Replacement of the anterior cruciate ligament

A comparison of autografts and allografts in dogs

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To compare the efficacy of allograft versus autograft replacement of the anterior cruciate ligament, 15 dogs had the ligament cut and replaced 1 month later: 11 dogs received a frozen bone-ligament-bone allograft cruciate ligament, while 4 dogs received a standard autogenous replacement with iliotibial band. Three of 11 allograft dogs developed postoperative infections and were removed from the study; and two of the remaining eight allograft ligaments were absent at autopsy. All the autograft ligaments were present.

From serial clinical and radiographic examinations, there were no differences observed in the two groups. Autopsy studies at 4 months, however, showed an increased inflammatory, pannus-like reaction about the origins and insertions of the six allograft ligaments as compared with the four autografts. The ligament hydroxyproline uptake was lower in the allograft group, averaging 60 percent of the contralateral unoperated on control versus equal to the control in the autograft group. The tensile strength of the allografts reached only 17 percent of the control value versus 41 percent for the autografts. Lymphocytotoxicity testing at 1 month revealed a donor-specific antibody response in 4 of 8 allograft dogs; however, no histologic evidence of immune response was observed in the ligaments. The synovial fluid leukocyte count was elevated in the allograft group at 4 months.

The increased synovial leukocyte counts and joint cartilage erosion, the decreased strength and metabolic activity of the grafts, and the evidence of an immune response in the allograft dogs do not support implantation of cadaver cruciate ligaments clinically at this time.

Various investigators have reported allograft anterior cruciate ligament replacements in dogs with inconsistent results. Shino (1984) and Arnoczky (1986) found that frozen patellar tendon allografts performed nearly as well as autogenous patellar tendon grafts. On the other hand, Curtis et al. (1985), using frozen fascia lata allografts, and Vasseur et al. (1987), using frozen anterior cruciate ligament allografts, found a poor mechanical result, presumably due to an immune response.

We have compared frozen anterior cruciate bone-ligament-bone allografts with fresh patellar tendon autografts as regards immune response, metabolic activity, and strength.

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Methods

Fifteen adult mongrel dogs, mean body weight 27 (22–29) kg, were subjected to two surgical procedures. Initially, each had the anterior cruciate ligament of one knee sectioned via a medial arthrotomy. The dogs were then allowed to bear weight for 4 weeks. At that time, the second surgery was performed: 11 dogs received an allograft ligament and 4 received an autograft tendon to reconstruct their ligament deficiency. All the operations were performed under standard aseptic technique. The opposite, unoperated on leg served as a control. All the dogs received a single 500-mg dose of cefazolin upon induction of anesthesia.

The allografts were stored at – 80 °C after removal under sterile conditions from dogs killed 1 to 2 h earlier. A portion of the spleen was removed to be processed and stored for immunologic assay.

The allograft ligaments were positioned after making a bony defect in the anterior tibial plateau and femoral notch using a small oscillating saw and osteo-

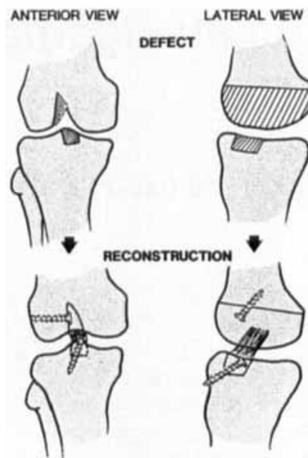

Figure 1. The allograft procedure.

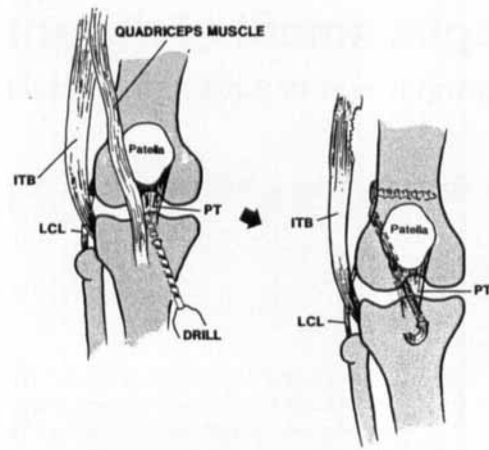

Figure 2. The iliotibial band and lateral patellar tendon blend into one structure. This was isolated and fashioned into a graft. The graft was passed through holes in the tibia and femur and attached to the femur by a screw and washer.

tomes (Figure 1). The bony insertions were then fixed by 3.5-mm cancellous bone screws. Small adjustments in tension were possible by varying the position of the femoral block in the sagittal plane.

