Histological Analysis of Human Meniscal Allografts : A Preliminary Report

Scott A. Rodeo, Aruna Seneviratne, Katsunori Suzuki, Kevin Felker, Thomas L. Wickiewicz and Russell F. Warren

J Bone Joint Surg Am. 2000;82:1071.

Histological Analysis of Human Meniscal Allografts

A Preliminary Report*

BY SCOTT A. RODEO, M.D.†, ARUNA SENEVIRATNE, M.D.†, KATSUNORI SUZUKI, M.D.‡, KEVIN FELKER, B.S.†, THOMAS L. WICKIEWICZ, M.D.†, AND RUSSELL F. WARREN, M.D.†

Investigation performed at the Laboratory for Soft Tissue Research, The Hospital for Special Surgery, New York, N.Y.

Abstract

Background: **Little is known about the biology of meniscal allograft transplantation in humans. In particular, little information is available about the phenotype of the cells that repopulate the allograft, whether an immune response is elicited against the graft, and whether the repopulating cells synthesize normal extracellular matrix components.**

Methods: **A small biopsy specimen of the meniscal allograft (twenty-eight menisci in twenty-five patients) and the adjacent synovial membrane (sixteen patients) was harvested during follow-up arthroscopy in patients who had undergone meniscal allograft transplantation at a mean of sixteen months earlier. Seventeen patients had undergone concomitant reconstruction of the anterior cruciate ligament with an allograft. Normal menisci (unimplanted allografts) and synovial specimens from age-matched controls were examined as well. All twenty-eight meniscal allografts were examined histologically. Immunohistochemical analysis was carried out on ten menisci and nine synovial specimens with use of monoclonal antibodies to class-I and class-II major histocompatibility complex antigens, CD-8, CD-11b, and CD-19 epitopes, as well as other epitopes, to demonstrate immunogenic macromolecules, cytotoxic T-lymphocytes, activated macrophages, and B-lymphocytes.**

Results: **Most of the specimens demonstrated incomplete repopulation with viable cells. The repopulating cells stained positively with phenotype markers for both synovial cells and fibroblasts. Polarized light microscopy demonstrated evidence of active remodeling of the matrix. The cells in frozen, unimplanted menisci stained positively for class-I and class-II human leukocyte antigens, indicating immunogenicity at the time of transplantation. Overall, nine of twelve specimens contained immunoreactive cells (B-lymphocytes or cytotoxic T-cells) in the meniscus or synovial tissue. However, only a small number of these cells was present. There was no evidence of frank immunological rejection. The clinical outcome (success or failure of the transplant) was not related to the overall histological score or to the presence of an immune response in the meniscal or synovial biopsy specimen.**

Conclusions: **Human meniscal allograft transplants are repopulated with cells that appear to be derived from the synovial membrane; these cells appear to actively remodel the matrix. Although there is histological evidence of an immune response directed against the transplant, this response does not appear to affect the clinical outcome. The presence of histocompatibility antigens on the meniscal surface at the time of transplantation (even after freezing) indicates the potential for an immune response against the transplant.**

Clinical Relevance: **Despite the absence of frank immunological rejection, a subtle immune reaction may affect the healing, incorporation, and revascularization of the graft. It is possible that the structural remodeling associated with cellular repopulation may render the meniscus more susceptible to injury.**

Both clinical and experimental investigations have clearly demonstrated the detrimental effects of meniscectomy[10,11]. These findings have provided the impetus for meniscal allograft transplantation in meniscus-deficient knees. The current indications for such transplantation include meniscal deficiency in patients with symptoms such as pain or swelling associated with early osteoarthritis (Outerbridge grades II and III[15]) in a stable and normally aligned knee. Currently, only limited information is available regarding the clinical outcomes of this procedure and little is known about the biology of meniscal allograft transplantation in humans[6,12,22]. The long-term function of a meniscal allograft transplant requires the presence of viable cells with the ability to synthesize extracellular matrix molecules. However, little is known about the phenotype of the cells that repopulate the allograft, whether an immune response is elicited against the allograft, and whether normal matrix components are synthesized by the transplanted meniscus.

