Proceedings from the Research Colloquium on Post-traumatic Arthritis

Friday, January 18, 2013

Sponsored by a Grant from the Duke University School of Medicine

Colloquium Director, Steven A Olson MD

The colloquium was attended by more than 40 participants from across Duke University Hospital, including Members of the Departments of Medicine, Orthopaedic Surgery, Physical Therapy and Radiology. This unique opportunity allowed investigators working in the area of arthritis after joint injury to share their own work, and learn of the ideas and efforts of others interested this growing area of clinical interest.

Arthritis is the nation's most common cause of disability. An estimated 50 million US adults (about 1 in 5) report doctor-diagnosed arthritis. An estimated 12% of all patients seeking intervention for symptomatic arthritis have an etiology of previous trauma to the involved joint. Compared to other forms of arthritis, post-traumatic arthritis (PTA) has a more rapid clinical onset. Recent evidence from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) indicated joint degeneration following injury is the most common cause of a soldier being unfit for duty. Therefore, PTA is a prime area for funding by the Department of Defense (DOD).

Further, the study of PTA has distinct advantages over other forms of arthritis—there is often a discreet known time of onset of the disease—the trauma itself. This unique feature allows investigators to begin to consider the time frame of the development of PTA after injury. Similarly, a better understanding of the natural history of events after joint injury will aid in the development of interventions to potentially prevent PTA.

Steven A Olson MD

Duke Post-traumatic Arthritis Colloquium

January 18, 2013

Searle Center Main Conference Room

8:00	Introduction and goals of the colloquium	Olson

Colloquium Goals

- Developing a shared knowledge of current research in the area of PTA
- Developing collaboration among investigators in the area of PTA at DUHS
- Indentifying strategies to mentor investigators in the field of PTA
- Identifying gaps in knowledge needed to advance the field of PTA
- Developing a DUHS Research Strategy to address key knowledge gaps in PTA

Session 1, Moderator: Guilak

Clinical Investigation

8:10	IL1-Ra and ACL injuries	Toth
8:30	PTA in the ankle	Adams
8:50	Discussion	

Opportunities for Collaboration

9:05	Gait lab opportunities for collaboration in PTA research	Queen
9:20	Radiology opportunities for collaboration in PTA research	Hash
9:35	Physical Therapy opportunities for collaboration in PTA research	Myers, Butler, Coeytaux
09:55	Break	

Session 2, Moderator: Toth

Basic Science Investigation—Potential for Translational Research

10:15	PTA work from DeFrate lab	DeFrate
10:35	Discussion	
10:45	PTA work from Kraus lab	Kraus
11:05	Discussion	
11:15	PTA work from Guilak Lab	Guilak
11:35	Discussion	
11:45	PTA work from Olson lab	Olson
12:05	Discussion	
12:15	Lunch?	

Session 3, Moderator: Kraus

12:45	PTA work from Setton lab	Setton
1:10	Overview—defining the need to know information to advance the field of PTA	
	Why is PTA important to the larger subject of osteoarthritis?	
	What are the key knowledge gaps in the field of PTA that limit progress for patient care?	
	What are the questions that will address these knowledge gaps?	
2:25	Break	

Session 4, Moderator: Olson

2:40	Mentoring investigators in PTA Research	
	Panel discussion: Garrett, Guilak, Kraus, Setton	
3: 10	Setting the future research agenda—where do we go from here?	
	Clinical Projects	
	What is the objective of the project?	
	Who will oversee hypothesis development?	
	What collaborations are needed?	

Timeline?
Potential funding sources?
Basic Science Projects
What is the objective of the project?
Who will oversee hypothesis development?
What is the translational potential for this project?
What collaborations are needed?
Timeline?
Potential funding sources?

4:30 End

Summaries of Active Research Efforts in PTA Ongoing at Duke University

IL-1Ra and ACL Injuries: Dr Allison Toth

Focus of Current Research Efforts: Evaluate the clinical effectiveness of intra-articular IL-1 receptor antagonist (IL-1Ra) for knee injury including anterior cruciate ligament (ACL) tear.
Primary Investigators: Alison P Toth, MD; Virginia Kraus, MD, PhD
Funding Sources: Women's Sports Medicine Fund Code

Background—Hypothesis: Joint injury triggers three phases of pathogenic events: The early (acute) phase involves joint swelling, hemarthrosis, expression of inflammatory cytokines [especially interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α)], and biomarkers of cartilage catabolism; an intermediate phase is characterized by reduction of joint inflammation, ongoing joint catabolism, but no evidence yet for typical features of radiographic osteoarthritis (OA); and a late phase characterized by radiographic OA. The catabolic state is accentuated by abnormal joint motion and loading after injury. There is considerable variation in response to surgical treatment and a lack of objective evidence to support a protective role of repair or reconstructive surgery of the ACL or meniscus against OA development. In part, we hypothesize that this may be due to the damage accrued by the joint during the first month after injury. Early biological response of the injured joint has a crucial impact on the long-term health consequences of the joint and, therefore, the early phase of acute injury may represent a window of opportunity for providing treatment to promote healing and to prevent the subsequent cascade of joint destructive processes following injury that often culminate in arthritis.

Methods/Protocols: We performed a placebo-controlled, randomized, double-blinded proof of concept pilot trial of intra-articular IL-1Ra (anakinra) for acute knee injury with ACL tear (NCT00332254). Eleven patients with acute ACL tear confirmed by magnetic resonance imaging (MRI) were randomized to receive a single intra-articular injection of IL-1Ra (anakinra 150 mg, n 1/4 6) or equal volume of saline placebo (1 ml, n 1/4 5). The double-blinded treatment was administered a mean 2 weeks after injury. Synovial fluid (SF) (n 1/4 9 patients) and sera (all patients) were available at baseline (prior to injection) and immediately prior to surgery (mean 35 days later) and analyzed for SF IL-1α, IL-1β, IL-1Ra and serum hyaluronan (HA), an indicator of synovial inflammation. The primary outcome (standardized KOOS questionnaire) was obtained at 0 (baseline), 4 and 14 days after injection.

