The Impact of Anterior Cruciate Ligament Injury on Lubricin Metabolism and the Effect of Inhibiting Tumor Necrosis Factor α on Chondroprotection in an Animal Model

K. A. Elsaid,1 J. T. Machan,2 K. Waller,1 B. C. Fleming,1 and G. D. Jay1

Objective. To examine the effects of anterior cruciate ligament transection (ACLT) in a rat model on lubricin metabolism and its relationship to markers of inflammation and cartilage damage, and to determine whether blocking the metabolic effects of tumor necrosis factor α (TNFα) by etanercept increases the chondroprotection provided by lubricin.

Methods. Unilateral ACLT was performed in Lewis rats. Levels of lubricin, TNFα, interleukin-1β (IL-1β), and sulfated glycosaminoglycans (sGAG) in synovial fluid (SF) lavage specimens and synovial tissue lubricin gene expression were evaluated at 1 week and 4 weeks following ACLT. Histologic evaluation of articular cartilage included staining with lubricin-specific monoclonal antibody 9G3 and Safranin O. The percentage of lubricin staining on the surface of articular cartilage in weight-bearing areas was estimated by digital imaging. Blocking of TNFα was performed using etanercept, which was administered subcutaneously at a dose of 0.5 mg/kg around the ACL-transected joints, using different dosing strategies. The ACL-transected and contralateral joints of these rats were harvested 4 weeks following surgery.

Results. Four weeks following ACLT, SF lubricin concentrations and the percentage of cartilage surface lubricin staining were significantly lower in the injured joints compared with the contralateral joints. A significant decrease in synovial tissue lubricin gene expression was associated with elevated TNFα and IL-1β concentrations in SF lavage samples. With all of the etanercept treatment strategies, blocking of TNFα significantly increased the amount of lubricin bound to cartilage, coupled with a significant decrease in sGAG release. However, changes in the concentrations of lubricin in SF were variable.

Conclusion. Blocking TNFα resulted in a chondroprotective effect, exemplified by increased lubricin deposition on articular cartilage and a decrease in sGAG release from articular cartilage in an animal model of posttraumatic arthritis.

Acute anterior cruciate ligament (ACL) injury is a significant risk factor for the development of secondary osteoarthritis (OA) (1,2). Many contributing factors such as joint instability, changes in kinematics, and tissue degradation pathways are postulated to play a significant role in the pathogenesis of OA following injury. Recently, our group reported that lubricin concentrations (3–5) in synovial fluid (SF) from the injured joints of patients with ACL injury were significantly lower than the concentrations in SF from the uninjured contralateral joints (6). Animal models of meniscectomy (7), medial collateral ligament and ACL disruption (8), and adjuvant-induced arthritis (9) have all shown an association of cartilage degeneration with a decrease in either SF or cartilage-bound lubricin. Lubricin gene expression is differentially regulated by a variety of cytokines, including tumor necrosis factor α (TNFα) (10), interleukin-1 (IL-1) (11), and transforming growth factor β (12), and by bone morphogenetic protein 7 (13), which can impact chondroprotection following recovery from an ACL injury (6).

The current study had 2 objectives. The first objective was to establish the early effects of acute ACL injury on lubricin metabolism and the resultant effects
on cartilage integrity and lubrication in the rat model. The second objective was to determine whether treatment with etanercept, a soluble TNFα inhibitor, could preserve lubricin and cartilage integrity. We hypothesized that early inhibition of TNFα would increase lubricin concentrations in the SF and on the surface of articular cartilage, providing better chondroprotection. Lubricin concentrations in SF lavage specimens, whole joint coefficient of friction values, sulfated glycosaminoglycan (sGAG) levels, and lubricin deposition on the surface of articular cartilage, as determined by histologic staining, were measured at 1 week and 4 weeks following ACL transection (ACLT) and following etanercept administration. Etanercept was administered as follows: every other day immediately following ACLT for 2 weeks (treatment A), on postoperative days 7 and 14 (treatment B), or on postoperative days 14 and 21 (treatment C). Lubricin gene expression in synovial tissue from ACL-transected and contralateral joints and TNFα and IL-1β concentrations in SF lavage samples from ACL-transected and contralateral joints at 1 week and 4 weeks following ACLT were determined. These measurements were correlated with concentrations of lubricin in SF lavage specimens, lubricin gene expression in synovial tissue, and the intensity of lubricin staining on the surface of articular cartilage.

