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The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse

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Summary

Objective: To evaluate anterior cruciate ligament transection (ACLT) and destabilization of the medial meniscus (DMM) surgical instability models of osteoarthritis (OA) in the 129/SvEv mouse knee joint.

Design: Micro-surgical techniques were used to perform ACLT or DMM under direct visualization. Histological scoring was performed on multiple sections to assess cartilage damage across the entire joint.

Results: The ACLT model gave severe OA, chondrogenesis of the joint capsule and, in some cases, severe subchondral erosion of the posterior tibial plateau. Surgical DMM was less invasive than the ACLT procedure and resulted in lesions primarily on the central weight-bearing region of the medial tibial plateau and medial femoral condyles. Lesions in the DMM model progressed from mild-to-moderate OA at 4 weeks, to moderate-to-severe OA at 8 weeks post-surgery. Destruction of the subchondral bone was never observed in the DMM model.

Conclusions: ACLT is not recommended in the mouse due to the high surgical proficiency required and the development of severe OA that may involve subchondral bone erosion. The severity and location of lesions following DMM are consistent with lesions observed in aged spontaneous mouse models of OA. The DMM model has sufficient sensitivity to show disease modification, as observed with the ADAMTS-5 knock out (KO) mouse. The DMM model should be a first choice to challenge mice with gene deletions of potential targets in OA.
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Key words: Animal models, Cartilage, Mouse, Surgery, Instability, Knee, Histology.

Introduction

Osteoarthritis (OA) is a slow progressing disease resulting in articular cartilage fibrillation and loss. Anterior cruciate ligament rupture results in altered biomechanics and joint instability, leading to OA 10−15 years later[1,2] in humans. Surgical instability models are the most common models of OA in laboratory animals, although it is unclear how well instability models resemble the predominantly idiopathic OA in the older human population. Models of OA utilizing surgical instability are widely accepted in dog[3], guinea pig, rabbit, rat, sheep and goat[4,5]. Advantages of surgical models over spontaneous models include a faster onset of disease, decreased variability, and decreased dependence on genetic background.

There are currently no approved disease modifying OA drugs (DMOADs), and therefore no animal model has been found to be predictive for human responses. Given the high cost of maintaining larger species, and their greater drug requirements, smaller animal models are preferred for preliminary screening. The mouse is the primary species for the generation of transgenic animals, including animals with gene deletions and gene over-expression. These features make the mouse the preferred species to characterize the *in vivo* significance of a particular gene. Disadvantages for the use of transgenic mice include long-generation times, great cost and poor accessibility of transgenics arising from other groups. Histologic evaluation of mouse joints is dependent on labor-intensive sectioning throughout the knee. Despite these known limitations, the potential for identifying key enzymes with transgenic animals make evaluation of OA models in mice particularly attractive.

This study demonstrated the feasibility of anterior cruciate ligament transection (ACLT), and destabilization of the medial meniscus (DMM) surgical instability models in the mouse. The ACLT model was evaluated because of its wide use in other species, and the DMM model was selected based on earlier unpublished studies in guinea pigs where this model induced OA with great ease and reproducibility. The severity of OA was compared for both models, with the aim of identifying a mild to moderate model of OA, so that the effects of disease modification with enzyme-deleted mice would not be overwhelmed by the greater biomechanic damage associated with more severe models. In addition, more severe models of OA were considered to have a greater association with iatrogenic damage, variability, biomechanic unloading, or regenerative changes (such as dramatic osteophyte formation or ankylosis) that could hamper the assessment of cartilage degradation. The DMM model provided extremely good reproducibility and a slower progression of disease, and was subsequently selected for evaluation of enzyme-deleted mice, where it

was instrumental in showing that ADAMTS-5, and not ADAMTS-4, was critical for the progression of surgically-induced mouse OA[6,7].

Methods

EXPERIMENTAL ANIMALS

All studies were performed following approval from the Wyeth Institutional Animal Care and Use Committee. Eighty male 129S6/SvEv mice (Taconic, Inc., Germantown, NY) were used since this is a common background strain for knock out (KO) generation, including the ADAMTS-4 and ADAMTS-5 KO mice. The DMM and ACLT groups had at least 10 animals per group, with the no surgery and sham groups having a minimum of six animals per group. Animals were housed in groups of five mice per cage on Alpha-Dri™ (Shepard Specialty Papers, Watertown, TN) bedding in 12.25 × 7.5 × 8 in. high cages (One Cage™ Housing System, Lab Products, Seaford, DE). Mice had free access to feed (Lab Diet # 5001 Rodent Diet, Purina Mills, Richmond, IN) and water (Edstrom Watering System, Edstrom Industries, Wallingford, WI). Mice were anesthetized with 300 mg/kg intra-peritoneal Tribromoethanol (Aldich, Milwaukee, WI) and knees were prepared for aseptic surgery. Buprenorphine (Buprenex®, Reckitt and Coleman Products, Kingston-Upon-Hull, UK) was provided peri-operatively at 0.09 mg/kg. Surgical equipment included a surgical microscope (Leica LZ-6, Leica Microsystems Inc., Bannockburn, IL); Sharpoint® 15° 5 mm blade micro-surgical knife; Absorption Spears (Fine Science Tools Inc., Foster City, CA); micro-iris scissors; micro-surgery needle holders; micro-corneal suturing forceps; Jewelers forceps (Miltex, Inc. York, PA); Vicryl® (Ethicon Inc., Somerville, NJ) suture; Nexaband SC tissue adhesive (Abbott, North Chicago, IL) and # 11 and # 15 blades.