Jackson et al.[8] used DNA probe analysis following fresh meniscal allograft transplantation in a goat model to demonstrate that, within one week, the donor cells in the allograft are replaced by host cells. By four weeks, no donor cells remained. Arnoczky et al.[1] examined meniscal allograft transplants in a dog model and reported that transplanted deep-frozen menisci were repopulated with cells that appeared to originate from the adjacent sy-

*No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article. No funds were received in support of this study.

†Laboratory for Soft Tissue Research, The Hospital for Special Surgery, 535 East 70th Street, New York, N.Y. 10021. E-mail address for S. A. Rodeo: rodeos@hss.edu.

‡Department of Orthopaedic Surgery, Oji General Hospital, 3-4-8 Wakakusa-cho, Tomakomai 053-8506, Japan.

TABLE I
CLINICAL DATA AND HISTOLOGICAL SCORES

Case	Gender, Age at Op. *(yrs.)*	Procedure*	Bone Plugs	Interval to Biopsy *(mos.)*	Histological Score† *(points)*	Immunological Score‡ *(points)*	Clinical Outcome§
1	M, 39	ACL, MM	+	13	4		Good
2	M, 24	LM	–	7	4		Good
3	F, 24	ACL, LM	+	27	NA	2	Good
4	F, 32	ACL, MM	+	47	2	1	Moderate
5	M, 39	ACL, MM	+	8	1		Moderate
6	M, 32	ACL, MM, LM	+	22	M-1, L-NA		Moderate
7	F, 33	ACL, MM	+	36	4		Moderate
8	M, 37	ACL, MM	+	12	2	1	Moderate
9	M, 29	ACL, MM	–	4	5		Moderate
10	M, 40	ACL, MM, LM	–	34	M-3, L-5	0	Moderate
11	M, 52	ACL, MM, LM	+	8	M-1, L-3		Moderate
12	F, 42	ACL, MM	+	12	3		Moderate
13	F, 25	LM	–	4	3	1	Moderate
14	M, 38	PTO, MM	+	4	3	0	Moderate
15	F, 25	LM	+	6	NA	1	Moderate
16	M, 17	ACL, MM	+	6	NA	1	Moderate
17	F, 23	ACL, MM, LM	–	21	M-5, L-5		Poor
18	M, 37	ACL, LM	–	4	2		Poor
19	F, 37	ACL, MM, LM	–	36	M-4, L-NA	0	Poor
20	M, 26	ACL, MM	+	6	3		Poor
21	M, 32	ACL, MM	+	5	0		Poor
22	M, 29	ACL, LM	–	21	5		Failed
23	M, 42	ACL, LM	–	4	1	1	Failed
24	M, 37	LM	+	6	2		Failed
25	M, 38	MM	+	4	2		Failed
26	M, 42	LM	–	40	3	1	Failed
27	F, 32	LM	+	26	3		Failed
28	M, 36	LM	+	21	2	2	Failed

*ACL = reconstruction of the anterior cruciate ligament, MM = medial meniscal transplantation, LM = lateral meniscal transplantation, and PTO = proximal tibial osteotomy.

†The histological scoring system is defined in a footnote in Table III. NA = no tissue available.

‡An immunological score of 0, 1, or 2 points was calculated by assigning 1 point for each cell type (B-lymphocytes or cytotoxic T-cells) in the biopsy specimen.

§Transplants graded as good or moderate were combined and classified as successful, and those graded as poor or fair were combined and classified as failed.

novial membrane. Cellular repopulation was first seen two weeks after transplantation. Although the degree of repopulation had progressed by six months, the central core remained acellular. By six months, the repopulating cells resembled meniscal fibrochondrocytes. It has been suggested that the repopulating cells are undifferentiated mesenchymal cells that eventually modulate into fibroblasts. It is not known whether the cells that repopulate the allograft function as normal meniscal fibrochondrocytes. There are very little biopsy data on human patients who have undergone meniscal transplantation[3,4].