Results: Compared with placebo, the IL-1Ra group had substantially greater improvement in key outcomes over 14 days (KOOS pain P 1/4 0.001; activities of daily living P 1/4 0.0015; KOOS sports function P 1/4 0.0026; KOOS quality of life (QOL) P 1/4 0.0048; and total KOOS ($p < 0.0001$). There were no adverse reactions in either group. SF IL-1α (P 1/4 0.05) and serum HA (P 1/4 0.03), but not IL-1α or IL-1Ra, decreased significantly in the IL-1Ra–treated patients, but not the placebo-treated patients. Compared with placebo, IL-1α was borderline significantly different in the IL-1Ra–treated group (P 1/4 0.06).

Discussion/Future Directions: Administered within the first month following severe knee injury, IL-1Ra reduced knee pain and improved function over a 2-week interval. This promising proof of concept study provides a new paradigm for studies of acute joint injury and suggests that a larger follow-up study is warranted. We have proposed a double-blind, placebo-controlled trial to be conducted with intra-articular administration of an IL-1 inhibitor after traumatic knee injury. The functional and metabolic state of the injured joint will be followed with time to assess the effect of blocking IL-1 during the initial pro-inflammatory state. A homogenous injury population with isolated ACL tears will be studied and functional outcome will be assessed clinically with patient-based outcome scores, pain assessment, ROM and functional recovery tools. Because the development of cartilage damage substantial enough to cause radiographic evidence of arthritis takes many years, the metabolic status of the articular cartilage will be followed with time with intra-articular and serum-based biomarkers of cartilage breakdown, such as serum HA. Intra-articular and serum cytokine profiles, including IL-1α, IL-1β, IL-1Ra and TNF-α, also will be followed with time to assess any change in response to administration of the IL-1 inhibitor. Finally, qualitative MRI, e.g., dGEMRIC and T2 mapping, will be used to assess for early matrix changes typical of arthritis.

Given the short half-life of anakinra (4 hours), we are considering studying longer acting IL-1 inhibitors, such as Rilonacept (Arcalyst, Regeneron Pharmaceuticals, Inc., Tarrytown, NY) an IL-1 tartrate-resistant acid phosphatase (TRAP) which targets the IL-1 receptor, or canakinumab (Ilaris, Novartis Pharmaceuticals, East Hanover, NJ), a human monoclonal antibody targeted

at IL-1β. It is possible that polymers, depots or nanoparticle preparations may be needed to provide sustained intra-articular delivery.

We also have experience with use of IL-1Ra in prevention and treatment of arthrofibrosis and adhesive capsulitis in hundreds of patients over the last 8 years.

PTA in the Ankle: Dr Sam Adams

Focus of Current Research Efforts: Metabolic profiling of biofluids and tissues can provide a panoramic view of the current physiologic state of a biological system, such as the intra-articular environment of a post-traumatic ankle joint. We envision the role of metabolomics in post-traumatic arthritis as a clinically applied diagnostic tool in which a sample of synovial fluid would be analyzed for a panel of metabolite biomarkers. Similarly to following serial values from a complete blood count, alterations in the metabolic profile could indicate disease progression or a therapeutic response at a resolution not possible with currently employed clinical techniques.

Primary Investigators: Samuel B Adams, MD, Dana L Nettles, PhD, Lori A Setton, PhD

Funding Sources: American Orthopaedic Foot and Ankle Society

Background—Hypothesis: Unlike other weightbearing joints, the majority (80%) of ankle arthritis is post-traumatic. Reports have estimated approximately a 20-year time period of progression from injury to end-stage ankle arthritis. Thus, there is the potential for many years of intervention with disease modifying agents if the correct targets are identified. However, very little is known about the disease process of post-traumatic ankle arthritis. Cytokine analysis, used as a tool to identify biomarkers, has been widely performed on knee joint synovial fluid but not in the ankle. Metabolic profiling is an emerging field of research concerned with the comprehensive characterization of the metabolites in biological systems. Metabolites are the end-products of cellular regulatory processes, and their levels can be regarded as the ultimate response of biological systems to environmental changes, such as those that might occur in the cartilage and synovium of post-traumatic arthritis. Metabolites have served as biomarkers in cardiovascular disease and prostate cancer. To date, there has been no literature identifying key metabolites or describing the metabolic state of post-traumatic ankle arthritis. The purpose of this study was to define the cytokine composition and metabolic profile of post-traumatic ankle arthritis, potentially identifying altered metabolic pathways that could be used as targets for disease modification or individual metabolites or cytokines that could serve as biomarkers of post-traumatic ankle arthritis (PTAA).

Methods/Protocols: Ankle joint synovial fluid was obtained from 20 patients with PTAA and 20 patients with no ankle pain or radiographic evidence of ankle arthritis (control group). Synovial fluid samples were analyzed for IFN-γ, TNF-α, MIP-1β, MCP-1, IL-1β, IL-1Ra, IL-4, IL-6, IL-8, IL-10, IL-13 and IL-15 using ELISAs. Additionally, synovial fluid samples were analyzed for more than 3000 metabolites using liquid and gas chromatography with mass spectroscopy. T-tests were used to identify cytokines and metabolites that differed between groups, and random forest analysis was performed on the metabolites to determine whether the control and PTAA samples could be differentiated based on their metabolic profile.

Results: IL-1Ra, IL-6, IL-8, IL-10, IL-15 and MCP-1 were significantly elevated in the PTAA group. Additionally, 107 metabolites were significantly altered in the PTAA group. These metabolites indicated derangement in amino acid, carbohydrate, lipid, energy metabolism, extracellular matrix turnover and collagen degradation. Additionally, they suggested an increased oxidative and inflammatory environment in PTAA. Random forest analysis yielded a predictive accuracy of 90% when using the metabolic profiles to distinguish between control and PTAA samples.

Discussion/Future Directions: With respect to arthritis research, the ankle joint has largely been ignored. Knowledge about arthritis in other joints, such as the knee or hip, cannot simply be inferred to the ankle joint, as the cause of ankle arthritis is largely (80%) post-traumatic, whereas only 10 and 2% of knee and hip arthritis, respectively, is post-traumatic. This study identified inflammatory cytokines and a distinct metabolic profile present in the synovial fluid of end-stage post-traumatic ankle arthritis. Several of the inflammatory cytokines have previously been implicated in rheumatoid arthritis and osteoarthritis in other joints. The RF analysis indicated that the identified metabolites could be used to identify synovial fluid from end-stage arthritic ankle joints with 90% accuracy. The identified cytokines and metabolites can be used as biomarkers for post-traumatic arthritis diagnosis or to monitor disease progression or therapeutic response. Metabolic profiling may have potential as a diagnostic tool for arthritis.

