Effects of Long-Term Antibiotic Treatment and LPS Induced Inflammation Pre-Injury on ACL Rupture Mouse Models of Post-Traumatic Osteoarthritis

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Introduction

Post-traumatic osteoarthritis (PTOA), is a secondary form of OA that develops in ~50% of people who have suffered a severe joint injury [1]. Studies have shown that modifications to the gut microbiome affect bone formation, bone mass, and susceptibility to inflammation [2]. It has been suggested that a state of inflammation prior to injury could contribute to more severe PTOA phenotypes [3]. Lipopolysaccharides (LPS) are microbial associated molecular patterns (MAMPS) which activate an inflammation cascade released by gram-negative bacteria [4]. The normal gut biome releases LPS as positive protective activation, but during this experiment we mimic a dramatic increase days prior to injury. Exposure to long-term antibiotics and LPS days before injury, mimics a state of acute inflammation, and may increase the severity of the PTOA phenotype.

Methods

C57Bl/6 mice were purchased from Jackson Laboratories. Mice were divided into four groups: untreated (VEH), antibiotic treated (+AB), untreated with LPS (+LPS), and antibiotic treated with LPS (+AB+LPS). AB and AB+LPS groups received antibiotics in their drinking water [ampicillin (1000mg/L); neomycin (500mg/L)] starting at weaning for six weeks. Five days prior to injury +LPS and +AB+LPS, received either a 1mg/kg of LPS subcutaneously in the abdominal area or a sham saline injection. Mice were injured at 10 weeks of age (N=5 per group), with a single-dynamic tibial compression load (1mm/s) to induce ACL rupture (~12N); some mice were not injured and served as controls (N=2 per group). Joints were collected at 6-weeks post injury, fixed in 10% neutral buffer formalin, decalcified using EDTA for four weeks with changes every other day, and embedded in paraffin wax. Sections (6mm) were stained with Safranin-O/Fast Green and imaged. Fluorescent immunohistochemistry was performed to determine the cellular infiltration in the sections.

Results

Figure 1. Histological Assessment of Male C57Bl/6J.

<u>Contralateral</u> +AB group shows a stronger stain compared to the rest of the groups while +LPS shows a decrease in staining and proteoglycan failure on the femoral head (A, E, I, M; numeral). Contralateral for VEH and +AB+LPS are the most similar.

<u>Injured</u> groups show proteoglycan failure (B, F, J, N; numeral and carat). +LPS group shows a decrease in mineralized surface compared to the other groups (B, F, J, N; asterisk). +AB+LPS shows a decrease in the mineralized surface area only when compared to VEH. +AB group shows stronger staining and a thickening of the meniscus along with cellular infiltration, cellular infiltration is present in +LPS and +AB+LPS (C, G, K, O; carat). On the femoral condyle the +AB group shows stronger staining and thicker cartilage while +LPS shows the thinnest cartilage (D, H, L, P; asterisk and arrow). +AB+LPS shows a consistent cartilage staining, similar to VEH.

<u>We can conclude</u> from this image that +AB group has the better cartilage phenotype while +LPS group has the worst. VEH and +AB+LPS are the closest to each other, suggesting that both treatments together restore the phenotype.

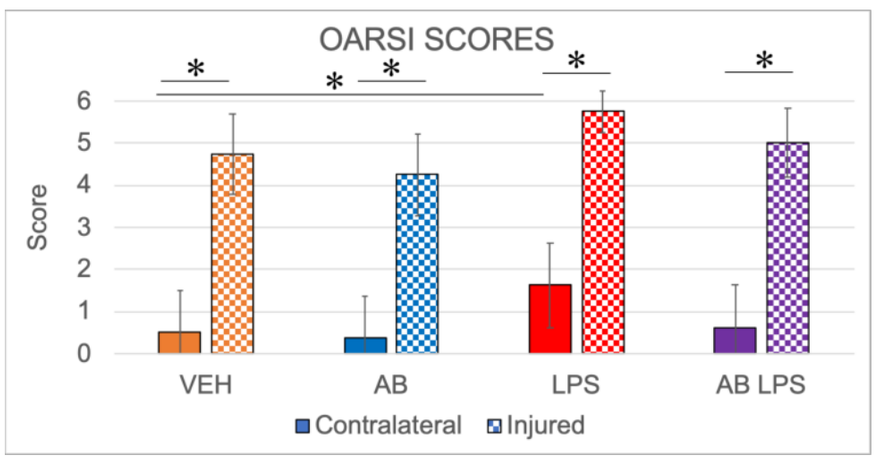


Figure 2. OARSI Scoring

There is a statistically significant difference between the contralateral and injured joints in each treatment group. There was a statistically significant difference between uninjured VEH and +LPS groups; additionally +AB and +LPS were statistically significant. The highest score was given to the +LPS group, suggesting the worst phenotype. The lowest score was for +AB, which suggests the best PTOA outcome of the treatment groups. VEH and +AB+LPS group scores had the smallest difference between them.

