REPLACING THE MEDIAL COLLATERAL LIGAMENT WITH AN ALLOGENEIC TENDON GRAFT

AN EXPERIMENTAL CANINE STUDY

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In order to determine whether an allogeneic tendon could be used to replace an extra-articular ligament, the right medial collateral ligament from 11 adult dogs was replaced with a fresh-frozen allogeneic patellar tendon. At each of 3, 6, 15, 30 and 52 weeks postoperatively, one dog was killed for micro-angiographical and histological studies; at 52 weeks the remaining six dogs were killed for tensile testing.

Micro-angiograms showed that the allogeneic tendon was revascularised with infiltration of the mesenchymal cells from the surrounding tissues and both ends of the graft. Histologically, the alignment of the fibroblasts and collagen bundles became more regular over time, without any immunological rejection. A biomechanical study performed at 52 weeks found no significant difference in stiffness or ultimate load between normal and reconstructed ligaments. Fresh-frozen allogeneic tendons are therefore considered useful for extra-articular ligament reconstruction.

Orthopaedic surgeons usually use autogenous tissue for reconstruction following chronic ligament insufficiency. This is often successful, but has disadvantages; normal tissue has to be sacrificed, and only limited amounts are available. Although problems of viral infection and storage remain unsolved, the use of allogeneic tendons for replacing injured ligaments has many advantages. The appropriate size and shape of graft can be chosen, there is no sacrifice of donor tissues, and the operating time is shorter.

Several clinical studies with allografts have been reported: on tendons of the hand (Peacock 1959; Peacock and Madden 1967); on rotator cuffs of the shoulder (Neviaser, Neviaser and Neviaser 1978); and on the anterior cruciate ligaments of the knee (Shino et al 1986). Several experimental studies of allografts have also been performed (Webster and Werner 1983; Shino et al 1984; Arnoczky, Warren and Ashlock 1986; Nikolaou et al

Fig. 1a

Fig. 1b

Operative procedures for replacing the MCL with an allogeneic patellar tendon: (a) After complete excision of the MCL, two bone tunnels have been made at the femoral and tibial insertion sites. The medial cortex above the tibial drill hole has been decorticated: (b) A graft with 2-0 monofilament nylon sutures has been passed through the holes and fixed with buttons.

1986; Jackson et al 1987), but there have been no reports on the use of an allograft for an extra-articular ligament in an animal model. The purpose of this present study was to determine whether an allogeneic tendon could be used successfully to replace an extra-articular ligament and to compare the results with those of our previous study on anterior cruciate ligament (ACL) replacement with an allogeneic tendon.

MATERIALS AND METHODS

Eleven skeletally mature mongrel dogs with body-weight ranging from 8 to 12 kg (10 ± 1 kg) were used. Allogeneic tendons (4 mm wide, 3 cm long) were obtained from the central part of the patellar tendons of the donor dogs

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under aseptic conditions. They were preserved at -20°C for at least 10 days.

All the dogs were anaesthetised with intravenous sodium pentobarbital. A medial skin incision was made to expose the medial collateral ligament (MCL) of the right knee by transecting the pes anserinus tendons near their tibial insertions. The MCL was completely excised from its insertion sites. With a 3 mm Steinmann pin, a tunnel was drilled through the femur at the femoral MCL insertion site; a second tunnel was made at the distal end of the tibial insertion site and the medial cortex above the tibial drill hole was decorticated (Fig. 1a). A preserved graft was thawed in saline at room temperature and a holding stitch was made in each end with 2-0 monofilament nylon thread sutures. Both ends of the allogeneic tendon were then passed through the holes and anchored with buttons under appropriate tension (Fig. 1b). Care was taken to ensure that the free ends of the graft were entirely within the drill holes. After the wound had been closed and the leg immobilised in plaster at 60° of knee flexion for four weeks, the dogs were permitted activity in a cage.