SURGICAL INDUCTION OF INSTABILITY: ACLT AND DMM

The medial meniscotibial ligament (MMTL) (Fig. 1) anchors the medial meniscus (MM) to the tibial plateau, while the
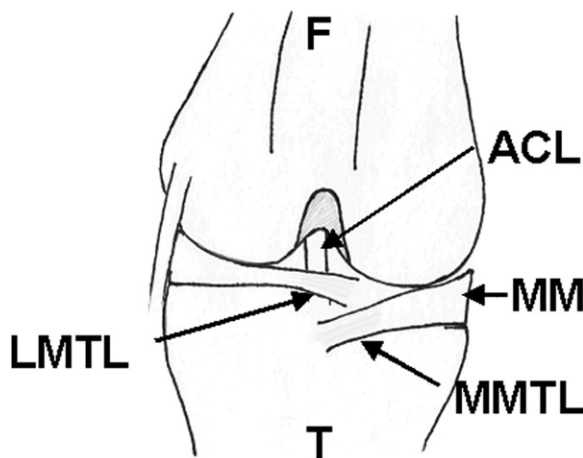

Fig. 1. Diagram of the right knee joint of the mouse. F = femur; T = tibia; MM = medial meniscus; ACL = anterior (cranial) cruciate ligament; MMTL = medial meniscotibial ligament; LMTL = lateral meniscotibial ligament. The MMTL is transected to generate destabilization of the medial meniscus (DMM). The ACL is transected in the ACLT model.

anterior cruciate ligament (ACL) restricts the tibia from moving anteriorly, relative to the femur. The surgical approach for both ACLT and DMM surgeries was similar with a 3 mm longitudinal incision over the distal patella to proximal tibial plateau [Fig. 2(A)]. The joint capsule immediately medial to the patellar tendon was incised with a # 15 blade and the joint capsule opened with micro-iris scissors [Fig. 2(A)]. Blunt dissection of the fat pad over the intercondylar area was then performed to expose either the intercondylar region, providing visualization of the anterior cruciate ligament [Fig. 3(A)] or the meniscotibial ligament of the medial meniscus [Fig. 2(B)]. Mild hemorrhage from the fat pad upon blunt dissection was controlled by pressure from absorption spears. Occasionally, application of one drop of epinephrine 1:1000 (AmVet™, Neogen Corporation, Lexington, KY) would assist with recalcitrant bleeding.

The anterior cruciate ligament (ACL) [Figs. 1 and 3(A–D)] originates from the posterio-lateral aspect of the intercondylar area and inserts anteriorly onto the central tibial plateau. The ACL is lateral to the posterior cruciate ligament (PCL), which was only rarely visualized in our surgical approach, in the posterio-medial intercondylar region. For the ACLT only, the patella was dislocated medially to give greater exposure of the femoral−tibial joint. When the patella was dislocated, the cartilage was kept moist with saline as required. The ACL was transected with a micro-surgical knife under direct visualization, avoiding the PCL, and complete transection confirmed by the presence of anterior drawer.

The medial meniscotibial ligament (MMTL) anchors the medial meniscus (MM) to the tibial plateau [Figs. 1 and 2(B)]. The fat pad over the cranial horn of the medial meniscus was dissected with Jewelers forceps. The MMTL was identified running from the cranial horn of the medial meniscus laterally onto the anterior tibial plateau. Care was taken to identify and avoid the lateral meniscotibial ligament (LMTL), which is posterior and has fibers running in a similar direction [Figs. 1 and 3(A)]. Occasionally there is a small band running between the MMTL and LMTL, and only in that case would it be transected. Sectioning of medial meniscotibial ligament with micro-surgical scissors, micro-surgical knife [Fig. 2(C)] or # 11 blade, with the blade directed proximo-laterally, gave destabilization of the medial meniscus (DMM). With the MM intact, there is a greater congruency and area of contact between the articulating structures, providing a larger region to transmit the weight-bearing forces. Following DMM, medial displacement of the MM [Fig. 2(D)] occurs, and weight bearing is focused across a smaller area, leading to increased local mechanical stress. Since the mouse knee is flexed during weight bearing, this results in greater stress on the posterior femur and central tibia, predominantly on the medial side. Control joints included no surgery and sham surgery in which the ligament was visualized but not transected.

The joint capsule was closed with a continuous 8-0 tapered Vicryl® suture and the subcutaneous layer with 7-0 cutting Vicryl®. The skin was closed by the application of tissue adhesive. Mice had excellent mobility within 2 h after either surgery.