The dogs receiving autografts had a 10-cm combined lateral patellar tendon and iliotibial band routed through drill holes through the tibia and femur to reproduce the normal position of the anterior ligament. After applying appropriate tension to the graft, it was fixed to the lateral aspect of the femur using a ceramic washer and cancellous screw (Figure 2).

The operated on legs were dressed and the dogs were allowed to bear weight in their cages. The dogs were moved to outdoor kennels at 10–14 days. Each dog was evaluated for limp, range of motion, and anterior-posterior stability at the following intervals: preoperatively, 2, 4, 8, and 16 weeks. The results were compared with the normal, unoperated on contralateral knee. Intraoperative range of motion was normal in all the dogs after positioning of the graft. An anterior-posterior tibial laxity of 3 mm was considered normal. All the dogs had AP and lateral radiographs at these time intervals, and had samples of serum and synovial fluid taken for immunologic testing and mucin-clot readings. The radiographs were evaluated for joint-space narrowing, subchondral cysts, screw migration, osteophyte formation, and bone resorption about the allograft block.

Three of 11 allograft dogs developed postoperative infections, and were removed from the study; and two of the remaining eight allograft ligaments were absent at autopsy. Of the 3 infected allograft dogs, 2 cultured *Staphylococcus aureus* and 1 cultured *Escherichia coli*. All the autograft ligaments were present at autopsy.

At 4 months, all the dogs not infected were killed, the legs were disarticulated at the hip and ankle, all soft tissue was removed from about the "stifle" joints on both the right and left sides, and the experimental side was completely dissected and photographed. The articular surfaces were evaluated for cartilage loss, pannus coverage, osteophyte formation, condition of the meniscus and of the ligament. All the dogs that had an intact ligament (6/8 allografts, 4/4 autografts) were submitted to mechanical testing.

After mechanical testing, approximately one half of each ligament was taken for routine hematoxylin and eosin staining. This portion was taken from the midsubstance and included the edge of the ligament, as well as the central region (Figure 3). The remaining half was divided into two parts. One of these was minced into small pieces, one half of which was used for determining H^3-proline uptake, whereas the other halfwas used for collagen typing. The remaining quarter of the ligament, which included an attached piece of bone, was used for autoradiography. The identical mechanical tests and preparation of specimens were carried out on the contralateral, unoperated ligaments as well.

Mechanical testing. Structural and material properties of the ligaments were measured using an Instron materials testing machine according to our protocols