Altered *in vivo* Cartilage Loading and Cartilage Degeneration

Focus of Current Research Efforts: Investigating relationships between altered *in vivo* cartilage loading and cartilage degeneration.

Primary Investigator: Lou Defrate, PhD.

Collaborators: Drs William E Garrett, Farshid Guilak, CT Moorman, James A Nunley, Steven A Olson, Robin M Queen, Charles E Spritzer, Dean C Taylor.

Funding Sources: National Football League Charities, Arthrex, NIH (R03 AR055659).

Background—Hypothesis: Altered cartilage loading after ligament injury is a contributing factor to the development of post-traumatic OA.

Methods/Protocols: We are using novel imaging techniques to measure *in vivo* cartilage deformation. High resolution magnetic resonance (MR) images are used to quantify joint geometry and *in vivo* joint kinematics are measured during weight-bearing loading conditions using biplanar fluoroscopy.[1] After registration of the MR-based 3D models to the radiographic images, cartilage strains are approximated from the overlap of cartilage layers.[2] In addition to these measurements, our laboratory has also developed techniques to measure cartilage strains using MR imaging before and after dynamic activities of daily living.[3,4] More recently, we have validated the use of T1-rho weighted MR imaging in our laboratory.[5] T1 rho-weighted imaging has been shown to be sensitive to early signs of cartilage degeneration. Specifically, changes in T1-rho weighted imaging have been correlated to changes in proteoglycan concentration.[5]

Results: These innovative imaging techniques have been used to demonstrate that nonanatomic graft placement during ACL reconstruction is associated with abnormal joint motion and localized cartilage thinning just 18 months after surgery.[6,7] In patients with anatomic graft placement, normal joint motion was restored, and no cartilage thinning was observed. We have also demonstrated that lateral ankle instability elevates cartilage strains during weight-bearing loading.[2] These changes were observed in a region of the joint associated with the development of post-traumatic OA.[2]

Discussion/Future Directions: In the future, our goal is to relate these changes in the mechanical environment of cartilage to degenerative changes to the joint. This could be accomplished through a long-term study evaluating alterations in cartilage composition using T1-rho weighted imaging and measuring cartilage loading using the advanced imaging techniques described above. Ultimately, understanding the relationships between changes in cartilage composition and loading may be critical to developing interventions aimed at slowing the onset and progression of post-traumatic OA.

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PTA Work from Kraus Lab: Dr Virginia Kraus

Focus of Current Research Efforts: Identifying biomarkers indicative of an osteoarthritis (OA) trajectory prior to radiographic manifestations of OA. Preclinical and clinical trials in animal models and humans to evaluate early treatment interventions to prevent an OA trajectory following post-traumatic OA.

Primary Investigators: Virginia Kraus, Janet Huebner, Thomas Stabler and collaborators Steve Olson, Bridgette Furman, Duke Sports Medicine physicians—Alison Toth, T Moorman, W Garrett, D Taylor, Christian Latterman (U Ky) and Kurt Spindler (U Vanderbilt).

Funding Sources: Arthritis Foundation, DOD

Background—Hypothesis: Acute interventions to prevent joint degradation in the immediate postinjury period will significantly decrease the risk of post-traumatic OA.

Methods/Protocols: We have ongoing efforts to develop an enlarging armamentarium of candidate biomarkers for evaluation of postinjury inflammation including markers of oxidant stress.

Results: We have completed a pilot study of intra-articular IL-1Ra in humans with ACL tear [Osteoarthritis Cartilage 2012 Apr;20(4):271-78. DOI: 10.1016/j.joca.2011.12.009. Epub 2012 Jan 10. Effects of intra-articular IL1-Ra for acute anterior cruciate ligament knee injury: A randomized controlled pilot trial (NCT00332254). Kraus VB, Birmingham J, Stabler TV, Feng S, Taylor DC, Moorman CT 3rd, Garrett WE, Toth AP]. This promising but small study showed a symptomatic and functional benefit of early anti-inflammatory biological intervention following joint injury. This laid the paradigm for subsequent studies to evaluate this and other anti-inflammatory therapies to prevent joint degradation following severe acute joint injury.

Discussion/Future Directions: We are supporting the ongoing efforts of the Arthritis Foundation to develop a national strategy to develop a clinical trials consortium to investigate new interventions in human acute joint injury to prevent post-traumatic OA.

PTA Work from Setton Lab: Dr Lori Setton

Focus of Current Research Efforts: Intra-articular and perineural drug delivery and noninvasive tracking of pathology development following joint injury.

Primary Investigators: Lori A Setton, Robert D Bowles, Kyle D Allen, Brian A Mata, Virginia B Kraus, Janet L Huebner, Jun Chen and Samuel B Adams.

Funding Sources: NIH R01AR047442, P01AR050245, F32AR063012, K99AR057426

Overview: Our group is committed to developing noninvasive means to characterize progressive osteoarthritis development in joints following joint injury. Our laboratory has established methods to evaluate parameters of gait in freely ambulating rodents, including measures of stride length, stride width, stance time, symmetry and foot splay, and has demonstrated their utility in documenting disease progression in a unilateral joint injury model. In addition, we have documented an ability to correlate these changes in gait parameters with measures of pain-related sensitivities (mechanical allodynia, thermal hyperalgesia) and serum or synovial fluid biochemical markers of osteoarthritis. Finally, we have developed new methods using transgenic mice carrying a luciferase reporter coupled to an inflammatory regulator, NF-κB, to document disease activity *in vivo* following joint injury. These studies are briefly described here.

Surgically-Induced Joint Injury: In one study, limb sensitivity, weight distribution, and kinematic and dynamic changes in gait were measured following injury to the medial collateral ligament (MCL) and medial meniscus (MM) in the rat knee. Rats underwent surgery for transection of the right MCL (MCL alone) or MCL transection followed by radial MM transection (MCL+MM). Behavioral metrics of weight bearing and mechanical sensitivity, gait kinematics and gait dynamics were obtained for all animals longitudinally following surgery. At sacrifice, serum and synovial fluid were collected for cytokine detection, and knees were processed for histological scoring using the OARSI histopathology assessment scheme.

