THE IMMUNE RESPONSE TO SMALL OSTEOCHONDRAL ALLOGRAFTS DOES NOT AFFECT GRAFT SURVIVAL

+*Hurtig, M (A-Ontario Ministry of Agriculture, Food and Rural Affairs); *Pearce, S; *Dickey, J; *Runciman, J; **Miniaci, A
+*University of Guelph, Guelph, Ontario, Canada. Department of Clinical Studies, University of Guelph, Guelph, Ontario, Canada, N1G 2W1, 1 519 823 8840, Fax: 1 519 767 0311, nhurtig@uoguelph.ca

Introduction
Arthroscopic mosaic osteoplasty is one option for management of patients with symptomatic chondral defects of the knee1. This type of small, cylindrical, heterotopic osteochondral autograft can be successful in carefully selected patients2, the donor site morbidity associated with harvesting osteochondral dowels from the femoropatellar joint or intercondylar notch is seldom acknowledged3. Furthermore, in experimental animal models of heterotopic osteochondral autografts, there is little evidence to show that cartilage grafts adapt to the new biomechanical environment at the recipient site4. One alternative is the use of fresh or refrigerated isotopic osteochondral allografts. In animal models, these osteochondral dowels can be harvested and banked for at least two weeks without significant loss of chondrocyte viability5. Long term survival of large osteochondral allografts is well documented6, but the immune response is detrimental to their incorporation and survival7. This experiment uses a large animal model to study the survival of small osteochondral grafts delivered arthroscopically to the femoropatellar joint. Articular tissues, synovial fluid and serum were used to characterize the immune response to these grafts.

Methods
Under general anesthesia and using aseptic conditions for bilateral arthroscopic knee surgery, three 6.5mm or 4.5mm osteochondral grafts were transplanted to the trochlear ridges of the femoropatellar joint in eight horses. Horses over 450kg received 6.5mm diameter grafts and horses less than this weight received 4.5mm grafts. Three horses received a total of 9 autografts from the contralateral joint and five horses received 15 allografts harvested from other donor horses within 12 hours of death. Horses receiving allografts had diagnostic arthroscopy of the contralateral joint. Serial synovial fluid and serum samples were obtained for quantitation of IgG at 10 days, 1, 6 and 12 months post-operatively. Six months postoperatively the horses were re-anesthetized for arthroscopic biopsies of the graft cartilage and synovial membrane. Nine months postoperatively the horses began an exercise regime that included running for 20 minutes three days per week. One year postoperatively the horses were sacrificed and synovial membranes were dissected for analysis of CD21, CD3, CD4, CD8, IgG, fibrin and fibrinogen. The synovial membrane, including the cartilage, was transferred to the tissue culture chamber and cultured for 12 days in conditioned medium with 10% fetal calf serum. Viability was determined by trypan blue exclusion and lactate dehydrogenase leakage.

Results
Cartilage biopsies 6 months after implantation showed that allografts, autografts and adjacent cartilage had comparable chondrocyte viability. Synovial membrane biopsies at this time revealed scant perivascular lymphocyte infiltration in 2 of the five horses that received allografts. After 12 months all allografts still had chondrocyte populations comparable to autografts. Technical errors at the time of implantation resulted in tipped or recessed grafts, cartilage flow and repair tissue over 3 grafts in the control group and 4 grafts in the allograft group. When there was accumulation of fibrin, debris or connective tissue around the perimeter of allografts, this material contained small clusters of lymphocytes. Statistical comparison of autograft and allograft histological scores, subchondral trabecular bone area, serum and synovial fluid IgG, time constants for biomechanical indentation, total collagen, and total glycosaminoglycan were not significantly different. Time constants for indentation testing and total glycosaminoglycans were significantly less (P<.05) in both types of grafts compared to surrounding and contralateral unoperated sites. Type 2 collagen content was >95% in both types of grafts. Resorption and remodeling of subchondral allograft bone was complete 12 months postoperatively. Three of 5 allograft recipients had subchondral bone cellular infiltrate containing small numbers of CD3 positive lymphocytes. The synovial membrane of these horses contained small groups of lymphocytes staining for both IgG and CD3 markers.

Discussion
We concluded that the immune response to small osteochondral allografts is present but there is little impact on the survival of grafts 12 months postoperatively. We noted that accumulations of leukocytes in debris around the perimeter of allografts were more common in grafts that were tipped exposing the bony sidewall of the recipient hole. We hypothesize that the main determinant in recruitment of immune response may be damage to, or malalignment of the cartilage surfaces, resulting in unmasking of chondrocyte antigens. Some variability arose from technical errors that reduced the statistical power of our analysis, but when compared to autografts, small osteochondral allografts retained similar chondrocyte populations, histological architecture and biochemical constituents after a 12 month period. Loss of glycosaminoglycans from auto- and allografts may be due to surgical trauma and our inability to produce perfect congruence with the recipient site. There may also be more topographical variation in articular cartilage and subchondral bone than expected, making it difficult to produce true isotopic transfers. Though aseptic harvesting and screening for infectious diseases are disadvantages, osteochondral allografts avoid donor site morbidity and may be an alternative to autografts. Immune response had less impact on outcome than accuracy of graft delivery and fit in this experiment.

References

**Toronto Hospital, Western Division, Toronto, Ontario, Canada.