MATERIALS AND METHODS

ACLT animal model. Twelve male Lewis rats (7–8 weeks of age) were assigned to an ACLT group, and another 12 were assigned to a sham group that underwent capsulotomy without disturbing the ACL. In both the ACLT and sham groups, the knee joints from 6 rats were harvested at 1 week (week 1 group), and the knee joints of the other 6 rats were harvested at 4 weeks (week 4 group). After the rats were anesthetized with intraperitoneally administered ketamine and xylazine, the skin was prepped with a topical antiseptic, and an incision was made in the skin laterally to the right knee joint. After the joint capsules were opened, the ACL was transected using a surgical scalpel. In all rats, the right knee joint underwent surgery, and the left knee was designated the contralateral joint. Sham surgery was performed in the same manner as the ACLT surgery, without transecting the ACL. All experimental procedures were approved by the Rhode Island Hospital Animal Welfare Committee.

Etanercept dosing. In a separate set of experiments, 24 rats underwent ACLT as described above. Eighteen rats received etanercept according to 3 dosing strategies (6 in each group), and 6 rats did not receive etanercept. The ACL-transected and contralateral joints of these rats were harvested 4 weeks following surgery. Etanercept was administered at a dose of 0.5 mg/kg by subcutaneous injection around the ACL-transected joints. Three different treatment regimens were used. In rats receiving treatment A, etanercept was administered 1, 3, 5, 7, 9, 11, and 13 days following ACLT. In rats receiving treatment B, dosing was performed only on days 7 and 14 following ACLT, and in rats receiving treatment C, dosing was performed only on days 14 and 21 following ACLT. As a measure of the effect of etanercept, the difference between the ACL-transected and contralateral limbs of etanercept-treated rats was compared with the difference between these limbs in rats in the week 4 ACLT group.

Histologic processing. Paraffin-embedded coronal sections were obtained from weight-bearing areas of the articular cartilage of the ACL-transected and contralateral joints of the rats described above. Histologic staining was performed using lubricin-specific monoclonal antibody 9G3 at 1:1,000 dilutions and using Safranin O–fast green stain for qualitative assessment of cartilage sGAG content.

Analysis of lubricin bound to the surface of articular cartilage. Representative sections of ACL-transected and contralateral joints, stained with monoclonal antibody 9G3, were imaged with an Olympus BX51 system (Olympus, Center Valley, PA) at 200× magnification. Images were captured using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD) with predetermined threshold parameters for lubricin staining. Regions of interest included surfaces of the tibial plateau and the femoral condyle cartilage between the tip of the meniscus and the origin of the cruciate ligaments in both joint compartments. The length of a contour drawn to follow the surface of the articular cartilage in the region of interest was calculated. The segmental length of lubricin immunopositivity on that same contour was calculated using Image-Pro Plus and was expressed as a percentage of the total length of normal histologic immunostaining.

Determination of whole joint coefficient of friction values. The whole joint coefficient of friction value was determined using a modified Stanton pendulum configuration, as previously described (9). The whole joint coefficient of friction value was determined for the ACL-transected and contralateral joints of rats in the week 1 and week 4 groups as well as etanercept-treated rats.

Lubricin concentrations in SF lavage specimens. Joints were lavaged by injecting 100 μl of normal saline in the joint capsule, then aspirating 30 μl of fluid. A sandwich enzyme-linked immunosorbent assay (ELISA) using peanut agglutinin (PNA; EY Laboratories, San Mateo, CA) and monoclonal antibody 9G3 was used to quantitate lubricin concentrations in SF lavage specimens. High-binding 96-well plates (Costar; Cole-Parmer, Vernon Hills, IL) were coated overnight with PNA in 50 mM sodium bicarbonate buffer, pH 9.5, at a final concentration of 10 μg/ml. Twenty-four hours following coating, serial dilutions of purified human lubricin (6) and diluted lavaged SF samples were incubated on the PNA-coated plates for 60 minutes at room temperature. The plate was then washed with phosphate buffered saline (PBS) plus 0.1% Tween 20. Subsequently, monoclonal antibody 9G3 was added at 1:10,000 dilution and incubated for 60 minutes at room temperature, followed by washing with PBS plus 0.1% Tween 20. Peroxidase-conjugated goat anti-mouse IgG (1:1,000 dilution; Invitrogen, Carlsbad, CA) was added to the plate for 60 minutes, followed by washing with PBS plus 0.1% Tween 20 and then with PBS alone. Finally, tetramethylbenzidine reagent (Pierce, Rockford, IL) was added, and the absorbance was measured at 450 nm.