Although most dense connective-tissue allografts are believed to be relatively "immunoprivileged" since the resident cells are embedded in a dense matrix and thus are not accessible to immunoreactive cells, histological and biochemical evaluations have demonstrated a subclinical immune response to connective-tissue allografts[5,19,20]. For example, a humoral response to both fresh-frozen and freeze-dried bone-patellar tendon-bone allografts has been demonstrated in patients[18,21]. Menisci are often transplanted with attached bone plugs; the attached bone probably increases the antigenic stimulus since frozen bone induces an immune response[2,5]. Furthermore, class-I and class-II major histocompatibility complex antigens have been shown to persist on the cells of a meniscus prepared for transplantation even after two freeze-thaw cycles, indicating that meniscal transplants are potentially immunogenic[9]. These observations suggest the possibility that an immune response in the host modulates the results of meniscal transplantation. Since synovitis, persistent effusions in the knee, delayed healing, and degeneration of the allograft and adjacent articular cartilage following meniscal transplantation have been observed in

TABLE II
PRIMARY ANTIBODIES USED FOR IMMUNOHISTOCHEMICAL STAINING

Antibody*	Specificity†	Clone (Source)
CD-3	T-lymphocytes	Clone SK7 (Becton Dickinson, San Jose, California)
CD-4	Helper T-lymphocytes	Clone SK3 (Becton Dickinson)
CD-8	Cytotoxic T-lymphocytes	Clone SK1 (Becton Dickinson)
CD-11b (C3bi receptor)	Activated macrophages	Clone 2LPM19c (Dako, Glostrup, Denmark)
CD-19 (leu 12)	B-lymphocytes	Clone 4G7 (Becton Dickinson)
CD-37	B-lymphocytes	Clone HH1 (Dako)
CD-43	T-cells, natural killer cells, granulocytes	Clone DF-T1 (Dako)
CD-57	Natural killer cells	Clone HNK-1 (Becton Dickinson)
CD-68	Macrophage/synoviocyte	Clone KP1 (Dako)
HLA-ABC	Class-I MHC antigens	Clone W6/32 (Accurate Chemical, Westbury, New York)
HLA-DR	Class-II MHC antigens	Clone FMC4 (Sera-Lab, Sussex, England)
5B5	β-subunit of prolyl 4-hydroxylase	Clone 5B5 (Dako)

*HLA = human leukocyte antigen.
†MHC = major histocompatibility complex.

some patients, it is possible that these phenomena are caused, at least in part, by an immune response against the allograft[6,12].

In the present study, we used histological and immunohistochemical techniques to examine biopsy specimens of human meniscal allografts and the adjacent synovial membrane to determine the phenotype of the cells that repopulate the transplant as well as whether there is an immune reaction against the transplant.

Materials and Methods

After obtaining informed consent, we took a small biopsy specimen from the transplanted meniscus and the adjacent synovial membrane of patients who had undergone meniscal allograft transplantation at our institution at a mean of sixteen months earlier. Normal menisci and synovial specimens from age-matched controls were examined as well. These tissues were processed for routine histological and immunohistochemical analysis with use of antibodies for specific cell types. The histological appearance of the meniscus and the presence of cells indicative of an immune response were correlated with the clinical outcome.

Patient Data

Between July 1989 and December 1995, forty-eight meniscal allografts were transplanted into forty-one patients at our institution. Fresh-frozen, nonirradiated, non-tissue-antigen-matched allografts were used in all patients. The allografts were obtained from the University of Miami Tissue Bank and were processed according to the standards of the American Association of Tissue Banks. Twenty-eight patients later underwent follow-up arthroscopy, either as part of the planned, routine evaluation of this new procedure (eighteen patients) or for the treatment of subsequent knee problems (ten patients). Biopsy specimens of the meniscus (twenty-eight menisci in twenty-five patients) and the synovial tissue adjacent to the meniscal transplant (sixteen patients) were obtained during follow-up arthroscopy (Table I).

