibit macrophage phagocytosis. Therefore tick saliva may down regulate proinflammatory molecules while at the same time increasing molecules that prevent the reparative process.