Micro-angiographic and histological studies. Five dogs were examined micro-angiographically and histologically. One dog each was examined at 3, 6, 15, 30 and 52 weeks postoperatively. The dogs were anaesthetised with pentobarbital and then heparinised with 500 iu/kg body-weight. The femoral artery of one or both legs was cannulated with a Teflon catheter. The dogs were then killed with an overdose of sodium pentobarbital and the hind limbs disarticulated at the hip and perfused with 500 ml of saline and heparin at a pressure of 60 cmH₂O. Radio-opaque suspension containing 50% barium sulphate, 2% Berlin blue and 2% gelatin was injected under a pressure of 150 mmHg. Less than 100 ml of the suspension filled each limb.

All soft tissues except the normal or reconstructed MCL were removed from the specimens which were then fixed in 10% neutral formalin. The specimens were

decalcified in hydrochloric acid, mounted in paraffin wax and sectioned into 2 mm blocks along the frontal plane; they were then radiographed with soft X-rays. Each block was recut into 5 μm sections and stained with haematoxylin and eosin.

Biomechanical studies. Six dogs were killed with an overdose of sodium pentobarbital 52 weeks after operation. The femur and tibia were cut 5 cm from the knee and stored at -20°C . On the day of the test, the specimen was thawed at room temperature. All soft tissues except the normal or reconstructed MCL were removed and the femur-MCL-tibia (or femur-reconstructed MCL-tibia) complex was mounted in a specially designed device (Fig. 2). The specimen was fixed with bone cement and pins so that a tensile load could be applied along the long axis of the normal or reconstructed MCL.

Fig. 3

Photograph of a reconstructed MCL (black arrow) 52 weeks after operation.

The shape of the ligament was assumed to be between a rectangle and an ellipse, and the cross-sectional area was calculated from the width and thickness of the ligament under no stress. The femur-MCL-tibia complex was tensile-tested at room temperature with a Shimazu autograph DSS-10-A. After application of 50 g preload, the specimen was cycled 10 times between 0 and 1 mm of deformation at a rate of 1 mm/min. Each specimen was then loaded at a rate of 500 mm/min until failure occurred. Load-deformation curves obtained during testing were recorded with an X-Y recorder. The stiffness,

Fig. 4a

Fig. 4b

Fig. 4c

Fig. 4d

Fig. 4e

Micro-angiographs of normal and reconstructed MCLs. (a) Normal: Main nutrient vessels run on the surface and form a fine network in the substance; (b) Reconstructed MCL at six weeks; vascular elements were present throughout the substance of the graft; (c) at 15 weeks; (d) at 30 weeks the graft was hypervascular; (e) at 52 weeks the graft remained slightly more vascular than the normal MCL.

which was measured at the linear portion of the load-deformation curve, the ultimate load and the peak stress were used as biomechanical parameters.

In order to investigate the mechanical properties of the graft before reconstruction, we performed tensile testing of nine 4 mm wide patellar tendon grafts without bone attachment. These grafts were obtained in the same manner as the allogeneic tendons for reconstruction and were frozen at -20°C . After being thawed, these grafts

were placed in a set of tendon clamps (Woo et al 1981). Because the patellar tendon graft did not include bones, the clamp was different from that for the femur-MCL-tibia complex. It had pivoted jaws providing a self-tightening effect on the graft to avoid slippage and failure of the graft within the clamps during testing. After applying a 50 g preload, each specimen was loaded at a rate of 500 mm/min until failure occurred. The ultimate load and peak stress were used as parameters.

Fig. 5

Fig. 6a

Fig. 6b

Fig. 6c

Fig. 7

Fig. 8

Figure 5 – longitudinal section of a normal MCL (haematoxylin and eosin, $\times 100$). Figure 6 – A reconstructed MCL at three weeks (haematoxylin and eosin); (a) the graft was necrotic and the collagen fibres were fragmented in the central area ($\times 50$); (b) there were many capillary buds with proliferation of mesenchymal cells ($\times 50$); (c) the bone tunnels are already filled with new bone ($\times 20$): graft (G), tibia (T) and bone tunnel (B). Figure 7 – At 15 weeks ($\times 100$) the bone graft junction has become nearly indistinguishable. Figure 8 – At 30 weeks ($\times 100$), although the graft was still hypercellular, the cells and collagen bundles were aligned longitudinally, resembling a normal MCL.