The mean age of the patients at the time of meniscal allograft transplantation was thirty-four years (range, seventeen to fifty-two years). There were nineteen male and nine female patients. The mean interval from allograft transplantation to follow-up arthroscopy was sixteen months (range, four to forty-seven months). There were sixteen lateral and seventeen medial meniscal transplants (five patients received both medial and lateral meniscal transplants). Nineteen patients underwent concomitant reconstruction of the anterior cruciate ligament with a patellar tendon allograft obtained from the donor of the meniscus. Eighteen menisci were transplanted with bone plugs attached to the anterior and posterior horns; the other ten transplants did not contain any bone. The meniscal transplantation was performed with an arthroscopic-assisted technique in twenty-five patients and with an arthrotomy in three patients. Most patients had areas of grade-II and grade-III chondral degeneration, with focal areas of grade-IV degeneration in the involved tibiofemoral compartment[15].

All of the patients underwent comprehensive evaluation with magnetic resonance imaging, arthroscopic inspection, radiography, physical examination, and completion of standardized knee-rating scales. Each allograft was assessed objectively with arthroscopy, and magnetic resonance imaging was used to evaluate twenty menisci in twenty patients. The outcome in these patients was graded as good, moderate, poor, or failed on the basis of the objective evaluation of the transplant[17]; for the present study, the allografts were broadly classified as either successful (good or moderate) or failed (poor or failed) in order to facilitate correlation of the histological data with the clinical outcome. The factors assessed during arthroscopy included healing at the horn and capsular attachments, the presence of degeneration or tears, and the presence of any extrusion from the tibiofemoral compartment. The factors assessed on magnetic resonance imaging included intrameniscal signal, healing at the horn and capsular attachments, the presence of degeneration or tears, and the presence of extrusion[16].

Specimen Collection

Two three-by-four-millimeter biopsy specimens were taken from normal-appearing regions of the inner portion of the meniscus with a small biopsy punch. Twenty-eight meniscal specimens from twenty-five patients were prepared for routine histological examination, and ten meniscal specimens were prepared for immunohistochemical evaluation. A biopsy specimen from the synovial tissue adjacent to the meniscal allograft (within eight millimeters of the meniscocapsular junction) was also taken from sixteen patients for routine histological examination and from nine patients for immunohistochemical evaluation. Immunohistochemical analysis was performed on either the meniscal or the synovial specimen from twelve different patients; these analyses were performed on only a subset of the patients because of limitations in the size of the tissue that was available for analysis. Normal synovial tissue was obtained from seven age-matched patients with no evidence of synovial inflammation or other synovial pathology who were undergoing an arthroscopic procedure on the knee for reasons unrelated to meniscal pathology. Nine normal menisci from age-matched individuals were provided by the University of Miami Tissue Bank for use as controls. These menisci had been processed and handled in exactly the same way as the implanted me-

nisci. The tissues were prepared for routine histological examination by fixation in 10 percent neutral buffered formalin followed by embedding in paraffin. Five-micrometer-thick sections were cut and stained with hematoxylin and eosin.