HISTOLOGIC EVALUATION

Mice were euthanized with carbon dioxide at 4 and 8 weeks post-operatively and the knee joints were fixed in 4% paraformaldehyde for 24 h. Whole joints were decalcified in EDTA for 6 days on a shaker. Joints were embedded in paraffin and 6 μm frontal sections were taken through the entire joint at 80 μm intervals. Slides were stained with Safranin-O
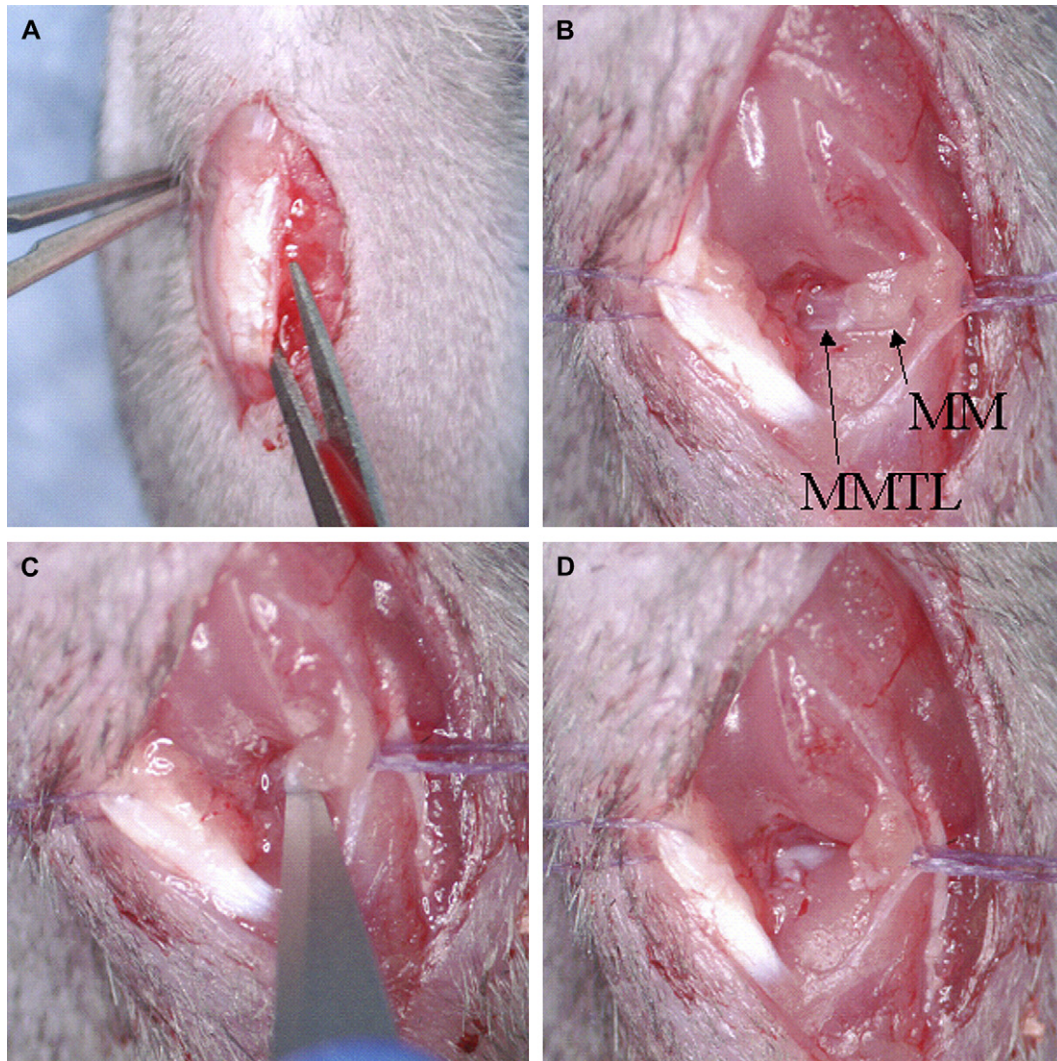
Fig. 2. A: Surgical approach to the right mouse knee joint with a medial para-patellar ligament incision; B: Following fat pad blunt dissection the medial meniscus (MM) and medial meniscotibial ligment (MMTL) are identified; C: Transection of the MMTL; D: Following transection, destabilization of the medial meniscus (DMM) is complete and the meniscus is free to displace medially. Note: Incisions are shown twice as long as usual in order to obtain good quality images.

and Fast green, Masson's Trichome and hematoxylin and eosin. Each knee yielded 13−16 slides for scoring by two blinded observers using a modified semi-quantitative grading scale[8], where 0 represented normal cartilage; 0.5: loss of Safranin-O with no structural lesions; 1: roughened articular surface and small fibrillations; 2: fibrillation below the superficial layer and some loss of lamina; 3: fibrillations extending to the calcified cartilage across less than 20% of the cartilage width; 5: fibrillation and erosions extending from 20 to 80% of the cartilage width; 6: cartilage erosion extending beyond 80% of the cartilage width. Blinded histological scoring was performed on the four quadrants: medial and lateral femoral condyles and medial and lateral tibial plateaus. Results were expressed as the mean, ±standard error of the mean (S.E.M.), of the maximum score or as the sum of all scores. Box and whiskers plots were generated to observe the distribution of scores of the twentififth, fiftitieth (median) and seventififth percentiles (boxed) as well as the total range of the scores (whiskers) (GraphPad Prism® version 4.0 for Windows, GraphPad Software, San Diego,

CA). Statistical analysis was performed with Prism® using a Kruskal−Wallis test with Dunn's post-analysis. In addition to evaluation of cartilage destruction, the joints were also examined for presence or sectioning of the ligaments, chondrogenesis, patellar dislocation, ectopic bone formation and osteophytes.