Quantification of sGAG, IL-1β, and TNFα in SF lavage samples. Quantification of the sGAG concentrations in SF lavage specimens from ACL-transected and contralateral
joints was performed using an Alcian blue binding assay (Alpeco Diagnostics, Windham, NH). The assay was based on the formation of an insoluble blue-colored complex between sGAG and Alcian blue that was quantified at a 590-nm absorbance wavelength. The sGAG concentrations were determined using serially diluted chondroitin 6-sulfate as a standard. Quantification of TNFα and IL-1β in rat SF lavage samples was performed using commercially available ELISA kits (Invitrogen). The reported minimum detection limits were 0.3 pg/ml and 0.1 pg/ml, respectively.

**Isolation of total RNA from the synovial tissue of ACL-transected and contralateral joints and real-time quantitative polymerase chain reaction (PCR) assay.** Synovial tissue specimens were removed from the ACL-transected and contralateral joints at 1 week and 4 weeks following ACLT (n = 6 at each time point) and were quickly snap-frozen in liquid nitrogen and stored at −80°C. Isolation of total RNA, conversion to complementary DNA, and quantitative PCR analysis of lubricin gene expression using primers complementary for exon 6 of the mucin domain in accession no. NW_047397 (National Center for Biotechnology Information, Bethesda, MD) were performed as previously described (9).

**Statistical analysis.** All statistical analyses were conducted using SAS software version 9.1.3 (service pack 4; SAS Institute, Cary, NC). Because the distributions of several variables were positively skewed, a base 2 logarithmic transformation of the coefficient of friction value, and the sGAG, IL-1β, and TNFα concentrations was applied prior to analysis. The values, means, and confidence intervals were back-translated for presentation.

Effects were tested by comparing the group differences in the ACL-transected and contralateral limb differential to adjust for overall differences between rats. The contralateral limb was used as a reference corrected for any innate differences between rats or day-to-day variation in instrumentation, thus decreasing the potential for bias and increasing statistical power. These comparisons were conducted using mixed linear models with correlated errors, similar to repeated-measures analysis of variance (ANOVA), containing fixed effects for group, limb (correlated errors within rat), and their interaction. An unstructured variance–covariance matrix was used for the correlated errors, and the model was fit using residual estimation of maximum likelihood. The Kenwood-Rogers method was used to calculate denominator degrees of freedom. Least-squares means within groups and their 95% confidence intervals are reported. When data from contralateral limbs were not available, these models were reduced to standard ANOVA or regression models. Planned comparisons were conducted using orthogonal contrasts to test the limb differentials within each group against zero, as well as comparing the differentials between all possible pairings of groups. Family-wise alpha was maintained at 0.05 across all planned comparisons, using the Holm’s test.

The relationship between observed measures of lubricin and coefficient of friction were also tested in correlated error (within animal across limbs) mixed linear models. Initially, a fixed effect for limb and its interaction with the lubricin covariate was run. If the interaction term was statistically significant, the relationships were tested within ACL-transected and contralateral limbs individually. If the interaction was not statistically significant, the fixed effects of limb and the interaction of limb with the covariate were removed, leaving only the fixed effect of lubricin and the correlated within-animal error.

**RESULTS**

**ACLT model in rats and effects on lubricin metabolism.** Safranin O and lubricin immunostaining of a representative ACL-transected joint and the contralateral joint at 1 week and 4 weeks following ACLT is depicted in Figure 1A. The ACL-transected and contralateral joints exhibited staining for proteoglycans (Figure 1, panels a and b, respectively) at 1 week following ACLT. Lubricin immunostaining in the ACL-transected joint at 1 week following ACLT exhibited immunopositivity on the surface of articular cartilage, with reduced staining in the superficial zone of articular cartilage (Figure 1A, panel c). The contralateral joint at 1 week following ACLT exhibited immunopositivity on the surface of articular cartilage and in the superficial zone of articular cartilage (Figure 1A, panel f). The ACL-transected joint at 4 weeks showed decreased proteoglycan staining (Figure 1A, panel e) compared with the contralateral joint (Figure 1A, panel d). There was no lubricin immunostaining either on the surface of articular cartilage or in the superficial zone of articular cartilage in the ACL-transected joint at 4 weeks following ACLT (Figure 1A, panel g). In contrast, the contralateral joint exhibited positive lubricin immunostaining on the articular cartilage surface and superficial zone (Figure 1A, panel h).

