Relation of Ligament Damage with Site Specific Cartilage Loss and Osteophyte Formation in Collagenase Induced Osteoarthritis in Mice

GERJO J.V.M. VAN OSCH, PETER M. VAN DER KRAAN, LEENDERT BLANKEVOORT, RIK HUISKES, and WIM B. VAN DEN BERG

ABSTRACT. Objective. To investigate the correlation between initial ligament damage and development of subsequent osteoarthritic changes.

Methods. Collagenase was injected intraarticularly into the knee joint of mice of strain C57B16 or C57B110. After 3 days, ligament damage was evaluated by measurements of knee laxity in the anterior–posterior direction as a measure of cruciate ligament function, and in the varus–valgus direction as a measure of collateral ligament function. The amount and location of cartilage loss and osteophyte formation were determined at Day 42.

Results. Significant correlations between the amount of laxity changes and the severity of cartilage loss (r = 0.78), the amount of laxity changes and the size of osteophytes (r = 0.87), and between the severity of cartilage loss and osteophyte size (r = 0.94) were demonstrated. The amount of cartilage loss and the degree of osteophyte formation at the medial side of the joint depended mainly on the severity of cruciate ligament damage. This is in contrast to changes at the lateral side of the joint, which appeared not to be associated with the severity of ligament damage.

Conclusion. A strong relationship exists between the severity of ligament damage and the severity of osteoarthritic changes on the medial side of the joint. In the lateral joint compartment, prone to spontaneous osteoarthritis in the mouse strain studied, this relation is absent. (J Rheumatol 1996;23:1227–32)

Key Indexing Terms:
OSTEOARTHRITIS CARTILAGE OSTEOPHYTES LIGAMENT DAMAGE JOINT LAXITY

Joint instability is a well known cause of secondary osteoarthritis (OA) of the human knee. Knees can become unstable after tears in ligaments or menisci. To study the pathogenesis of instability induced OA, animal models are frequently used. These models show OA changes that mimic those in human OA, such as cartilage damage, fibrosis, osteophyte formation, and sclerosis of subchondral bone. In dogs and rabbits, instability is induced surgically by transection of one or more ligaments (mostly the anterior cruciate ligament), (partial) meniscectomy, a combination of both, or transection of tendons and muscles. In mice, OA of the knee can be induced by intraarticular injection with highly purified collagenase. Collagenase is able to induce increased joint laxity with minimal inflammation.

We are interested in the relation between initial ligament damage and development of osteoarthritic changes. Although consensus exists about ligament damage as a cause of OA, the relation between severity of ligament damage and severity of osteoarthritic changes in the progression of OA is not frequently studied. Kannus and Järvinen performed clinical studies on this topic. They showed that the amount of radiological osteoarthritic changes depends on the severity of ligament damage. For the score of radiological changes they used a combination of scores of different features (cartilage damage, osteophyte formation, ligament calcification, and subchondral scleroses). Ligament damage was scored as grade I, II, or III lesions. We developed methods to quantify ligament damage, cartilage loss, and osteophyte formation separately, on a continuous scale in a mouse model for OA.

Our objective was to evaluate the correlation between the magnitude of the initial ligament damage and the progress of the osteoarthritic process, quantified by the severity of cartilage loss and osteophyte formation, irrespective of the initial cause of the onset of the osteoarthritic process.

MATERIALS AND METHODS
Experimental design. The onset of the osteoarthritic process was induced by injection of collagenase into the knee joint of mice, of which the main effect is to cause severe damage to the ligaments. Two strains of mice were

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used, C57Bl6 and C57Bl10. In each strain, either 3 or 10 units of collagenase were used for injection, giving 4 different groups with a large range in severity of changes. With these groups, 3 experiments with identical design were done. In the first experiment, 16 mice in each group were used for ligament testing and 10 mice in each group were used for histology. The 2nd experiment used 12 mice in each group for ligament testing and 10 mice for histology. In the last experiment, we tested ligaments in 6 mice per group and used 6 or 10 mice per group for histology.

The study was composed of 12 experimental groups. Mice from each group were selected at random for measurements of joint laxity on Day 3 and for histology to measure cartilage loss and osteophyte size at Day 42. The mean laxity and the mean histological changes were calculated for each group. This resulted in 12 pairs of laxity and histological changes. These pairs were used for correlation studies.