Results

The post-operative period was unremarkable with no infections developing. At 4 weeks post-operatively, low levels of OA were observed in the no surgery and sham surgery groups utilizing the maximum scores method (scores of 1.1 ± 0.1 and 1.0 ± 0.3 out of a maximal score of 6.0) [Fig. 4(A)]. When the summed score was utilized, both groups showed negligible OA (2.7 ± 0.6 and 1.4 ± 0.6 out of a maximal score over 300) [Fig. 4(C)]. At 8 weeks, the scores were similar (maximum scores of 1.2 ± 0.3 and 1.2 ± 0.2 and summed scores of 4.1 ± 1.9 and 4.9 ± 1.1) for the no surgery
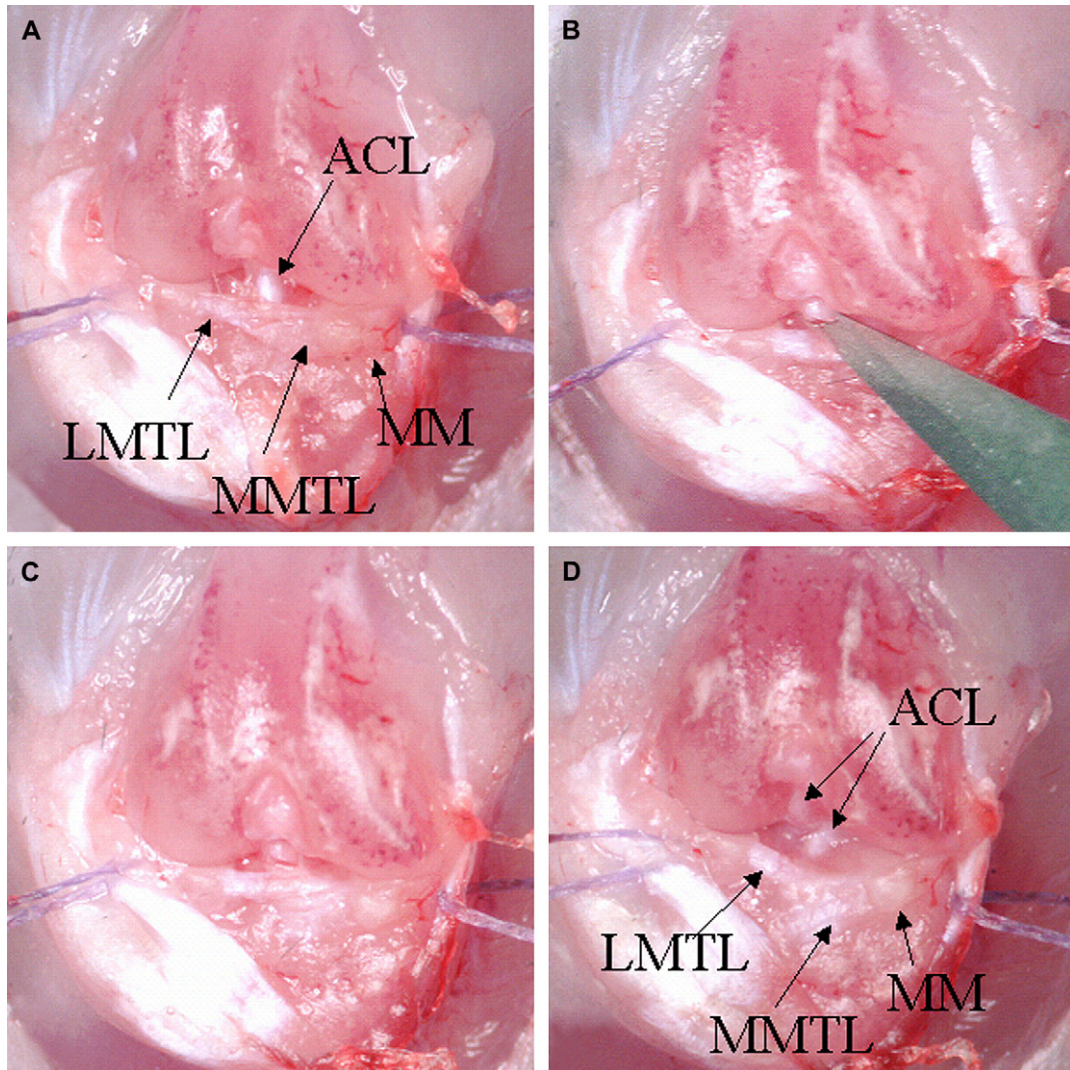
Fig. 3. A: Extensive dissection of the right knee joint to expose the anterior cruciate ligament (ACL), medial meniscus (MM), medial meniscotibial ligament (MMTL) and lateral meniscotibial ligament (LMTL); B: ACL transection; C: ACLT prior to testing anterior drawer; D: ACLT following confirmation of anterior drawer, showing intact MMTL and LMTL.

and sham groups [Fig. 4(B,D)]. Chondrogenesis, erosion and free cells were never observed in the control groups.

The DMM model had significantly more OA than the controls and increased scores were observed, from 4 to 8 weeks, using the maximum score method (3.7 ± 1.5, $P < 0.001$, Fig. 4(A)) and 4.1 ± 0.3, $P < 0.01$, Fig. 4(B)) and the summed score method (25.2 ± 4.7, $P < 0.01$, Fig. 4(C) and 35.7 ± 3.8, $P < 0.01$, Fig. 4(D)). This represented a progression from mild-to-moderate to moderate OA. Lesions were most apparent on the medial side of the joint on both the femur and tibia, but slightly more common on the anterio-central tibia [Fig. 5(A–D)]. Chondrogenesis, patellar dislocation, free cells in the synovial cavity and ectopic bone formation were not observed in this model.