The lubricin concentration in SF lavage samples from the ACL-transected joints of rats in the week 1 group was significantly lower (adjusted \( P < 0.001 \)) compared with that in the contralateral joints of rats in the week 1 group (Figure 1B). The concentration of lubricin in SF lavage samples derived from the ACL-transected joints of rats in the week 4 group was significantly lower (adjusted \( P < 0.001 \)) than that in samples from the contralateral joints of rats in the same group. There was no significant difference between week 1 and week 4 after adjustment for multiple comparisons (adjusted \( P = 0.0605 \)). At both week 1 and week 4, the effects exhibited by rats in the ACLT group were larger than those exhibited by their sham-operated controls (adjusted \( P < 0.001 \) for both); in both groups, no significant between-limb differences were observed.

The percentage of lubricin staining on the surface of select cartilage areas of ACL-transected and contralateral joints at 1 week and 4 weeks is shown in Figure 1C. At 1 week following ACLT, lubricin staining was significantly lower (adjusted \( P < 0.001 \)) in the ACL-transected joint than in the contralateral joint. At 4 weeks following ACLT, lubricin staining remained sig-
significantly lower (adjusted $P < 0.001$) in the ACL-transected joint compared with the contralateral joint. Additionally, lubricin staining in the ACL-transected joints at 1 week was significantly higher (adjusted $P = 0.0002$) than that in the ACL-transected joints at 4 weeks, after adjusting for the contralateral limbs. At both week 1 and week 4, the effects exhibited by rats in the ACLT group were larger than those exhibited by their sham-operated controls (adjusted $P < 0.001$ for both); in both groups, no significant between-limb differences were observed.

The sGAG concentrations in SF lavage samples from ACL-transected and contralateral joints at 1 week and 4 weeks following ACLT are shown in Figure 1D. The concentrations in samples from ACL-transected joints were significantly higher than those in samples from the contralateral joints of rats in both the week 1 and week 4 groups (adjusted $P = 0.0003$ and adjusted $P < 0.0001$, respectively), with a statistically significantly greater fold increase in the week 4 group (adjusted $P = 0.0099$).

No TNFα or IL-1β was detectable in the contralateral joints at either week 1 or week 4. In ACL-transected joints, TNFα and IL-1β concentrations were significantly higher ($P < 0.0001$ for both) at week 1 compared with week 4 (Figure 2A). Lubricin messenger RNA (mRNA) expression in synovial tissue from ACL-transected joints relative to that in synovial tissue from the contralateral joints at 1 week and 4 weeks following ACLT is shown in Figure 2B. In control rats, there was no difference in lubricin gene expression between sham-operated and contralateral joints, as represented by a ratio of 1.0. At 1 week following ACLT, lubricin mRNA expression in the ACL-transected joints relative to that in the contralateral joints was significantly lower (adjusted $P < 0.001$) compared with that in control joints. Similarly, at 4 weeks following ACLT, the ratio of lubricin mRNA expression in the ACLT joints to that in

Figure 1. Rat model of anterior cruciate ligament transection (ACLT) at 1 week and 4 weeks following transection. A, Safranin O (a–d) and lubricin immunostaining using lubricin-specific monoclonal antibody 9G3 (e–h) of ACL-transected and contralateral (CL) joints of representative rats at 1 week and 4 weeks following ACLT. Panels a and e show ACL-transected joints at 1 week; panels b and f show the contralateral joints at 1 week. Panels c and g show ACL-transected joints at 4 weeks; panels d and h show the contralateral joints at 4 weeks. Arrows indicate decreased proteoglycan staining in the ACL-transected joint at 4 weeks (c), lubricin staining on the surface of articular cartilage in the ACL-transected joint at 1 week (e), and lack of lubricin staining on the surface of articular cartilage in the ACL-transected joint at 4 weeks (g). B, Lubricin concentrations in synovial fluid lavage specimens from ACL-transected and contralateral joints at 1 week and 4 weeks following transection. C, Percentage of lubricin staining of select cartilage surfaces of ACL-transected and contralateral joints at 1 week and 4 weeks following transection. D, Sulfated glycosaminoglycan (sGAG) concentrations in synovial fluid lavage specimens from ACL-transected and contralateral joints at 1 week and 4 weeks following transection. Bars in B–D show the mean ± SD. * = $P < 0.001$ versus CL.
the contralateral joints was significantly lower (adjusted \(P < 0.001\)) than that same ratio in control joints.