**Induction of joint laxity.** Abnormal joint laxity was induced in male mice at an age of 12–16 weeks. Animals were kept in cages with wood chip bedding in a room air conditioned at a constant temperature. They were given a standard laboratory diet (Hope Farms, Deventer, The Netherlands) and acidified water ad libitum. Highly purified bacterial collagenase (type VII, *Clostridium histolyticum*, Sigma, St. Louis, MO, USA) was dissolved in saline and 6 μl was injected into one of the knee joints. (Half the mice had injection in their right and half in the left knee joint).

**Laxity testing.** Laxity tests indicated the function of the ligaments. Laxity in the anterior–posterior (AP) direction was a measure of the function of the cruciate ligaments; laxity in the varus–valgus (VV) direction determined the function of the collateral ligaments. Joint laxity was measured in the AP direction and in the VV direction using 2 specially developed testers, as described. The tests were performed on Day 3 after collagenase injection, when the maximal degree of damage was reached (data from pilot experiments). At later time points, tests tend to produce complex results, because joints can be stabilized by the formation of osteophytes and destabilized by the loss of cartilage (data not shown).

Before laxity testing, mice were killed by cervical dislocation. Their hindlimbs were isolated and all soft tissues were removed from the femoral head and tibial diaphysis, but leaving tissue around the knee joint to prevent dehydration of joint structures. Use was made of anatomical reference points on the bones to position the limbs firmly and reproducibly in the test device, with the knee in 60° flexion and the tibia in neutral rotation. The method we used to test laxity of knees had been validated and appeared to be highly reproducible.

In the AP test, displacement between proximal tibia and distal femur was measured. In the VV test, rotation of the proximal tibia with respect to the distal femur was measured. In both tests, 3 full cycles were performed in 100 s. For the AP test, a full cycle means forces were applied from 1 Newton (N) anterior to 1 N posterior and back. For the VV test, a full cycle means a moment of force was applied from 5 Nmm varus to 5 Nmm valgus and back. Load and displacement data were sampled at the rate of 1 Hz and stored on a personal computer. Load displacement curves of a normal murine knee in the AP and VV direction are shown in Figure 1.

**Translations.** The tibia at forces of 0.8 N anterior and 0.8 N posterior (indicated as ±0.8 N) and rotations at moments of 4 Nmm varus and 4 Nmm valgus (indicated as ±4 Nmm) were calculated, using distribution-free estimation methods. To calculate overall laxity, the laxity values at the forces and moments indicated above were first expressed in percentages relative to the standard normal values, which were 0.28 mm anterior, 0.19 mm posterior, 5.5° varus and 11.8° varus, and then averaged. The limits of the testers were ±1 mm displacement in the AP directions and ±45° rotation in the VV directions. For laxity values exceeding these maxima, the maximum value was assigned.

**Quantification of histological indexes.** Osteoarthritic changes visible on histology were cartilage loss and osteophyte formation. Osteophytes were visible from the 2nd week after collagenase injection, cartilage loss developed after a month. Knee joints were isolated for histology on Day 42 after injection of collagenase when osteoarthritic changes were clearly visible on histology.

Knees were fixed in phosphate buffered formalin (pH 7.4), decalcified in 5% formic acid, and embedded in paraffin for histology. Frontal whole knee joint sections were made (7 μm) and stained with safranin O and fast green. An example of a frontal knee section of a C57Bl6 mouse 42 days after injection of 10 units collagenase is shown in Figure 2. For each knee joint, cartilage loss and osteophyte formation were measured using an image analysis system (VIDAS, Kontron Image Analysis Division, Zeiss, The Netherlands) on 2–4 histological sections from the central part of the joint, spaced 133 μm apart. This appeared to give a good indication of the mean changes of the entire joint. The contours of cartilage and osteophytes were indicated manually using a digitizing tablet, and graphically displayed on a monitor.

**Cartilage loss.** On each section, an estimation was made of the virtual original cartilage contour before damage had occurred and of the area of cartilage that was lost. The area of lost cartilage was divided by the original total area and multiplied by 100. The average of the different sections of one joint was calculated to get one figure for the whole joint, a “joint score.” In this way, figures for cartilage loss were obtained for the medial and lateral side of the joint separately. Total cartilage loss was presented as the mean of the percentages of cartilage loss in the lateral and the medial tibial plateau.

**Osteophytes.** Osteophyte formation was quantified by measurements of

![Figure 1. Load displacement curves of a normal murine knee joint. (A) AP force displacement curves; (B) VV moment rotation curves.](image-url)
osteophyte areas at 6 different sites, i.e., the margin of medial and lateral femur and tibia and the insertion of medial and lateral collateral ligament to the femur. At each site, the osteophyte area was determined as the mean area of newly formed cartilage and bone over the histological sections measured.