The most severe OA was observed in the ACLT group, with maximum scores of 4.3 ± 0.4 [Fig. 4(A)] and 5.0 ± 0.4 [Fig. 4(B)], and summed scores of 37.2 ± 7.1 [Fig. 4(C)] and 58.0 ± 10.6 [Fig. 4(D)] at the 4- and 8-week time points, respectively. The ACLT group was significantly different from the no surgery and sham surgery groups ($P < 0.01$), but did not demonstrate a significant

statistical difference to the DMM group. Destruction of the posterior tibial plateau through the subchondral bone to the growth plate was not observed at 4 weeks but was observed in 30% of the 8-week knees [Fig. 6(A,B)]. In knees with destruction of subchondral bone, free bone marrow cells could be observed within the synovial cavity (not shown). Chondrogenesis and proteoglycan deposition were prominent on the medial and lateral aspects of the joint. Ectopic bone formation in the medial joint capsule was not present at 4 weeks, but was present in 40% of the 8-week joints. PCL integrity was confirmed histologically in 19/20 knees from this study. Patella dislocation was not observed in this study.

An earlier ACLT study (unpublished results), when the feasibility of surgically-induced models of OA was being explored in the mouse, exhibited much more severe pathology. There was no progression over time due to the lesion severity reaching maximal scores with our scoring system (maximum scores at both 4 and 8 weeks were 6.0 ± 0.0 and summed scores were 133.0 ± 20.1 and 144.4 ± 27.3). Destruction of the posterior tibial plateau
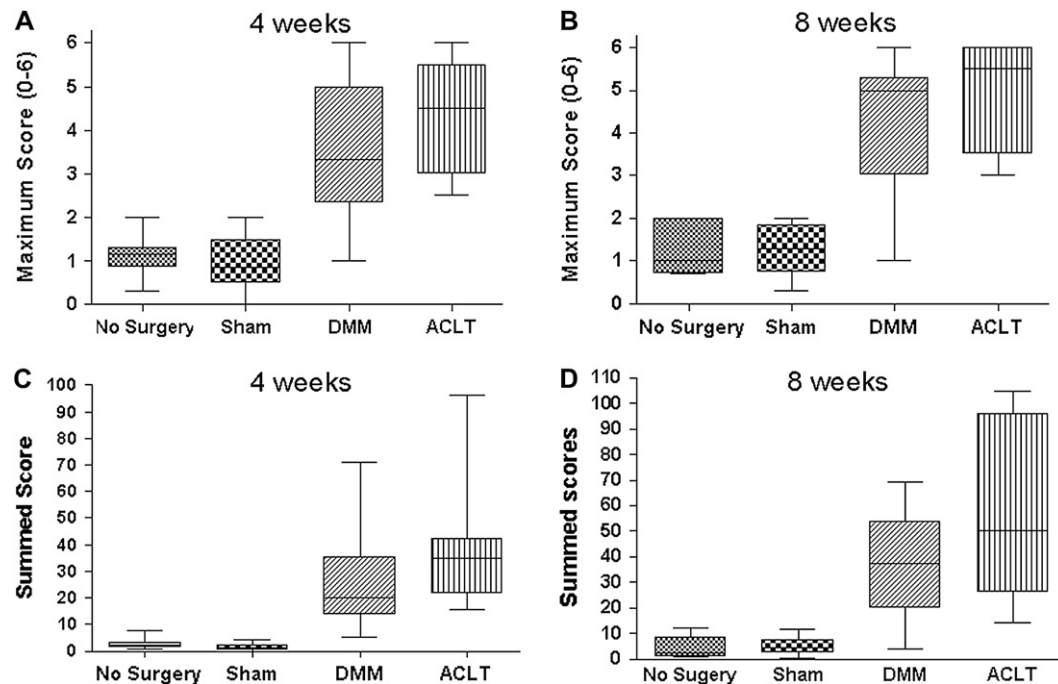
Fig. 4. Box and whisker plots showing the twentififth through seventififth percentiles boxed and the central horizontal line the median score. The data range is represented by whiskers. A: The mean maximum histologic scores at 4 weeks post-operatively ($\pm$s.e.m.) for the No surgery, Sham surgery and DMM and ACLT groups were 1.1 ± 0.1, 1.0 ± 0.3, 3.7 ± 1.5 and 4.3 ± 0.4. B: At 8 weeks the mean maximum scores were 1.2 ± 0.3, 1.2 ± 0.2, 4.1 ± 0.3, 5.0 ± 0.4. C: At 4 weeks the mean summed scores were 2.7 ± 0.6, 1.4 ± 0.6, 25.2 ± 4.7, 37.2 ± 7.1 and at 8 weeks (D) 4.1 ± 1.9, 4.9 ± 1.1, 35.7 ± 3.8, 58.0 ± 10.6.

through the subchondral bone to the growth plate occurred in the majority of joints. Ectopic bone formation was observed in the medial joint capsule of half the joints at both 4 and 8 weeks, and was not restricted to the medial collateral ligament. PCL integrity could only be confirmed histologically in 29% of the joints from that study. Patella dislocation was observed in 1/14 knees and that joint was not associated with a greater development of OA lesions.