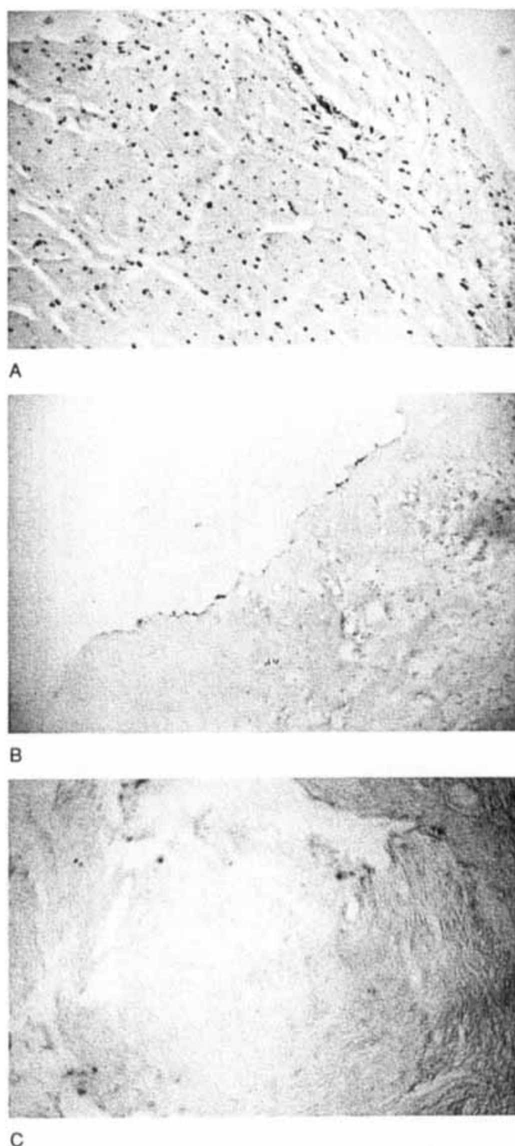

Figure 3. Autoradiograph of a normal, unoperated on ligament (A) shows cellular activity throughout the specimen. Autoradiography of an autograft (B) shows cellular activity principally at the periphery of the tissue. An autoradiograph of an allograft (C) shows cellular activity at the periphery, but at a lower level than is seen in the autograft.

described previously (Vasseur et al. 1987). All the values are expressed as a percentage of the unoperated on contralateral leg.

Hydroxyproline uptake, autoradiography, and collagen typing. These procedures were performed according to our protocols described previously (Vasseur et al. 1987). For hydroxyproline uptake and autoradiography, ligaments were labeled with H^3 proline. Collagen types were separated electrophoretically on an SDS-Page gel, stained with Coumassie Blue stain, and measured with a photodensitometer.

Immunologic studies. At the time of obtaining the donor ligaments, a portion of the donor spleen was removed. The tissue was processed according to the protocol for HLA typing used by the Sacramento Medical Foundation Blood Center and Bodmer and Bodmer (1979). The fluorescein-labeled cells were stored at – 80 °C until used in the lymphocytotoxicity assay.

At the time of testing, the cells were removed from the – 80 °C storage tank, thawed by rolling the tubes between the fingers, and the cells resuspended in the medium. The cells were tested for percentage viability using eosin. The cells were then pipetted into tissue-typing plates containing mineral oil in all the wells. The animal's serum or synovial fluid was added to the wells containing the cell suspension and allowed to incubate for 15 min, after which rabbit complement was added. The cell-serum-complement mixture was allowed to incubate for 30 min, after which the wells were read under transmitted fluorescent light. All the wells were made in duplicate. The control wells included cells only and cells plus complement to monitor poor cell viability and complement toxicity. The results were given a score recorded on the basis of the negative control: 0, no cell kill; 1, up to 25 percent cell kill; 2, 25–50 percent cell kill; 3, 50–75 percent cell kill; 4, 75–100 percent cell kill. If the well showed greater than 50 percent cell kill, a "positive" immune response score was given to that well, and a "negative" score was given to any well with less than 50 percent kill.

Results

There was no difference between the two groups for the mean values in limp, range of motion, or anterior-posterior laxity throughout the study, and no difference in range of motion, although both groups lost about 4° of extension; the flexion loss was minimal in both groups. At death, 5 of 8 allograft dogs limped, versus 1 of 4 of the autograft dogs.

In the synovial fluid, the mean leukocyte count was increased in the allograft group as compared with the autograft group at 16 weeks (Table 1). The mucin clot quality showed no difference between the two experimental groups.

At autopsy, two of eight allografts had ruptured; no autograft had completely ruptured, although one was

Table 1. Synovial fluid leukocyte counts. 10^9 cells/L

Dogs	Preop	2	4	8	16 weeks
Autograft					
1	0.39	0.69	0.20	3.3	0.93
2	0.36	1.3	–	3.4	1.0
3	0.43	0.36	1.3	1.6	1.0
4	0.65	0.76	2.5	1.5	1.5
Mean SD	0.46 *0.13*	0.78 *0.39*	1.3 *1.2*	2.7 *0.84*	1.1 *0.27**
Allograft					
5	1.4	0.91	1.9	1.8	2.3
6	1.2	52	10	4.0	1.7
7	0.20	0.70	0.58	0.58	2.2
8	1.6	0.44	1.0	0.93	4.7
9	0.050	1.4	1.0	1.3	–
10	1.7	–	3.5	1.0	2.3
11	2.2	5.2	1.3	3.1	4.6
12	0.15	1.1	1.9	1.1	0.98
Mean *SD*	1.07 *0.75*	8.8 *19*	2.7 *3.2*	1.7 *1.2*	2.7 *1.4**

* $P < 0.05$, Student's independent t-test.