Immunohistochemical Analysis

The tissue was embedded in OCT tissue-embedding medium (Tissue Tek; Miles Laboratories, Elkhart, Indiana), frozen in liquid nitrogen, and stored at –80 degrees Celsius until sectioning. Six-micrometer-thick frozen sections were cut onto glass microscope slides and fixed in 100 percent acetone at room temperature for ten minutes. Immunohistochemical analysis was performed with standard immunoperoxidase techniques. The slides were air-dried for five to ten minutes and then rehydrated with 0.05 percent Tween 80-Tris-buffered saline solution (NaCl, Trizma base [Tris(hydroxymethyl)-aminomethane], and 0.1 percent Tween 20 [polyoxyethylene sorbitan monolaurate]; Sigma Chemical, St. Louis, Missouri) at a pH of 7.4 for five minutes. Endogenous peroxidase activity was blocked with 0.3 percent hydrogen peroxide in methanol for twenty minutes, and nonspecific binding sites were blocked with 3 percent normal goat serum (Vector Laboratories, Burlingame, California) in 0.1 percent Tween 80-Tris-buffered saline solution for ten minutes. The tissue section was then incubated for fifty minutes with the appropriate dilution of the primary antibody at room temperature. Negative control sections consisted of serial sections in which 3 percent normal goat serum in 0.1 percent Tween 80-Tris-buffered saline solution was substituted for the primary antibody.

After rinsing off the primary antibody with 0.05 percent Tween 80-Tris-buffered saline solution, the sections were incubated for twenty-five minutes at room temperature with a biotinylated secondary antibody (Dako, Glostrup, Denmark). The slides were then incubated with ABC (streptavidin/biotinylated horseradish peroxidase complex; Dako) for twenty-five minutes at room temperature, and the reaction product was detected with the addition of 0.1 percent 3,3′-diaminobenzidine tetrachloride containing 0.018 percent hydrogen peroxide for two serial incubations of seven minutes each. The slides were washed three times with 0.05 percent Tween 80-Tris-buffered saline solution between each step. The sections were counterstained with Meyer's hematoxylin for two minutes and rinsed in tap water until clear, followed by dehydration with a graded series of alcohol. The alcohol was cleared with two washes of xylene, and then a coverslip was applied with Permount (Fisher Scientific, Pittsburgh, Pennsylvania). The slides were examined with light microscopy, and photomicrographs were made. Specific antibodies were used to identify cell types and components of an immune response (Table II). Appropriate positive control tissue was used for each antibody to establish the specificity, appropriate dilutions, and optimal incubation times for each antibody. Positive and negative control sections were run in parallel with the experimental sections to confirm the specificity of the staining results.

Specimen Evaluation

The sections subjected to routine histological analysis were examined under light and polarized light microscopy to identify the cell types (fibrochondrocytes, fibroblasts, mononuclear synovial cells, and inflammatory cells), the overall matrix organization, and signs of matrix degeneration (disorganized collagen and chondromucoid appearance). Newly synthesized collagen fibrils were detected, with use of polarized light microscopy, as thin, fine fibrils compared with the thicker fibers of native meniscus. For the immunohistochemical analysis, sections from both the synovial and the meniscal specimens were examined for specific staining in cells and blood vessels. Cellular staining was graded as positive if specific staining was seen for a given antibody. Positive staining around blood vessels was recorded if there was staining around more than 50 percent of the blood vessels per medium-power field (magnification, × 200).

Each specimen was given a histological score of 0 to 6 points according to a scale in which cellularity, predominant cell type, collagen organization, and matrix morphology were considered (Table III). The presence of an immune response was noted by identification of B-