Discussion

The development of surgical models of OA in the mouse has lagged many years behind the description of surgical models in larger species due to the technical challenge[9] and concern regarding uncontrolled damage to other tissues[10]. Instability models, widely used prior to the development of micro-surgical techniques, include the intra-articular (IA) collagenase model, and a closed method utilizing a 25-gauge needle[11] to induce cruciate transection. The closed 25-gauge needle technique has the limitation of unknown amounts of iatrogenic damage, uncontrolled IA bleeding and variable transection of one or both cruciates. IA injection of type VII bacterial collagenase has been used as a surrogate to surgically-induced instability models for many years[9,10,12,13]. Direct cartilage damage via type II collagen cleavage is theoretically possible with the supra-physiological doses that are utilized as a bolus. However, bacterial collagenase is inefficient at cleaving type II collagen of cartilage[14], and immunohistochemistical staining for type II collagen neoepitopes have not been observed in the early stages of the IA collagenase model (personal communication, W. van den Berg). The slow development of lesions[15] in vivo is consistent with damage occurring secondary to

ligamentous damage. Variability in this model may arise from different degrees of collagenase-mediated destruction of multiple ligaments, rather than total transection of a single ligament as in surgical models. Both anterior and posterior cruciate ligaments, as well as the patellar tendon, medial and collateral ligaments may be degraded. While increased macroscopic instability has been reported as soon as 1–3 days after collagenase injection[14], it is not clear if this represents partial or complete destruction of the ligaments. Ligament destruction may also occur at different times after collagenase injection, further contributing to variability. Posterior erosion of the subchondral bone of the tibial plateau[13] is reported following IA collagenase injection and resembles the lesions observed following erosion in our ACLT model. The collagenase model is associated with chondrogenesis of the collateral ligaments with progression to bone formation that is more apparent on the medial than the lateral side of the joint. Patella dislocation is described in 33%[16]–80%[15] of collagenase-injected joints, although it is not clear if this represents complete or partial dislocation. The contribution of patella dislocation alone to the development of murine OA is unknown.

Spontaneous models of mouse OA[5,17,18] develop over long periods of time as in idiopathic human OA. Backcrossing a KO mouse onto a strain with a genetic predisposition to OA takes 6–10 generations. This may take 2 years, with a further 6 months to 1 year to age the mice before effects can be assessed. Large numbers of mice are usually required for such an evaluation due to the variable induction of OA in spontaneous models. These factors make spontaneous models less attractive for initial target evaluation and inhibitor studies.

The first surgical model of OA described in the mouse, was the murine partial medial meniscectomy (PMM) and
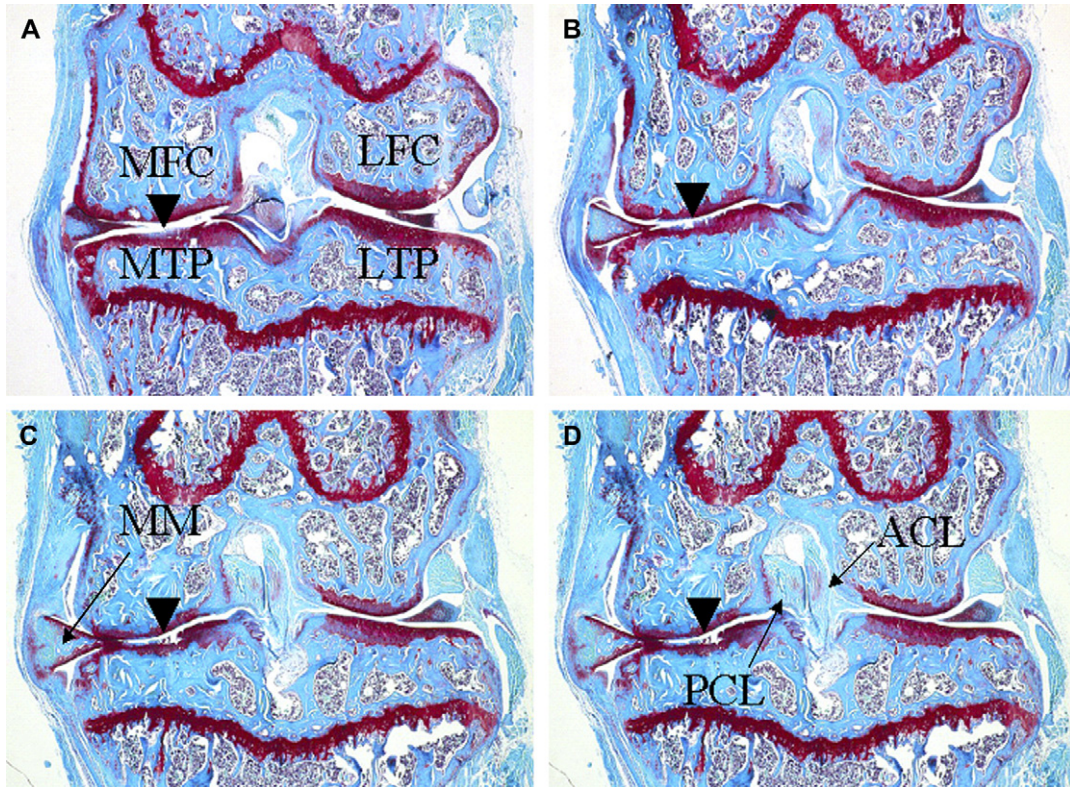
Fig. 5. Four sequential histologic levels from posteriorly (A) to anteriorly (D) at 8 weeks following DMM (maximal score = 5). Histology following DMM often resulted in medial displacement (B–D) of the medial meniscus. The cruciate ligaments were always intact (C,D) and no ectopic chondrogenesis or bone formation was present. A consistent feature of the DMM model was loss of Safranin-O, fibrillation and cartilage loss on the medial tibial plateau (arrowhead, A–D) and medial femoral condyle. Erosion through the sub-chondral bone was not observed. MFC = medial femoral condyle; LFC = lateral femoral condyle; MTP = medial tibial plateau; LTP = lateral tibial plateau; MM indicates a medially displaced medial meniscus.