Table 2. Mechanical properties of anterior cruciate ligament grafts tested to failure 4 months postimplantation. Values given as experimental side/control side, percentage in brackets

Dogs	Stress (MPa)		Strain (%)		Modulus (MPa)		Load (kN)	
Autograft								
3	7.9/65	(12)	30/34	(89)	26/190	(14)	150/1500	(10)
4	18/40	(44)	44/31	(140)	40/130	(31)	750/940	(80)
5	7.8/88	(9)	39/43	(92)	20/160	(13)	340/1100	(32)
Mean % *SD*		22 *19*		110 *29*		19 *10*		41 *36*
Allograft								
5	3.2/110	(3)	38/39	(98)	8.2/290	(3)	50/1600	(3)
6	11/66	(17)	47/44	(110)	23/150	(15)	330/150	(22)
7	3.4/59	(6)	73/35	(210)	4.7/170	(3)	60/1300	(5)
8	8.3/98	(8)	29/40	(72)	29/240	(12)	290/1400	(20)
9	9.9/60	(17)	32/49	(64)	46/160	(29)	470/1400	(34)
Mean % *SD*		10 *6.3*		110 *59*		12 *11*		17 *13*

attenuated and could not be tested mechanically. In mechanical tests of the unoperated on knees, the ligament always failed through the substance near the tibial insertion, often avulsing a small portion of bone with the ligament. However, both the autografts and the allografts failed near either of the insertions of midsubstance, without a discernible pattern in either group. All the grafts had pannus tissue, especially about the notch region, and in some cases extending into the patellar groove. The pannus tissue covered wider areas in the allografts. In addition, the allograft group had more injury of the articular surface, mainly on the femoral side. Half of the allografts were associated with moderate to severe articular cartilage erosion versus none in the autograft group. Each group had one minor meniscal tear, both on the lateral side. Radiographically, there was mild bony resorption about the graft site in 4 of 8 allograft dogs, predominantly on the tibial side.

Mechanical testing. The allograft group showed a mean load to failure of only 17 percent of the contralateral control ligament versus 41 percent in the autograft group (Table 2). Other material properties showed a similar trend with both groups markedly below their control-side values, and the allograft group inferior to the autograft in every parameter.

Hydroxyproline uptake at 4 months was equal to the controls in the autografts, but reduced by 39 percent in the allografts ($P = 0.059$, Table 3).

Collagen typing. Acrylamide gel protein electrophoresis failed to demonstrate any difference in the amount of Type III collagen contained in the allograft (26 percent) versus the autograft (33 percent) liga-

Table 3. Labeled hydroxyproline uptake by ligament grafts; 10^3 counts per min/mg tissue

Dogs	Experimental side	Control side	Percent
Autograft			
1	1.9	1.9	99
2	1.4	0.091	150
4	2.0	1.9	80
13	1.1	1.5	74
Mean *SD*			100 *36**
Allograft			
5	2.6	2.4	110
6	1.2	2.1	60
7	1.7	1.5	110
8	0.59	2.7	22
9	2.4	3.7	65
10	0.80	2.7	30
12	0.61	2.0	30
Mean *SD*			61 *38**

**P* = 0.05, Student's independent *t*-test.

Table 4. Percentage of Type III collagen in graft tissue

Dogs	Experimental side	Control side	Percent
Autograft			
1	35	15	230
2	27	25	110
4	38	18	210
13	32	14	240
Mean *SD*	33 *4.7*	18 *5.0*	200 *60**
Allograft			
5	30	11	270
6	24	8.0	300
7	26	3.6	300
8	20	17	120
9	21	7.5	280
10	26	14	190
12	38	8.7	440
Mean *SD*	26 *6.2*	10 *4.5*	330 *200**

**P* = 0.12, Student's independent *t*-test.

ments (Table 4). Both groups had much greater percentage of Type III collagen than their controls, which averaged 13 percent.

Immunology. Fluorescein lymphocytotoxicity testing at 1 month postreconstruction could be done in 8 of the 11 allografted dogs. In the other 3 dogs, the long-term storage of the donor lymphocytes failed, as indicated by low viability of the frozen cells upon thawing. All 8 dogs were tested preoperatively and at 2 and 4 weeks. Three dogs had viable cells remaining at 8 weeks, and these were tested at that interval. All the dogs tested negative on preoperative samples, and 1 dog tested positive at 2 weeks in both the serum and synovial fluid.