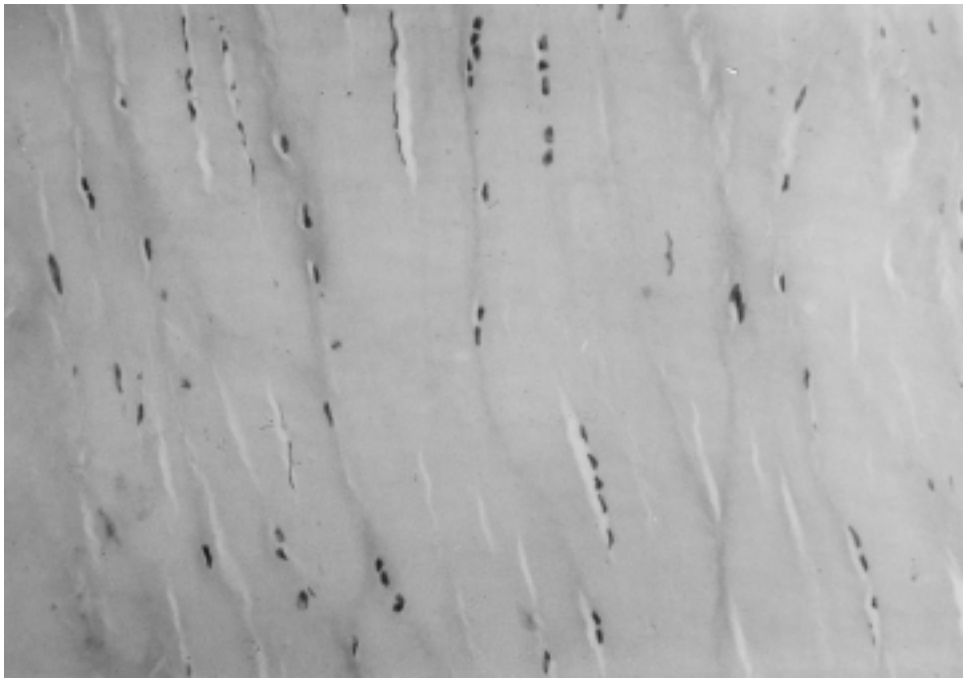

FIG. 1-A

Figs. 1-A, 1-B, and 1-C: Case 24. Meniscal and synovial biopsy specimens (hematoxylin and eosin, × 200).

Fig. 1-A: Photomicrograph of a meniscal allograft biopsy specimen before transplantation, showing meniscal fibrochondrocytes embedded in a dense matrix. These cells were nonviable because of the rapid freezing process, and it was presumed that they would disappear shortly after implantation.

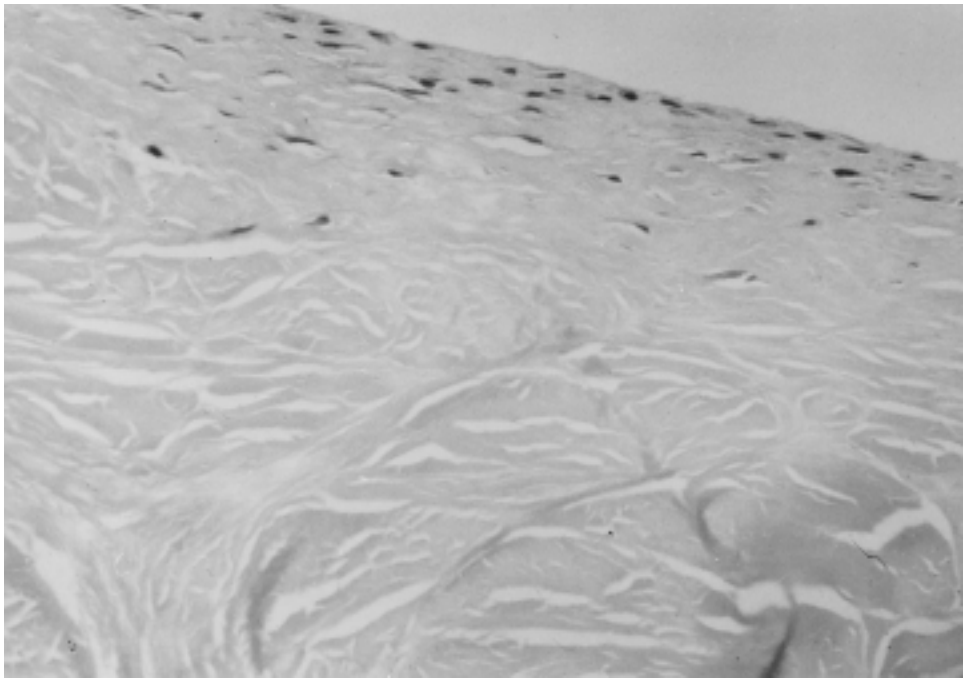

FIG. 1-B

Photomicrograph of a meniscal allograft biopsy specimen six months after transplantation, showing cells at the edge of the tissue. The deeper tissue remained acellular.

lymphocytes or cytotoxic T-lymphocytes in the meniscal or synovial sections. An immunological score of 0, 1, or 2 points was calculated by assigning 1 point for each cell type present. The specimens were examined in a blinded fashion by two examiners (S. A. R. and A. S.). The Mann-Whitney rank-sum test was used to compare histological scores between different clinical groups, and the Spearman rank-order correlation test was used to correlate the histological grade with the duration between meniscal transplantation and biopsy.