During locomotion, the gait of rats with MCL+MM transection became increasingly asymmetric, significantly different from a symmetric gait pattern by day 23 (Fig. 1: top, *, $p = 0.018$). Moreover, rats receiving MCL+MM transection tended to have stance time imbalances, spending more time on their contralateral limb than the operated limb while walking (Fig. 1.

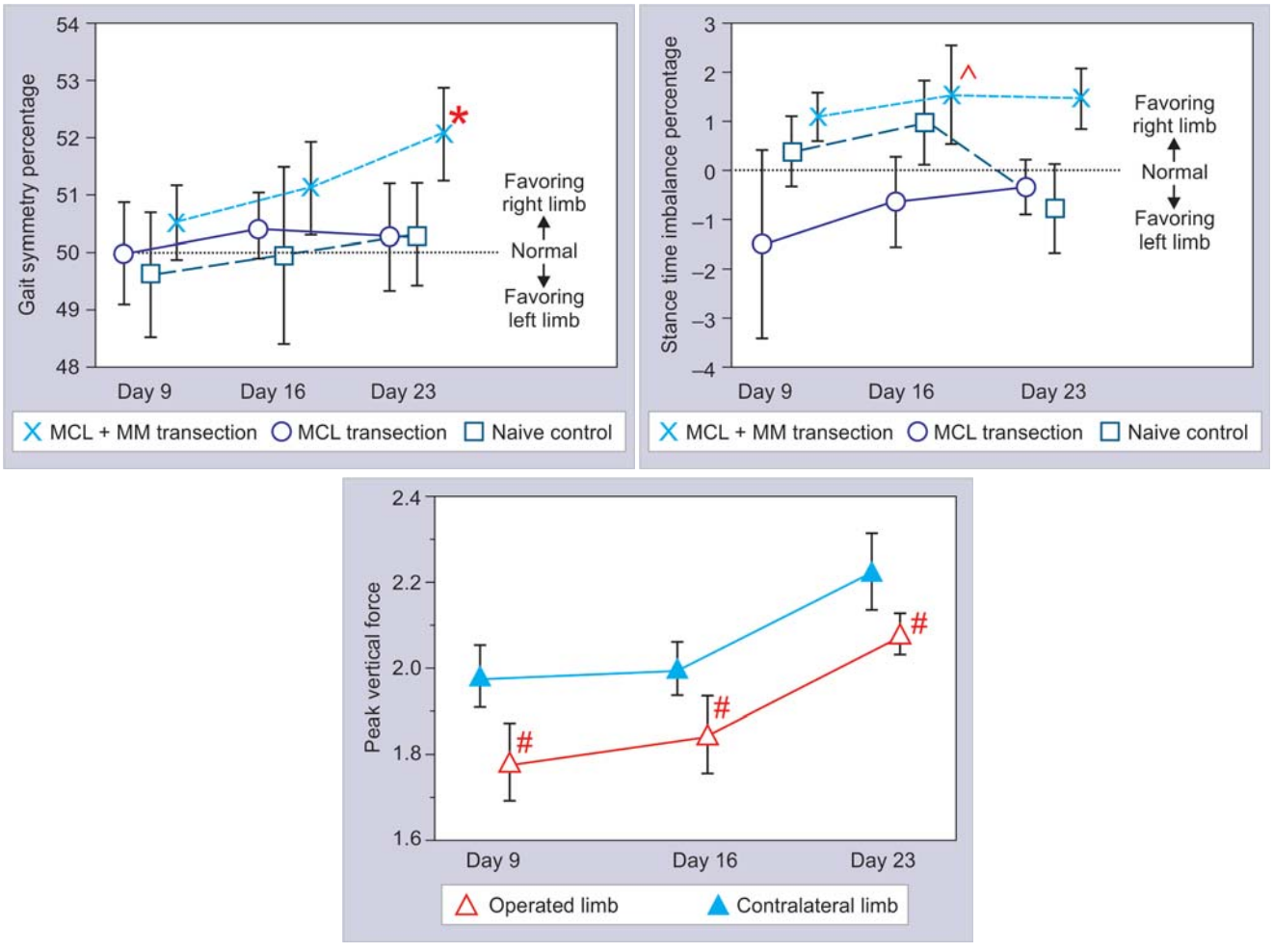
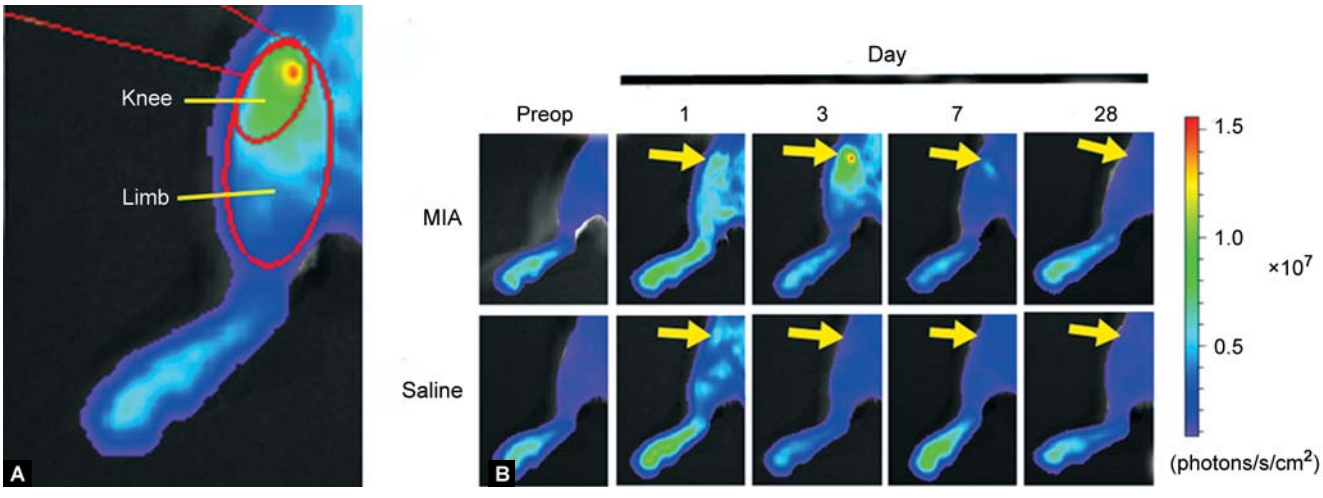
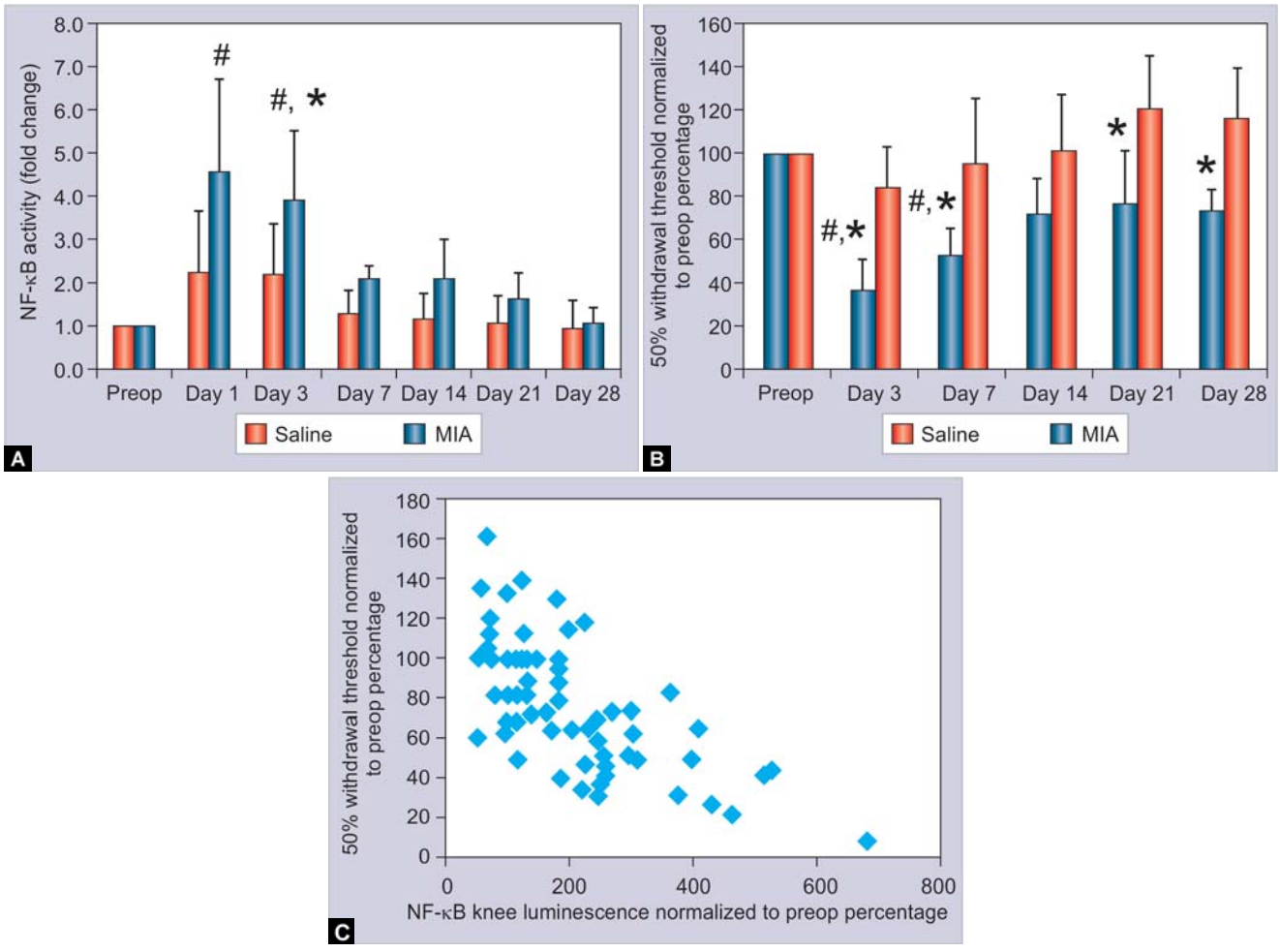