Two other dogs showed a positive response at 4 weeks, and 1 additional dog showed a positive response when tested at 8 weeks, making a total of 4 dogs of 8 with a positive immune response in the synovial fluid and 3 of 8 with a positive immune response in the serum.

Histology. We saw no regions of lymphocytic infiltration, chronic inflammatory cells, or perivascular cuffing. The bone blocks incorporated well into the recipient bone. The cartilaginous portion of the allograft block remained intact within the deeper layers, but produced less proteoglycan material, indicated by a decrease in safranin-O staining. The ligament-bone junction appeared disorganized, with some vascular ingrowth present in both allografts and autografts. The orientation of fibers in the normal undulating pattern was seen only sporadically in both the allograft and the autograft ligaments. The autoradiographs of allografts in particular seemed to have areas of acellularity.

Discussion

The operative procedures for allografts and autografts were not identical, but roughly comparable. The autograft procedure is in routine clinical use, and the allograft procedure could be adopted clinically if it proved efficacious.

Even though the allografts consistently performed worse than the autografts in all the mechanical and biochemical parameters studied, they were repopulated with living cells and had substantial metabolic activity, suggesting that the incorporation of the allografts was simply delayed.

With regard to clinical parameters, both groups appeared to function equally well, but allograft dogs had more infections. This could reflect contamination of the implanted specimen or may be due to immunologic reasons. The uninfected allograft dogs functioned as well as the autografts in the clinical measurements of limp, laxity, range of motion, and mucin clot.

The apparent function of both allografts and autografts in the knees could be due in part to the dog's prominent ability to mount a massive fibrous-tissue response in the knee-joint capsule following arthrotomy. This fibrosis provides external support and stability to the knee, even when tested under anesthesia. Marshall and Olsson (1971) observed this in a study of the natural history of untreated ligament lesions in dogs. This would provide protection for the healing ligament and would minimize the effects of ligamentous instability. This fibrosis was marked after the two arthrotomies in our experiment: the anteromedial joint capsule was up to 1.2 cm thick in some dogs.

Shino (1984) found that allograft patellar tendon achieved approximately 30 percent tensile strength of unoperated on controls at 30 weeks. Webster and Werner (1983) found a similar level of strength at 8 months after implantation of freeze-dried allograft flexor tendons. Curtis et al. (1985) documented a gradual decline in strength of freeze-dried fascia lata allograft cruciate ligament replacements, with the mean load to failure dropping to only 14 percent of control at 12 weeks. Our finding that allograft ligaments had 17 percent of the control load to failure at 16 weeks is consistent with these studies.

The autografts in our group either did not drop in strength to such a marked degree, or regenerated more quickly. It is impossible to predict from our results at a single point of time (16 weeks) whether the allografts would ultimately obtain tensile strength equal to that of an autogenous replacement. Nikolaou et al. (1986) noted a similar, but less marked, lag in strength and histologic appearance of allografts at 4 months. Thus, it appears that it is necessary to protect allograft replacements far longer than one would protect a reconstruction using autogenous tissue. In addition, our allograft procedure substituted a relatively small amount of ligament; if we used more allogenous material, such as could be provided by an iliotibial band, the strength at autopsy might have been greater as suggested by other studies (Nikolaou et al. 1986, Shino et al. 1984).

Other investigators (Shino et al. 1984, Curtis et al. 1985, Arnoczky et al. 1986, Nikolaou et al. 1986) have failed to show any evidence of immune response in knee ligament allografts based on histologic and gross observation, as well as vascular-injection studies. We, too, found no histologic evidence of an immune reaction or chronic inflammation in either allografts or autografts, but we did find evidence of such a reaction by serum and synovial fluid antibody studies.

The failure of an immune response to occur in 4 of the 8 allograft dogs is unlikely to be due to genetic similarity between donor and host. Preoperative studies of mixed lymphocyte cultures would be necessary to verify this supposition. Vasseur et al. (1987) found a higher percentage of dogs exhibiting an immune response to ligament allografts.

The increased synovial leukocyte counts and joint cartilage erosion, decreased mechanical strength and metabolic activity, and evidence of immune response in our allograft dogs certainly do not support implantation of cadaver cruciate ligaments clinically.

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