The variability in whole joint coefficient of friction measures was high. The whole joint coefficient of friction value for the ACL-transected limbs at 4 weeks was \(\sim 36\%\) higher than that for the contralateral limbs (adjusted \(P = 0.0247\)). By comparison, the whole joint coefficient of friction value for ACL-transected joints at 1 week following ACLT was \(\sim 11\%\) higher than that for the contralateral joints, but this difference did not reach statistical significance (adjusted \(P = 1.0\)), nor did the value differ from that at week 4 (adjusted \(P = 0.9590\)). The whole joint coefficient of friction value for the limbs from sham-operated controls in the week 1 and week 4 groups did not differ (adjusted \(P = 1.0\) and adjusted \(P = 0.8852\), respectively), nor did the value in rats that underwent ACLT differ from that in their sham-operated controls (adjusted \(P = 1.0\) and adjusted \(P = 0.4255\), respectively).

The TNF\(\alpha\) concentrations in SF lavage specimens at week 1 and week 4 following ACLT were significantly negatively related to lubricin gene expression (by quantitative PCR analysis) in synovial tissue from these joints (\(P = 0.006\), adjusted \(R^2 = 0.594\)) (Figure 2C). Similarly, the IL-1\(\beta\) levels at weeks 1 and 4 following ACLT were significantly negatively related to...
lubricin expression in tissue from these joints ($P = 0.0001$, adjusted $R^2 = 0.833$) (Figure 2C). Conversely, there were no significant relationships either between TNFα concentrations in SF lavage specimens and lubricin expression ($P = 0.35$) or between IL-1β levels in SF lavage specimens and lubricin expression ($P = 0.632$). There was a significant positive relationship between the percentage of lubricin staining and IL-1β concentrations in SF lavage specimens ($P = 0.0005$, adjusted $R^2 = 0.817$) and between the percentage of lubricin staining and TNFα concentrations in SF lavage specimens ($P = 0.0002$, adjusted $R^2 = 0.852$) (Figure 2D).

**Effect of etanercept on lubricin metabolism.** The lubricin concentrations in SF lavage specimens from the ACL-transected joints of both treated (treatments A, B, and C) and untreated rats were significantly lower (adjusted $P < 0.001$) than those in specimens from the contralateral joints (Figure 3A). When adjusted for
lubricin SF concentrations in the contralateral joints, the SF lubricin concentrations in the treatment A group were significantly higher (adjusted \( P = 0.021 \)) than those in the untreated ACLT group. Similarly, SF lubricin concentrations in the treatment B group were significantly higher (adjusted \( P = 0.025 \)) than those in the untreated ACLT group. There were no differences in SF lubricin concentrations between the treatment C group and the untreated ACLT group (adjusted \( P = 1.0 \)). There was no significant difference (adjusted \( P = 0.762 \)) between the adjusted SF lubricin concentrations in rats receiving treatment A and those receiving treatment B.

The percentage of lubricin staining on the surface of articular cartilage from the ACL-transected joints of rats receiving treatment A and untreated rats was significantly lower (adjusted \( P < 0.001 \)) than that on cartilage from the contralateral joints, while no significant difference in the percentage of lubricin staining between treatments B or C and the contralateral joints were observed (Figure 3B). When adjusted for the percentage of lubricin staining in the contralateral joints, the percentages of lubricin staining after treatments A, B, and C were significantly higher (adjusted \( P < 0.001 \)) than that in untreated ACL-transected joints. The percentages of lubricin staining in rats receiving treatments B and C were significantly higher (\( P < 0.001 \)) than that in rats receiving treatment A, with no significant difference in the percentages between treatments B and C.

The sGAG concentrations in SF lavage specimens from the ACL-transected joints of untreated and etanercept-treated rats are shown in Figure 3C. The sGAG concentrations in SF lavage specimens from rats receiving treatment A (adjusted \( P < 0.0001 \)), treatment B (adjusted \( P = 0.0001 \)), and treatment C (adjusted \( P = 0.0055 \)) were significantly lower than those in specimens from the ACL-transected joints of untreated rats. The sGAG concentrations in the treatment A group were significantly lower than those in the treatment C group.
(adjusted \( P = 0.0117 \)), and the concentrations in the treatment B group did not differ significantly from those in either the treatment A group (adjusted \( P = 0.1358 \)) or the treatment C group (adjusted \( P = 0.1448 \)).