medial collateral ligament transection (MCLT) model, developed by Visco *et al.*[19]. This model was used to evaluate the interleukin-1 beta (IL-1β), MMP-3 (Stomelysin-1), interleukin-1 converting enzyme (ICE), and inducible nitric oxide synthase (iNOS) KO mice[20]. Surprisingly, disease was not abrogated in any of these KO mice and more severe OA was observed in the contralateral limbs of the IL-1β KO. This result could indicate that other catabolic pathways were upregulated[20], increased weight-bearing was occurring on the non-operated limb, or that alternate models or scoring systems may be more appropriate. In our laboratory, DMM challenge of the same MMP-3 and IL-1β KO mice (Taconic) resulted in no differences between MMP-3 and WT controls, while the IL-1β KO was significantly protected at 4 and 8 weeks[21]. This contrasting result may support the hypothesis of Visco *et al.*[22] that mechanical instability created in the PMM + MCLT model may be too severe to overcome, and may also be impacted by the differences in histologic sectioning and scoring between the two laboratories.

Kamekura *et al.*[23] have recently described four surgical models of OA including ACLT ± complete medial meniscectomy (MM) ± posterior cruciate and patellar ligament transection, as well as MM + medial collateral ligament transection. Subchondral bone erosion of the posterior tibial plateau (in some cases reaching the growth plate) was demonstrated in histologic photomicrographs from the ACLT combination surgeries, similar to our observations with the ACLT, but free marrow in the joint was not described. Less

severe histologic changes were reported for their ACLT-alone model than observed in our ACLT study, and may reflect differences in surgical technique, background strains of mice used (C57/Bl6 compared to 129S6/SvEv in our study), different scoring systems and histologic sectioning. In both laboratories, a lack of synovial inflammation was observed after surgery. Our study differs from Kamekura's by comparing the ACLT model to the DMM model, and providing photographs detailing the precise anatomical location for ligament transection. The histologic evaluation of the ACLT model is also different, with incorporation of all the scores from four quadrants of the joint using frontal sections in this study. Kamekura's evaluation used frontal sections for the medial model, and sagittal sections of the medial side of the joint (no analysis of the lateral side), for the other three models. This difference in sectioning makes it impossible to blind the medial model group. When evaluating new models or transgenics, the region of joint damage cannot be assumed to be restricted to the medial side of the joint, as DMM surgery in the hMMP-13 over-expressing transgenic resulted in maximal cartilage destruction in the lateral joint (unpublished observations). The investigation of disease modification of MMP-13 and RunX2 KO mice[23] in the models of Kamekura *et al.* is eagerly awaited.

The ACLT model in our laboratory gave minimal OA in the anterior region of the joint with moderate-severe OA in the central weight-bearing region. Lesions following ACLT were often severe in the most posterior aspects of the tibial plateau, due to the anterior drawer of the tibial plateau
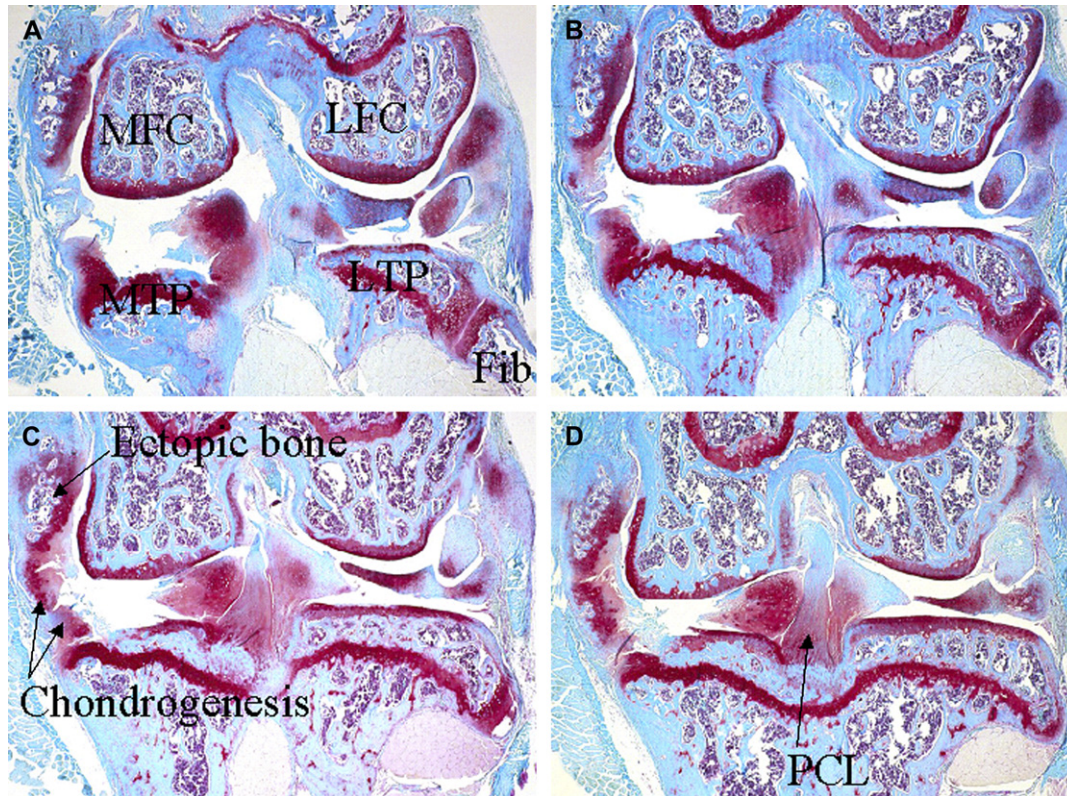
Fig. 6. Four sequential histologic levels from posteriorly (A) to anteriorly (D) at 8 weeks following ACLT (maximal score = 6). Erosion to the growth plate (A, B), chondrogenesis and ectopic formation of new bone (A–D) in the medial synovium and joint capsule was a feature of the ACLT model. Damage was more severe in the posterior aspect of the joint and was not restricted to one area of the joint. Free bone marrow cells (not shown) were often observed in the joint when severe erosion was present following ACLT. The posterior cuciate ligament (PCL) remained intact (D). MFC = medial femoral condyle; LFC = lateral femoral condyle; MTP = medial tibial plateau; LTP = lateral tibial plateaul; Fib = Fibula.