Fig. 1: Gait abnormalities following MCL + MM transection



Figs 2A and B: (A) Knee and limb ROI location on luminescence image and (B) *in vivo* imaging of NF-κB activity (yellow arrows indicate injection site)

middle). Thus, rats with MCL+MM transection spent less relative time on their operated limb (^, stance time imbalance > 0.0) and had a foot strike spacing indicative of right (operated) limb injury (*, gait asymmetry > 0.5). Ground reaction force analysis confirmed gait abnormalities. MCL+MM also resulted in heightened operated limb sensitivity relative to contralateral control. Histology confirmed the presence of OA-like lesions on the medial tibial plateau of rats with MCL+MM transection. Taken together, these data indicate MCL+MM transection causes a subtle, but detectable, limp in rats that is similar to that observed for humans. Follow-on studies can be performed to use gait as a screening tool for novel therapeutics in this animal model.



Figs 3A to C: (A) NF-κB activity, (B) mechanical allodynia over 28 days [$p < 0.05$ compared to preop (#) and sham (*)], and (C) mechanical allodynia plotted against NF-κB activity

***In vivo* Imaging of Joint Inflammation in an Injury Model:** The goal of this study was to test for relationships between NF-κB activity and painful symptoms and impaired function in a murine model of arthritis. Transgenic mice engineered to carry cDNA for luciferase downstream of NF-κB response elements [Tg(NF-κB-RE-luc)] received a 5 μl intra-articular injection of normal saline or monoiodoacetate (MIA). Mice underwent *in vivo* imaging of luminescence to measure NF-κB activity (IVIS100, Perkin Elmer) before and after the procedure, along with simultaneous measures of mechanical allodynia and weight distribution.