**Relationships between whole joint coefficient of friction values, the percentage of cartilage surface lubricin staining, and lubricin concentrations in SF lavage specimens from ACL-transected and contralateral joints.** The relationship between cartilage surface lubricin staining and the concentration of lubricin in SF lavage specimens from the ACL-transected joints of rats that were or were not treated with etanercept is shown in Figure 4A. The contralateral joints of the rats in both of these groups exhibited the combination of a high percentage of lubricin staining and high concentrations of lubricin in SF lavage specimens. In contrast, the untreated ACL-transected joints exhibited a combination of low lubricin staining and low lubricin concentrations in SF lavage specimens. None of the etanercept-treated joints fully recapitulated lubricin in both locations to the levels observed in the contralateral joints. However, rats in the treatment B and treatment C groups demonstrated more lubricin staining compared with rats receiving treatment A, which had higher lubricin concentrations in SF lavage specimens compared with rats receiving treatment C.

The relationships between the percentage of cartilage surface lubricin staining and lubricin concentrations in SF lavage samples as a function of coefficient of friction values for the ACL-transected and contralateral joints of etanercept-treated and untreated animals are depicted in Figure 4B. The lowest coefficient of friction values clustered around a combination of high lubricin staining of cartilage and high concentrations of lubricin in SF lavage samples. The significant negative relationship between lubricin concentrations in SF lavage specimens and sGAG concentrations is shown in Figure 4C (\( P = 0.0095 \)). The relationship between the percentage of cartilage surface lubricin staining and sGAG concentrations in SF lavage specimens is presented in Figure 4D. There was a statistically significant negative relationship between sGAG concentrations in SF lavage specimens and lubricin staining of the cartilage surface in the ACL-transected limbs (regression line and 95% confidence interval plotted adjusted \( P = 0.015 \) but not in the contralateral limbs (adjusted \( P = 0.074 \)).

**DISCUSSION**

ACL injury is an acute traumatic injury leading to an increased risk of the long-term development of degenerative joint diseases. Following ACL injury, SF concentrations of proinflammatory cytokines such as IL-1\( \beta \), TNF\( \alpha \), and IL-6 have been shown to be highest within 24 hours (14) and associated with an increase in the levels of SF proteoglycans (15). These findings corroborated our study of SF from patients with an acute unilateral ACL injury (6).

In the present study, we examined the time course of the impact of an ACL injury on cartilage chondroprotective abilities. Following an ACL injury in a rat model, an early increase in the levels of proinflammatory cytokines in the SF was detected at week 1 and also at week 4 (Figure 1). The elevated levels were significantly related to decreased levels of synovial tissue lubricin gene expression and with lubricin deposition on the articular cartilage surface. This association is consistent with previous reports that the proinflammatory cytokines TNF\( \alpha \) and IL-1 significantly decrease lubricin gene expression in synovocytes and superficial zone chondrocytes (10,11). However, the detection of larger quantities of lubricin on the cartilage surface as the levels of IL-1\( \beta \) and TNF\( \alpha \) increased (Figure 2D) suggests that lubricin that is already present on the surface has a long half-life. This has been observed in other investigations in which interaction of labeled lubricin with the cartilage surface increased its half-life to 6.3 days (16).

At 1 week following ACLT, concentrations of lubricin in SF lavage samples were significantly lower in the ACL-transfected joints than in the uninjured joints (Figure 1C). This decrease was also paralleled with a significant decrease in lubricin deposition on weight-bearing areas of cartilage surfaces. These results, coupled with the observation that the coefficient of friction value was significantly elevated in the ACL-transsected limb relative to the contralateral limb in the untreated ACLT group at week 4 (worst damage), indicate compromised joint lubrication at a relatively early stage following an ACL injury. The compromised lubrication may be attributable to a significant reduction in lubricin gene expression leading to decreased SF lubricin concentrations and decreased lubricin deposition on the articular cartilage surface. These results corroborate earlier findings that demonstrated an early decrease in SF lubricin concentrations in joints with ACL injuries compared with uninjured joints in humans (6) and another animal model (8).