relative to the femoral condyles. The increased concentration of weight bearing on the posterior aspect of the posterior tibial plateau resulted in destruction of the cartilage and subchondral bone to the level of the growth plate in 30% of 8-week ACLT knees. The subchondral erosion may allow marrow and blood cells within the synovial cavity. Repeated bleeding into the joint is a known risk factor for OA in patients with hemophilia[24] and free blood in murine knees may also contribute to a greater progression of OA. PCL integrity was confirmed in 95% of the joints in the current ACLT study, confirming the accuracy of our ACLT single ligament transection method. The impact of chondrogenesis and ectopic bone on joint stability is unknown, and it is possible that ankylosis could occur at later time points in the ACLT model.

Our first experience with micro-surgical induction of OA in the mouse knee was with the ACLT model (unpublished results) where we observed more severe OA, chondrogenesis, and subchondral bone erosion, than reported in this study. We attribute the decrease in OA over time to improved technique and equipment. The quality and depth of field of our micro-surgical scopes improved, providing better visualization of the ACL. Incisions became smaller, possibly resulting in decreased drying of the articular cartilage and decreased hemorrhage into the joint. It is possible that PCL transection occurred in addition to ACLT in the earlier study (due to poor visualization of deeper structures) and may have contributed to the greater pathology

observed in that study. Therefore, we recommend that groups embarking upon micro-surgical studies make investments in equipment, micro-surgical training, and extensive practice, especially for the ACLT model. Initial and subsequent studies using the DMM model (unpublished results) have not shown the dramatic decreases in OA scores that were observed with the ACLT model, and may thus be a preferred model to embark upon. The work described here is limited to the 129S6/SvEv strain, and while we do know that strain-specific differences occur for the DMM model[25], this has not been evaluated in the ACLT model.

In the DMM model, mild to moderate OA was present with lesions on the central weight bearing area of the MFC and MTP. Lesions increased in severity through 2-, 4-, 8-[26], and 26-week (unpublished results) time points. Posterior erosion of the tibial plateaus was never observed in the DMM model and chondrogenesis was negligible. Lesions were primarily located on the anterior-central portion of the medial joint, with lesions on both the tibial plateau and femoral condyles. In mice euthanized immediately following surgery, no cartilage injury was observed (unpublished results), supporting our hypothesis that the negligible level of OA in the 4- and 8-week sham-surgery groups reflects a lack of iatrogenic damage, rather than an ability of murine cartilage to spontaneously repair.

Neochondrogenesis of the medial and lateral joint capsule and synovia was significant in the ACLT model. In

contrast, sham and DMM operated joints had no evidence of neochondrogenesis nor free cells in the synovial cavity. The ACLT model did not resemble spontaneous OA of mice and the severe lesions at the posterior portion of the joint would be missed if sections were not examined throughout the depth of the joint. Therefore, caution must be taken to interpret studies that evaluate only the central portion of the joint as entirely different conclusions could be reached than from the whole joint. The ACLT surgery was much more difficult to perform and required a greater exposure than the DMM surgery. The potential for iatrogenic damage was much higher in the ACLT model as ACLT requires deeper access in the intercondylar area while the DMM exposure was more superficial to the weight-bearing regions of the joint.

Animal models of OA need to balance the demands for rapid screening of targets and DMOADs with their similarity to human OA. It is possible that the murine ACLT model is too severe to model human OA, and that regenerative features such as dramatic chondrogenesis may complicate the evaluation of cartilage destruction. In our laboratory, the DMM model is preferred over the ACLT model, as it more closely resembles the more slowly-progressive human OA and should allow for evaluation of DMOADs. The DMM model can be used for target validation studies and has been shown to be sufficiently sensitive to show significant protection against OA progression in ADAMTS-5[7] and IL-1β[21] KO joints at 4 and 8 weeks following DMM. Investigations using this model may provide impetus for moving potential DMOADs to the clinic, while final confirmation of molecular targets and DMOADs for human OA will require positive results in phase III clinical trials[27].

Acknowledgements

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