Results demonstrated significant increases in NF-κB activity in the knee joints receiving an MIA injection on days 1 and 3 after injection, as compared to preprocedural values and saline values (Figs 2A, 2B and 3A). Mechanical allodynia was significantly decreased for MIA injection animals (Fig. 3B), while weight distribution significantly shifted to the contralateral limb for MIA injection animals at similar timepoints. NF-κB activity in the knee joint was found to linearly correlate with mechanical allodynia (Fig. 3C) (Spearman $\rho = -0.69$) and weight distribution (ρ of –0.39), suggesting that *in vivo* luminescence imaging of NF-κB activity has definite relationships to pain-related parameters. In summary, we have demonstrated a novel use of *in vivo* luminescence imaging to longitudinally measure NF-κB activity and study its relationship to developing pain sensitivities in a model of chemically-induced joint injury. We expect that this method can be applied to an expanded array of joint injury models in order to obtain noninvasive and longitudinal parameters of pathology that better relate to human dysfunction and pain.

Obesity and Post-traumatic Arthritis

Focus of Current Research Efforts: The primary focus of our work has been to examine the interactions among biomechanical loading, inflammation and obesity[1] as they relate to the onset and progression of post-traumatic arthritis

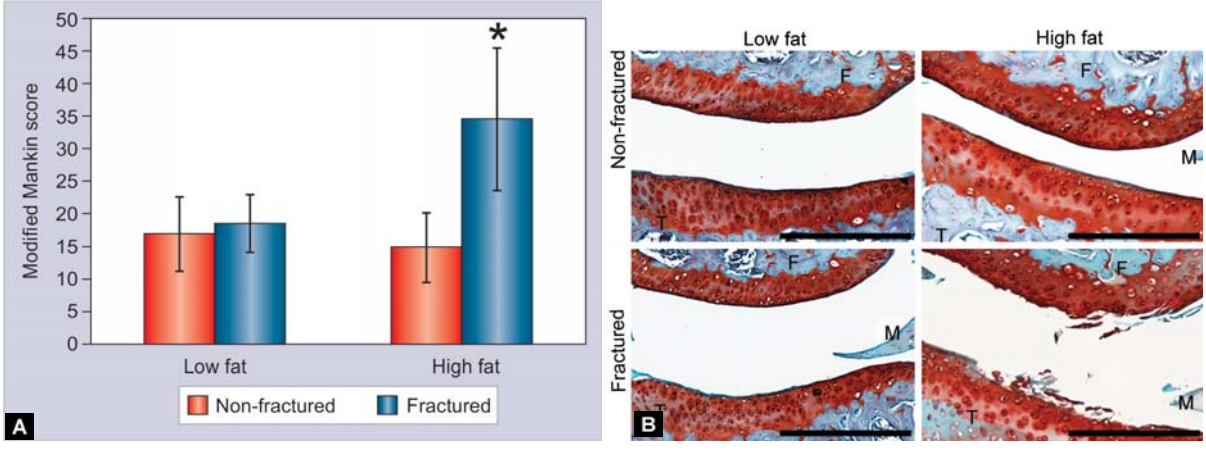
(PTA). Furthermore, we are examining a variety of cellular, molecular and physical approaches for the development of new therapies for PTA.[2]

Primary Investigators: Farshid Guilak and Steven Olson; Collaborators: Craig Louer, Bridgette Furman, Janet Huebner, Virginia Kraus, Chia-Lung Wu, Deeptee Jain, Brian Diekman.

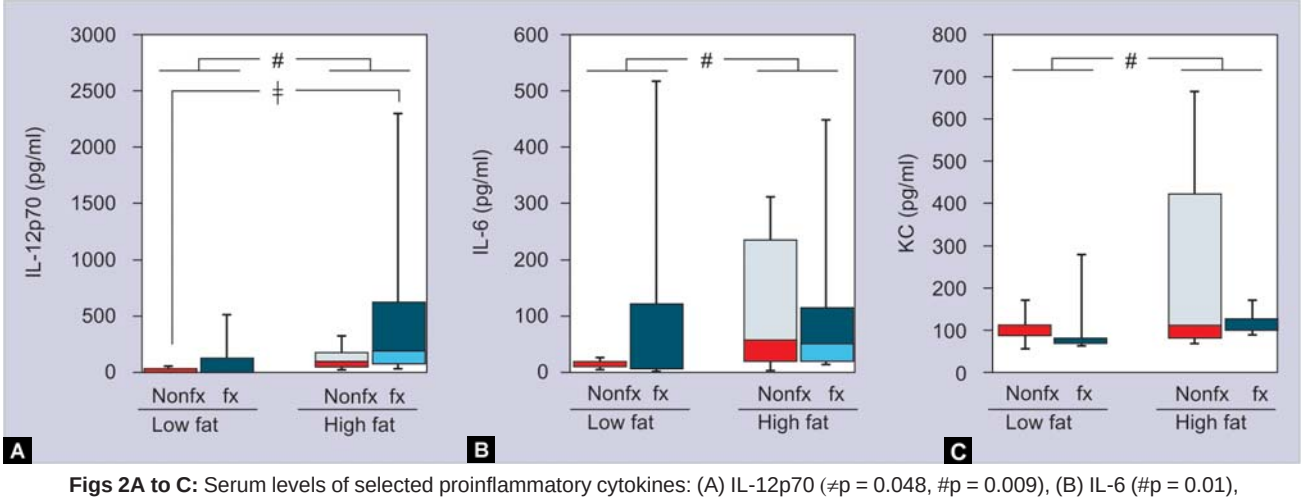
Funding Sources: Arthritis Foundation, NIH grants AR50245, AG15768, AR48852 and AR48182, Department of Defense.

Background—Hypothesis: Obesity and joint injury are both primary risk factors for osteoarthritis (OA) that involve potential alterations in the biomechanical and inflammatory environments of the joint. PTA is a frequent long-term complication of intra-articular fractures. Obesity has been linked to primary OA and may potentially contribute to the development of PTA by a variety of mechanisms. The objectives of this study were to determine if diet-induced obesity influences the severity of PTA in mice and to examine inter-relationships between joint degeneration and serum levels of inflammatory cytokines and adipokines in this response.