RESULTS

Macroscopically, all the reconstructed MCLs appeared viable and functional (Fig. 3). No significant degeneration of the articular cartilage nor meniscal tears were seen in any of the specimens. None of the postoperative knees had excessive valgus laxity.

Micro-angiograms. Several main vessels supply the normal MCL; they run on its surface and form a fine network in its substance (Fig. 4a). At three weeks, the graft at both the femoral and tibial attachments showed some vascularity, but there was little in the substance. By six weeks, vascularity was present throughout the substance of the graft (Fig. 4b). At 15 and 30 weeks, the graft was abundant with vessels (Figs 4c and 4d). At 52

weeks, vascular ingrowth in the graft had subsided, but the graft remained slightly more vascular than the normal MCL (Fig. 4e).

Histology. In the normal MCL, the fibroblasts and collagen fibres are aligned longitudinally, while a normal crimp pattern is also seen (Fig. 5). Three weeks after operation, the graft was necrotic and the collagen fibres were fragmented in the central area (Fig. 6a); infiltration of mesenchymal cells was observed in the central area. At the bone attachment site, there were many capillary buds with proliferation of mesenchymal cells (Fig. 6b); the bone tunnels were already filled with new bone (Fig. 6c). At six weeks, fibroblasts were present throughout the graft substance but the fibre orientation was irregular. At 15 weeks, the graft appeared hypercellular, and the collagen fibres were aligned longitudinally. Drill holes could not be recognised and the bone-graft junction had become nearly indistinguishable (Fig. 7). At both 30 and 52 weeks, cells and collagen bundles were aligned longitudinally, resembling a normal ligament (Fig. 8). However, the reconstructed MCL was not exactly the

Fig. 9a

Fig. 9b

Photomicrographs of normal and reconstructed MCLs at 52 weeks (haematoxylin and eosin, polarised light, $\times 50$) (a) polarised light demonstrates the high-frequency crimp pattern of a normal MCL; (b) the fibre orientation of a reconstructed MCL at 52 weeks was irregular and the crimp pattern was different from that of a normal MCL.

same as the normal one; the fibre orientation of the reconstructed MCL was looser and less regular than in a normal MCL, while the graft was relatively hypercellular (Figs 5, 8 and 9). There was no evidence of immunological rejection in any of the specimens.

Biomechanics. All normal and reconstructed MCLs failed in the midsubstance during the tensile testing of the femur-ligament-tibia complex. Although the tendon clamps for 4 mm-wide patellar tendon grafts were specially designed to avoid graft failure at the clamped sites, five specimens failed within the clamps. Since the ultimate load for these failed grafts did not reflect their mechanical properties, data obtained from four grafts which had been torn in midsubstance were used for comparison.

The cross-sectional areas of the normal MCLs, the reconstructed MCLs and the 4 mm-wide patellar tendon grafts before reconstruction were 5.3 ± 0.5 , 10.1 ± 2.0 , and 5.2 ± 0.6 mm², respectively. The values for the reconstructed MCL were thus significantly higher than those for the normal MCL ($p < 0.001$, reconstructed compared with normal MCLs, paired *t*-test). Furthermore, an unpaired *t*-test demonstrated that the cross-sectional area of the graft became significantly larger after reconstruction ($p < 0.01$, reconstructed MCLs at 52 weeks compared with 4 mm-wide patellar tendon grafts before reconstruction).

The mean stiffness of the normal and reconstructed MCLs was 68 and 54×10^3 N/mm, respectively (Fig. 10). A paired *t*-test demonstrated no statistically significant difference between the stiffness of the normal MCLs and that of the reconstructed MCLs ($p > 0.05$). With regard to the ultimate load, the average values for the normal and reconstructed MCLs were 351 and 247 N respectively (Fig. 11), not a significant difference (paired *t*-test, $p > 0.05$). The ultimate load for the 4 mm-wide patellar tendon grafts before reconstruction averaged 415 N. Statistical analysis demonstrated that the ultimate load for the reconstructed MCL at 52 weeks dropped

significantly ($p < 0.01$) to approximately 60% of that for the 4 mm-wide patellar tendon graft before reconstruction.