Etanercept is a soluble TNF\( \alpha \) receptor that competes with endogenous TNF receptors for binding to TNF\( \alpha \), resulting in attenuation of the effects of TNF\( \alpha \). Along with other TNF\( \alpha \)-targeted therapies, etanercept has significantly advanced the treatment of rheumatoid arthritis (17–20). Clinically, etanercept has been used in
other clinical conditions in which TNFα plays a significant role in pathogenesis (21,22). Dosing schedules of etanercept were aimed to examine the effects of early versus late blocking of the effects of TNFα as well as the effects of high-dose versus low-dose etanercept. Given the effect of TNFα on lubricin expression (10), we anticipated that etanercept administration would increase the levels of lubricin in both SF and cartilage. Across all treatment strategies, the level of cartilage-associated lubricin in the injured joints was higher than that in the joints of untreated animals. However, elevations in the levels of lubricin in SF lavage samples were variable. Although treatments A and B resulted in higher levels of lubricin in SF lavage samples than those in samples from untreated joints, treatment C did not result in any significant difference in the levels of lubricin in SF lavage samples (Figure 3A). Furthermore, the percentage of cartilage surface staining for lubricin was higher in rats that received treatments B and C compared with treatment A (Figure 3B).

Taken together, these results indicate that blocking the effects of TNFα leads to an increase in total lubricin in the joint, suggesting an improved chondroprotective activity. Treatment B may have provided the best combination of an increased concentration of lubricin in SF lavage specimens and an increased percentage of cartilage surface lubricin staining, though whole joint lubrication was still compromised.

The relationship between SF and cartilage lubricin is complex and is not entirely understood (23). Nugent-Derfus et al (24) examined the effect of bathing fluid conditions on the lubricin concentration at the articular surfaces and concluded that the articular surface lubricin concentration was independent of the lubricin concentration in the fluid phase. Following removal of lubricin from the surface of articular cartilage, lubricin in the fluid phase saturated the articular surface. These results may be consistent with findings from our model, in which at 1 week following injury, the decrease in lubricin was greater in the SF than at the surface of articular cartilage (Figure 1). As the posttraumatic period progressed, the level of surface lubricin decreased and was associated with cartilage damage. Conversely, in etanercept-treated joints, lubricin on the surface of articular cartilage was replenished independently of or preferentially to SF lubricin.

ACL injuries, meniscus tears, and meniscectomies are examples of acute joint injuries that lead to the development of OA and involve down-regulation of lubricin expression as a part of the disease process (7,25). Relevant to these observations is the fact that meniscus cells also secrete lubricin (26). Intraarticular lubricin supplementation has been advocated as a potential new therapeutic modality for these conditions (27,28). In our study, inhibiting the effects of TNFα was investigated as an alternative approach to intraarticular lubricin supplementation. To the extent of the measured effects, TNFα inhibition increased the levels of both SF and cartilage surface lubricin, and it also decreased proteoglycan release from articular cartilage.

At an early stage following an acute ACL injury, lubricin gene expression is down-regulated by the effects of the proinflammatory cytokines IL-1β and TNFα. This down-regulation results in decreased lubricin concentrations in SF and on the surface of articular cartilage, compromising joint lubrication and increasing the risk of wear-induced damage that may exceed the limited regenerative ability of cartilage. Early inhibition of the effects of TNFα restores lubricin in the SF and on the surface of articular cartilage, lowers whole joint coefficient of friction values, and limits cartilage damage. We acknowledge that in this model it is difficult to differentiate this effect of TNFα inhibition from more general degradative enzyme activity upon cartilage itself. In assessing lubricin staining, areas were selected that were likely weight-bearing regions and unsupported by menisci. However, because the entire articular cartilage surface was not examined for lubricin coverage, the results should not be generalized to the entire surface. The lack of surface lubricin in some joints served to validate the lack of recoverable lubricin in SF lavage samples. Due to the method of quantification, we did not factor in the intensity of lubricin staining at areas of measurement but relied solely on the segmental length of the immunostained surface.

ACKNOWLEDGMENTS

We thank Dr. Mathew Warman from Boston Children’s Hospital for providing lubricin-specific monoclonal antibody 9G3 and Ms Margaret Case for reviewing the manuscript.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Jay had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Elsaid, Fleming, Jay.

Acquisition of data. Elsaid, Fleming, Jay.

Analysis and interpretation of data. Elsaid, Machan, Waller, Fleming, Jay.
REFERENCES