Methods/Protocols: All procedures were performed in accordance with an IACUC-approved protocol. Four-week-old mice (male, C57BL/6) were started on the following diets: 13% kcal fat normal/ 'low-fat' diet (n = 18; 5001, Picolab, MO) or 60% kcal fat 'high-fat' diet (n = 12; D12492, Research Diets, NJ). At 16 weeks of age, half the mice from each group were randomized to receive moderate fractures of the proximal tibial plateau under anesthesia using a previously described custom apparatus.[3] Mice were weighed weekly and sacrificed at 8 weeks after fracture. Body fat mass and % were determined by DEXA (PIXImus, GE Healthcare) at fracture and sacrifice. Hind limbs from all animals were dissected, fixed in formalin, and imaged using microCT (μCT 40, Scanco). Bone mineral density and cancellous bone fraction (bone volume/total volume) were measured in the distal femur. Bone mineral density and bone volume were measured in the tibial epiphysis and metaphysis.



Figs 1A and B: (A) Mankin scoring of cartilage degeneration, (B) representative slides of the tibiofemoral contact regions, where the Mankin scores were assessed (F: Femur; T: Tibia; M: Meniscus; Scale bar: 100 μm)



Figs 2A to C: Serum levels of selected proinflammatory cytokines: (A) IL-12p70 (≠p = 0.048, #p = 0.009), (B) IL-6 (#p = 0.01), (C) KC (#p = 0.03) (Nonfx: non-fractured; Fx: fractured; min/25%/median/75%/max)

Joint sections (8 μm thick) were prepared for histology and stained with either Safranin-O and fast green or H&E. Arthritis severity was assessed using a modified Mankin score[3] for structural damage and loss of staining in the articular cartilage (maximum score: 108) by 3 blinded graders. Synovitis was assessed using a modified standardized scoring system for synovial lining thickness and cellular density in the synovial stroma (maximum score: 24) by 3 blinded graders. Serum was collected from mice at the time of sacrifice. Mouse multiplex ELISA (MSD, MD) was used to assess levels of interleukin(IL)-1β, IL-6, KC (human IL-8 analog), IL-10, TNF-α, IFN-γ. Data for bone morphology and cartilage degeneration were analyzed with a multifactorial ANOVA for diet, fracture, and diet+fracture effects. Data for synovial inflammation and cytokine levels were analyzed with a Kruskal-Wallis ANOVA for diet, fracture and diet+fracture effects. A Spearman test was used for cytokine correlations, while a multiple regression model was used for synovitis score determinants. Significance was reported at the 95% confidence level.

Results: Mice on a high-fat (HF) diet gained more weight and body fat ($p < 0.001$) compared to mice fed the normal, 'low fat' diet. Induction of intra-articular fracture at week 16 did not affect weight or body fat mass within diet groups ($p > 0.05$). Fracture significantly decreased cancellous bone fraction in the femoral condyles of HF mice (fx 0.497 ± 0.20 *vs* nonfx 0.687 ± 0.14, $p = 0.001$), though not in LF mice (fx 0.580 ± 0.06 *vs* nonfx 0.611 ± 0.10). In HF mice, fracture significantly increased cartilage degeneration scores, but LF mice showed no statistically significant increase with fracture (Figs 1A and B). HF fractured (HF-fx, 8.6 ± 4.3) mice had significantly increased synovitis scores compared to LF nonfractured (LF-nonfx, 2.9 ± 1.7) mice ($p = 0.006$), while LF-fx (4.1 ± 2.0) and HF-nonfx (4.9 ± 1.6) mice did not differ from LF-nonfx mice. Synovitis also increased overall with a HF diet, regardless of fracture status ($p = 0.02$). HF diet resulted in significant elevation of the proinflammatory cytokines interleukin (IL)-12p70, IL-6 and KC (Figs 2A to C). IL-12p70 levels were also significantly higher in HF-fx mice compared to LF-nonfx mice (see Fig. 2A). Synovitis scores positively correlated with body fat mass at the time of fracture ($r = 0.40$). This association was maintained even after correcting for Mankin score in the multivariate fit model, indicating that both body fat and cartilage degeneration independently contribute to synovial inflammation. None of the tested cytokines were associated with either body fat or Mankin score in the multivariate fit model.

Discussion/Future Directions: The findings of this study support the hypothesis that obesity contributes to the pathogenesis of PTA and leads to more severe joint degeneration. After 8 weeks of healing, obese mice on a high-fat diet subjected to an intra-articular fracture showed significantly more cartilage degeneration and adaptive bone changes compared to nonfractured mice on the same diet, while normal diet mice showed minimal joint degeneration. Synovial inflammation was likewise increased in the high-fat fractured mice when compared to nonfractured normal-diet controls. These findings indicate that diet-induced obesity is a critical risk factor that worsens PTA severity after intra-articular fracture. Multivariate analysis of synovial inflammation supports the hypothesis that increased systemic inflammation associated with obesity contributes to synovitis and may, by extension, increase cartilage damage. While IL-12p70, IL-6 and KC emerged as potential mediators of such a response based on their associations with a high-fat diet, there are potentially other factors involved. Further investigation into the mechanisms accelerating the development of PTA with obesity may provide new insights into the prevention and treatment of this debilitating disease.

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The Natural History of an Untreated Intra-articular Fracture: A Study of Post-traumatic Arthritis

Focus of Current Research Efforts: Evaluate the intra-articular and systemic changes in mice that develop post-traumatic arthritis (PTA) following intra-articular fractures (IAF) and mice that do not develop PTA after IAF.

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Funding Sources: Arthritis Foundation, DOD; Synthes USA Research Support Agreement; Bioventus; NIH Core Program Project.

Background: Arthritis is the nation's most common cause of disability. An estimated 50 million US adults (about 1 in 5) report doctor-diagnosed arthritis. An estimated 12% of all patients seeking intervention for symptomatic arthritis have an etiology of previous trauma to the involved joint. Compared to other forms of arthritis, PTA has a more rapid clinical onset. Recent evidence from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) indicated joint degeneration following injury is the most common cause of a soldier being unfit for duty. This rapid onset of degenerative arthritis is occurring following joint injuries in a younger population with very high levels of baseline physical function.

Hypothesis: Close observation of a closed IAF of the tibial plateau in the knee of a mouse will provide insight into the development of PTA following IAF.