The medial osteophyte index was the sum of the areas of the medial osteophytes; the lateral osteophyte index that of the lateral osteophytes. The total "osteophyte index" was calculated as the sum of the areas of the 6 separately determined osteophytes.

Statistical analysis. To calculate the correlations, use was made of the means of laxity and histological variables for each of the 12 experimental groups. A laxity and a histological variable from each experiment can be considered as a pair. Linear correlations (Pearson) were calculated between laxity variables, cartilage loss, and osteophyte sizes from the data of the 12 experiments. Partial correlations between laxity and histological changes were calculated to correct for influences of laxity in other directions than the direction of interest. As a rule of thumb, correlations above 0.75 were considered very good to excellent and \( p < 0.01 \) was considered statistically significant.

Differences between variables were evaluated using Wilcoxon's signed rank test; \( p < 0.05 \) was considered statistically significant.

RESULTS
The overall severity of ligament damage was associated with the total amount of cartilage loss (Figure 3A; \( r = 0.78 \), \( p < 0.003 \)) and with the total size of osteophytes (Figure 3B; \( r = 0.87 \), \( p < 0.0002 \)).

Cartilage loss and osteophyte formation were usually visible at both medial and lateral plateaus. The prevalence of osteophytes was higher than the prevalence of cartilage loss (\( p < 0.01 \)). The severity of total cartilage loss and total osteophyte size (Figure 3C) correlated excellently (0.94; \( p < 0.0001 \)). Both cartilage loss (\( p < 0.05 \)) and osteophyte formation (\( p < 0.02 \)) were more severe on the medial than on the lateral tibial plateau. The amount of cartilage loss in the lateral tibial plateau was small, never exceeding 20%, while medial cartilage loss could be as much as 100%. Severe cartilage loss medially was frequently accompanied by medial dislocation of the femur and a varus position of the tibia.

For more precise analysis of the relation between laxity and the location of changes, differentiation was made between medial and lateral osteoarthritic changes. Furthermore, laxity was divided into the anterior, posterior, varus, and valgus directions. Laxity changes in different directions seem to be related; therefore calculations of partial correlations were necessary to correct for the influences of laxity changes in other directions than the direction of interest. In most occasions, laxity changed in more than one direction.

Correlation coefficients (Table 1) and scatter diagrams (Figures 4 and 5) of medial and lateral changes against total AP and total VV laxity showed that the severity of lateral cartilage loss and lateral osteophytes appeared to be independent of the amount of ligament damage. Although significant correlation existed between lateral osteophyte size and severity of AP laxity, mainly caused by laxity in the anterior direction (\( r = 0.84 \)), this was not significant after correction for laxity in other directions.

In contrast to lateral osteoarthritic changes, the severity
of cartilage loss at the medial tibial plateau correlated significantly with the amount of laxity in the anterior and in the valgus direction. Controlled for laxity in other directions, a significant correlation remained only between medial cartilage loss and laxity in the total AP direction. Although the size of medial osteophytes correlated significantly with laxity in all 4 directions, after control for laxity in other directions, a significant correlation remained only with the severity of AP laxity.

Table 1. Correlation coefficients of laxity in the anterior, posterior, varus, and valgus directions with severity of cartilage loss and osteophyte formation in the medial and lateral tibial plateau.

<table>
<thead>
<tr>
<th></th>
<th>AP Laxity</th>
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<th>VV Laxity</th>
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<td>Pearson</td>
<td>Corrected for VV Laxity</td>
<td>Pearson</td>
<td>Corrected for AP Laxity</td>
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<tr>
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<td></td>
<td></td>
<td></td>
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<td>0.80*</td>
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<tr>
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<tr>
<td>Osteophytes</td>
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<tr>
<td>Medial</td>
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<td>0.78*</td>
<td>0.76*</td>
<td>0.01</td>
</tr>
<tr>
<td>Lateral</td>
<td>0.82*</td>
<td>0.69</td>
<td>0.62</td>
<td>-0.20</td>
</tr>
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*p < 0.01.

Figure 4. Scattergrams of AP laxity (mm) on the horizontal axis and severity of medial and lateral cartilage loss (%) and osteophytes (μm²) on the vertical axis.

Figure 5. Scattergrams of VV laxity (degrees) on the horizontal axis and severity of medial and lateral cartilage loss (%) and osteophytes (μm²) on the vertical axis.