Results

Histological Analysis (Tables I and III)

Normal menisci contain two populations of cells: spindle-shaped, fibroblastic cells on the meniscal surface and rounder fibrochondrocytes in the interior. Unimplanted menisci that have been frozen and then thawed usually contained normal-appearing cells on both the

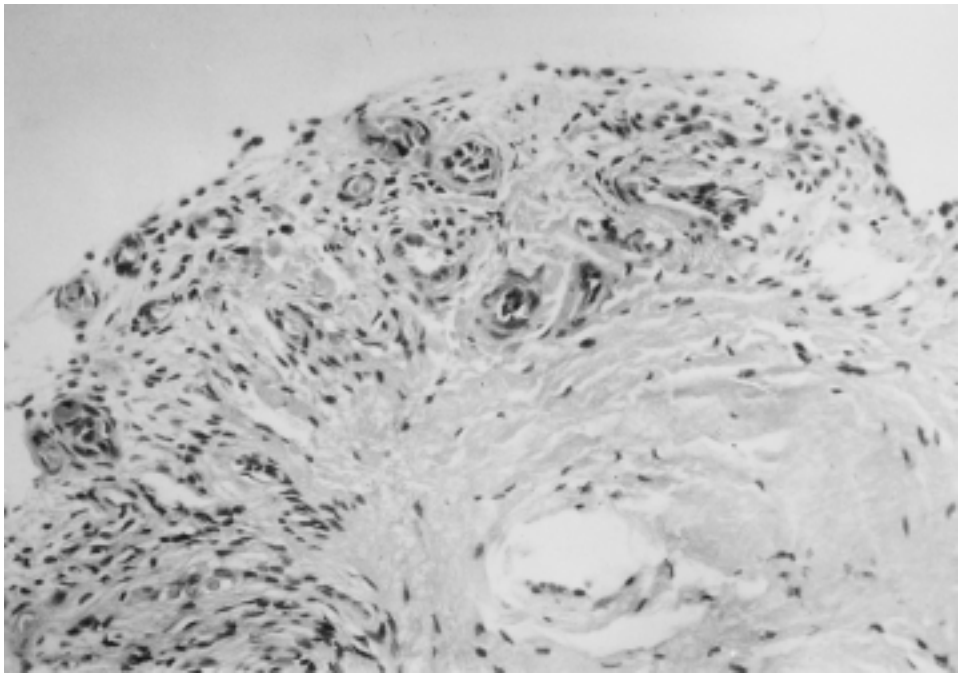

FIG. 1-C

Photomicrograph of a synovial biopsy specimen six months after meniscal transplantation. There is moderate hyperplasia with proliferation of the lining cells, which is consistent with nonspecific synovitis and chronic inflammation.