Timeline

- 2005: Experimental model developed by Jens Strand (3rd year student) and Bridgette Furman
- 2007: Furman et al—Proof of concept paper published in Journal of Orthopaedic Research
- 2008: Ward et al—Proof of concept that MRL-MpJ Mice are protected from PTA
- 2008: Seifer et al—Novel technique to recover synovial fluid from the knee of a mouse
- 2011: Lewis et al—Synovial inflammation is related to severity of fracture
- 2012: Louer et al—Obesity increases severity of PTA after fracture
- 2012: Diekman et al—Intra-articular stem cells reduce severity of PTA after fracture
- 2012: Lewis et al—Genetic and cellular evidence of inflammation in PTA—see below

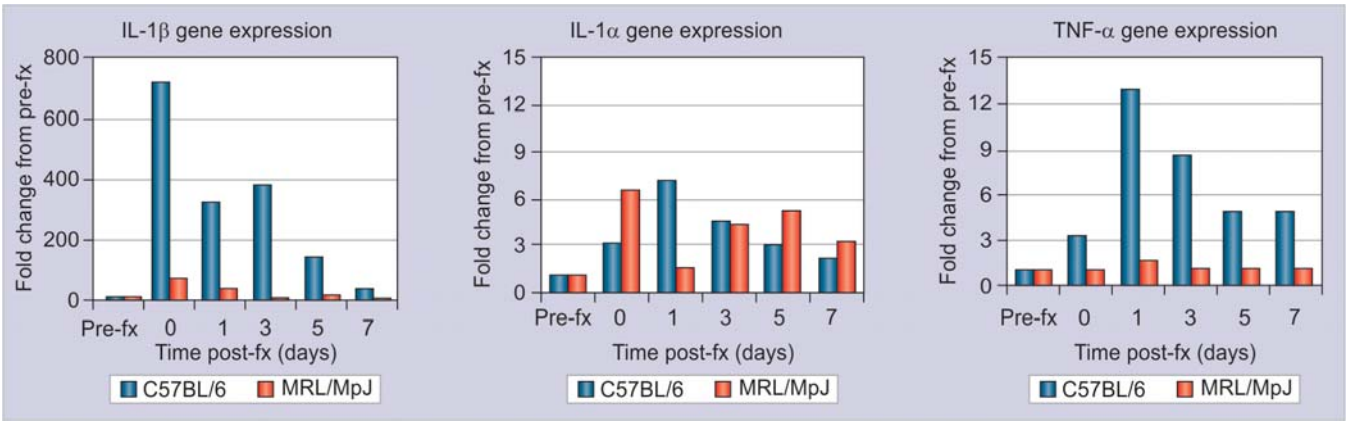

Fig. 1: Gene expression of synovial tissue in C57BL/6 and MRL/MpJ mice following articular fracture of the knee compared to prefracture (3-fold higher or lower relative mRNA expression considered significant)

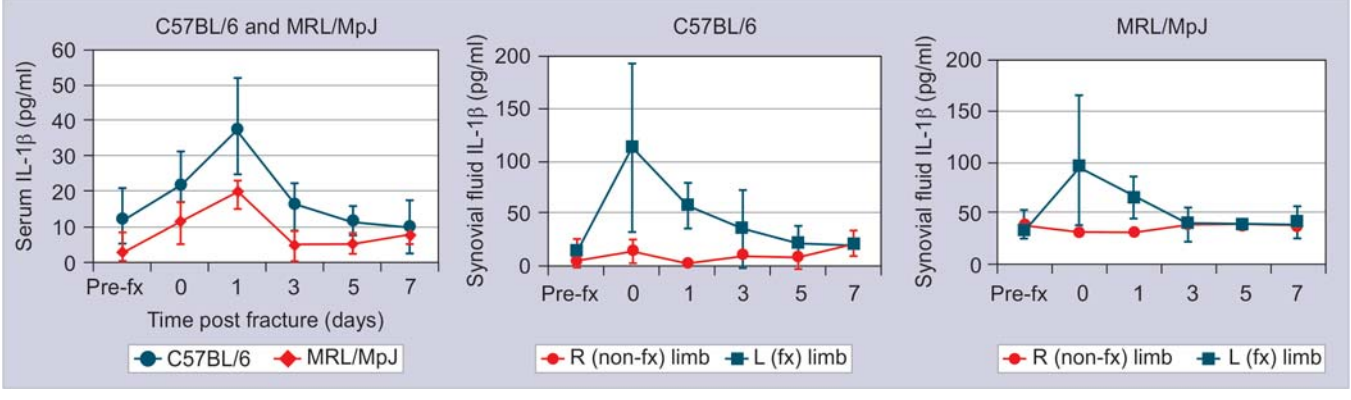

Fig. 2: IL-1β (left) serum levels of IL-1β in C57BL/6 and MRL/MpJ (middle) C57BL/6 and (right) MRL/MpJ synovial fluid levels of IL-1β for right non-fractured (non-fx) and left fractured (fx) limbs

Cellular and Molecular Evidence for the Role of Reduced Joint Inflammation in the Prevention of Post-traumatic Arthritis in MRL/MpJ mice: The objective of this study was to examine cellular and molecular differences between the MRL/MpJ and C57BL/6 strain that may explain the absence of post-traumatic arthritis in MRL/MpJ mice. MRL/MpJ (n = 36) and C57BL/6 (n = 36) mice were subjected to simple fractures with our protocol. Mice were sacrificed prior to fracture (pre-fx), and at 0, 1, 3, 5 and 7 days postfracture. At sacrifice, serum from each animal, synovial fluid (SF) and joint capsule tissue from both hind limbs of each animal were harvested.

- MRL/MpJ mice showed significantly reduced intra-articular and systemic inflammation following articular fracture compared to C57BL/6 mice.
- C57BL/6 mice showed greater significant increases in synovial gene expression of IL-1β and TNF-α (Fig. 1) following articular fracture.
- IL-1β levels were elevated following articular fracture in a pattern indicating first a local intra-articular response followed by a systemic elevation in the serum (Fig. 2).
- IL-1α levels were elevated following fracture. The response appears to be systemic in nature as serum levels rise first, followed by elevated levels in synovial fluid from both knee joints.

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