DISCUSSION

In humans and in animal models, evidence exists for a relation between the kind of instability and the location of cartilage damage\[^2,4,5,13,15–17,22\]. Complete insufficiency of the medial collateral ligament was shown to lead to cartilage damage on the medial side\[^4\]. Complete insufficiency of the lateral collateral ligament provokes cartilage damage on the lateral side\[^6\] or on the lateral and medial sides\[^22\]. Partial or complete insufficiency of the anterior cruciate ligament causes cartilage damage predominantly on the medial side, but the lateral side can be affected also\[^5,13,15–17\].

In contrast to previous studies, we did not obtain ligament damage in only one ligament. Although it is a very clean method, surgical transection of ligaments in mice was technically impossible. Therefore, we used injection of collagenase to obtain ligament damage. Injection of collagenase gave diffuse damage of all the ligament structures. This made it difficult to draw conclusions about the relation of damage of one specific ligament and the occurrence of osteoarthritic changes on the medial or lateral side of the joint. It was possible to make a statistical correction for influences of laxity in other directions than the direction of interest, using partial correlations. In this way, we found that the amount of cruciate ligament damage correlated well
the amount of medial osteoarthritisic changes. This does not mean that changes of the collateral ligaments are not important. Without collateral ligament damage, cartilage loss would probably be less obvious. It was, however, not possible to find a correlation between the amount of collateral ligament damage and the severity of osteoarthritisic changes. This could be due to the large effects of the cruciate ligaments, so the correlation between collateral ligaments and osteoarthritisic changes may be weakened by correction of the correlations for the influence of cruciate ligament damage.

Our study showed a relationship between the amount of laxity increase and the development of cartilage loss and osteophytes at the medial side of the joint. The severity of lateral damage, however, did not correlate with the amount of ligament damage. Lateral cartilage loss has been shown to develop spontaneously in C57 Black mice. Injection with collagenase may accelerate the process of spontaneous OA in the lateral plateau. Only a small change in laxity in any direction may be enough to cause this acceleration.

The difference between changes in medial and lateral tibia suggests that the cartilage changes are not caused simply by direct effects of collagenase on cartilage. Although direct effects of collagenase on cartilage cannot be totally excluded, it was demonstrated that bacterial collagenase cannot easily degrade anatomically intact (uncut) cartilage, suggesting a protective layer covering intact cartilage or shielding of collagen by proteoglycans. But independent of the initial cause of OA, a larger laxity increase will cause a faster progression of the OA damage on the medial side of the joint.

Our results support the hypothesis that joint instability leads to the development of OA or at least accelerates the progress of the OA. The limited amount of lateral cartilage loss and the lack of correlation between the severities of ligament damage and lateral cartilage loss could also be explained by joint stabilizing capacity. The severity of medial changes, however, was related to the severity of laxity changes, indicating that at least no big influence of stabilizing capacity existed for these changes. Damaged ligaments will cause joint instability depending on the capacity of the remaining structures, including the muscles as active stabilizers, to keep the joint within its normal range of motion. Joint instability leads to altered loading of the cartilage, which will be damaged when the mechanical properties of the cartilage remain unaltered or cannot adapt sufficiently to withstand the new loading conditions. By this hypothesis, the lack of correlation between the severity of ligament damage and the progress of OA on the lateral side can be explained by the small effect on cartilage loading of the instability that was created. If the murine knee joint is predominantly medially loaded, then the effects of ligament damage should be highest on the medial compartment if the additional functional stabilizers cannot maintain normal loading conditions. This theory was supported by the high correlation between the amount of ligament damage and the progress of OA in our study.

Another possible cause for the development of cartilage damage is a change in mechanical properties of the cartilage, reducing the resistance of the cartilage to normal loading. Altered loading can cause altered biosynthesis or biodegradation of cartilage matrix components. Such a mechanism for the pathogenesis of OA after joint trauma was suggested by the finding of an imbalance of enzymes and enzyme inhibitors and increased concentration of proteoglycans in traumatically injured knees. This may suggest increased degradation of cartilage components by enzymes released after trauma of the ligaments or menisci. By this hypothesis, cartilage damage and laxity changes would be related, but cartilage damage would not be induced directly by altered joint loading. However, because in our study medial OA changes correlate with laxity changes and lateral OA changes do not, it seems likely that in our study changed loading conditions are more important than effects of released mediators, at least for the speed of the progress of OA.

We concluded that in the lateral joint compartment, prone to spontaneous OA in C57 Black mice, there is no relation between severity of laxity and histological changes. The severity of laxity and histological changes on the medial side of the joint correlate, indicating that, in this model, the extent of medial osteoarthritic changes is determined by the magnitude of the ligament damage.

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REFERENCES

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