The average peak stresses of the normal MCL, the reconstructed MCL and the 4 mm-wide patellar tendon graft before reconstruction were 70 ± 10 , 27 ± 6 , and 94 ± 12 MPa, respectively. Statistical analysis demonstrated that the peak stress of the reconstructed MCL was significantly lower than that of both the normal MCL and the patellar tendon graft before reconstruction ($p < 0.05$).

DISCUSSION

In the early stages, the central area of the graft was necrotic and the graft was revascularised from the femoral and tibial attachment sites. Fibroblasts were thought to have infiltrated from the surrounding soft tissues and both ends of the graft. After six weeks, fibroblasts were seen throughout the graft; by 15 weeks the bone-graft junction was considered to have matured. Alignment of the fibroblasts and collagen bundles became more regular over time. In summary, the fresh-frozen allogeneic tendon graft was revascularised by invasion of the mesenchymal cells, followed by reorganisation of the collagen fibres over time, without any immunological rejection.

A comparison with our previous study on ACL replacement using the same materials (Shino et al 1984) showed that the remodelling process of the allogeneic tendon for the MCL was similar to that for the ACL. However, the remodelling rate was somewhat different. Allogeneic tendons used for MCL reconstruction showed maturation of the bone graft junction by 15 weeks and of the graft substance by 30 weeks postoperatively; the grafts for ACL reconstruction took approximately twice as long.

A paired *t*-test demonstrated no significant difference in the ultimate load and stiffness between the normal

Fig. 10

Fig. 11

Figure 10 – Histogram of average stiffness of the femur-MCL-tibia and femur-reconstructed MCL-tibia complexes. No statistically significant difference was found between the stiffness of the normal MCLs and that of the reconstructed MCLs ($p > 0.05$). Figure 11 – Histogram of average ultimate loads for the normal, the reconstructed MCLs and the central part of the patellar tendon before reconstruction. No significant difference was found between normal and reconstructed MCLs (paired t -test, $p > 0.05$), but the values for the reconstructed MCL at 52 weeks and those of the 4 mm-wide patellar tendon graft before reconstruction were significantly different.

and the reconstructed MCL at 52 weeks ($p > 0.05$). However, peak stress for the reconstructed MCLs was less than for normal MCLs ($p < 0.05$, paired t -test). Histologically, the reconstructed MCL had become similar to the normal by 30 weeks, but not identical. Histological differences in the number of cells, crimp pattern and collagen fibres may be one of the explanations for the inferior mechanical properties of the reconstructed MCLs.

According to our previous study (Shino et al 1984), the average ultimate tensile load for the reconstructed ACLs at 52 weeks was about 35% of that for normal ACLs, and 27% of that for the grafts before reconstruction. On the other hand, the ultimate load for the reconstructed MCLs at 52 weeks was 75% of that for the normal MCLs and 60% of that for the grafts before reconstruction. There are several possible reasons why allogeneic tendons for MCLs showed better ultimate load than those for ACLs. One is the difference in ligament fibre orientation; ACL fibres are twisted and have complex functions. Since the patellar tendon is a single strand, it may not be able to duplicate the complicated anatomy and function of the ACL. On the other hand, MCL fibres are generally parallel; therefore, the patellar tendon can duplicate the anatomical functions of the MCL more easily than that of the ACL. Another possible explanation is the difference in the environment around the graft. The histological study showed that fibroblasts infiltrate from surrounding soft tissues and both ends of the graft. Because the graft for the MCL is surrounded with soft tissues, fibroblasts can infiltrate it more rapidly than is the case with the ACL; this may result in more rapid remodelling of the graft and lead to better biomechanical results.

In conclusion, fresh-frozen allogeneic tendons are thought to be useful for extra-articular ligament reconstruction. They are not rejected immunologically, but the mechanical properties of the reconstructed MCLs at 52 weeks are inferior to those of normal MCLs.

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