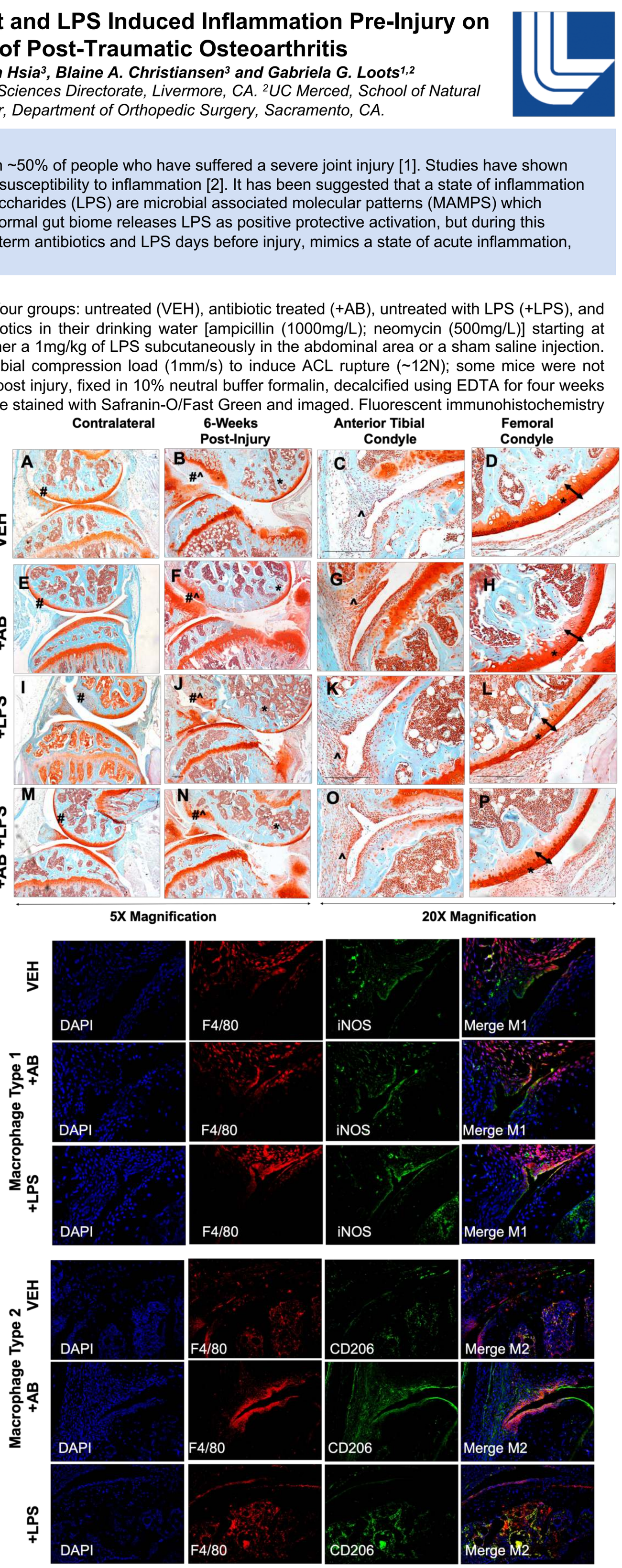
Scoring was done using the OARSI guidelines. Statistical analysis was done using Student T-test. (=p>0.05)*

Figure 3. Macrophage Population Immunohistochemistry Analysis

Due to the cellular infiltration we were able to see during the Safranin-O/Fast-Green staining we wanted to identify the cell populations present in the joint. We wanted to identify the macrophage populations present. Macrophages type 1 are considered to be pro-inflammation while macrophages type 2 are associated with healing.

<u>M1 populations are increased in +LPS group.</u> Present in all treatment groups but there was an increase in staining for F4/80 and iNOS in +LPS group.

<u>M2 populations are increased in +AB group.</u> The thickening of the meniscus on +AB group could suggest an inflammatory state, however we found that there is an increase in M2 population. They were present in VEH and +LPS, however we could mainly found M2 in the bone marrow.



Future Direction

Healing an orthopedic injury depends on many factors, including the overall health of the immune system. While clear correlations have been established between chronic state of inflammation and osteoarthritis, it is less clear how acute immune activation the disruption of the gut microbiome can impact joint healing. This study suggests that a transient inflammatory response, similar to a mild bacterial or viral infection can dramatically impact joint healing. In similar manner, removing the positive protective activation of MAMPS which train naïve immune cells, seem to reduce the response to injury, and in turn, give a better outcome after injury. In future experiments, we will characterize the subchondral bone phenotype in order to determine how the bone phenotype changes in the different treatment groups. Additionally we will add female cohorts to determine how changes may affect females.

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