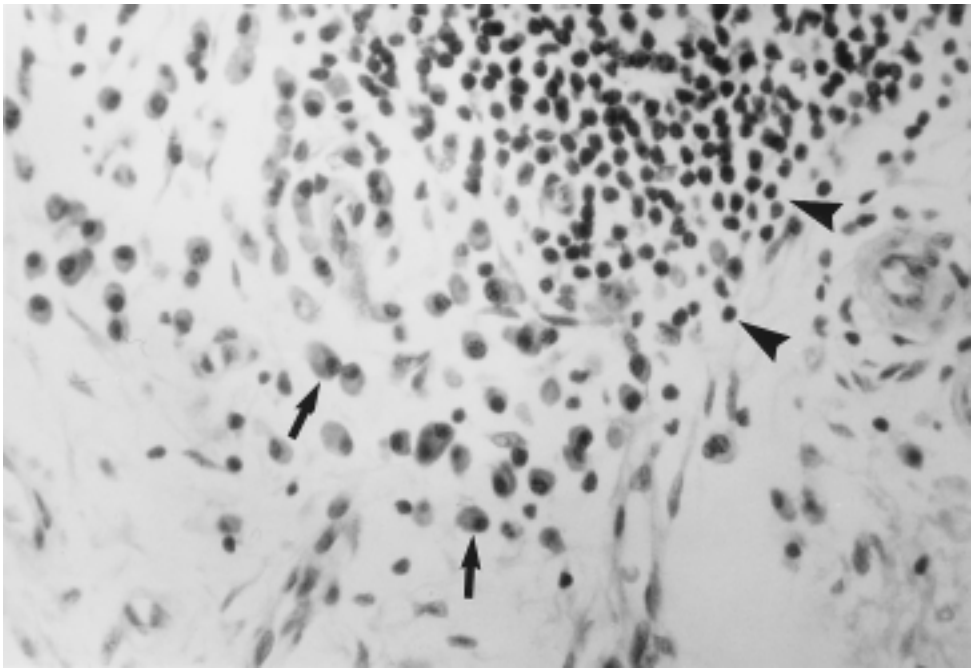

FIG. 2

Case 23. Photomicrograph of a synovial biopsy specimen four months after meniscal allograft transplantation, showing lymphocytes (arrowheads) and plasma cells (arrows) (hematoxylin and eosin, × 400).

surface and the deeper layers (Fig. 1-A). However, despite their normal appearance these cells are nonviable because of the rapid freezing process.

Most (twenty-five) of the twenty-eight implanted meniscal allograft specimens had viable-appearing cells in the meniscal tissue. There was incomplete cellular repopulation, with more cells at the periphery. The central area was often hypocellular or acellular. Regions with normal cellularity were found adjacent to acellular areas (Fig. 1-B). The repopulating cells resembled mononuclear synovial cells, fibroblasts, or fibrochondrocytes. All three cell types were sometimes found in a single specimen. The overall appearance of the specimens suggested repopulation with synovial cells.

The synovial biopsy specimens demonstrated moderate hyperplasia with proliferation of the lining cells, which is consistent with nonspecific synovitis and chronic inflammation (Fig. 1-C). Localized infiltrates of inflammatory cells (lymphocytes and plasma cells) were found in two meniscal specimens and in two synovial specimens (Fig. 2). The plasma cells identified in one of these synovial specimens contained Russell bodies, indicating immunoglobulin production. The three patients from whom these specimens were obtained all had a failed transplant according to clinical criteria. A few foreign-body giant cells were seen in one meniscal specimen and two synovial specimens. There was variable collagen-fiber disorganization and focal areas of chondromucoid degeneration. Poorly organized matrix was usually observed in areas with cellular repopulation, which is consistent with matrix remodeling. Polarized light microscopy demonstrated fine, newly synthesized collagen fibrils in areas with cells; there were coarser collagen fibers (presumably native collagen structure) in acellular areas (Fig. 3).

Immunohistochemical Staining

Normal, unimplanted menisci: Normal, unimplanted menisci stained positively with CD-68 (a stain for macrophages and macrophage-derived synoviocytes) and 5B5 (a fibroblast marker) only in cells at the peripheral capsular attachment, while cells in the interior of the meniscus were negative. There was no B-cell staining (CD-19 and CD-37), but there were a few cells around vessels and a few synovial cells at the capsular attachment that stained with T-cell markers (CD-3, CD-4, and CD-43). These cells appeared to be helper T-cells (CD-4-positive). There was no staining for CD-8 cells (cytotoxic cells) or CD-57 cells (natural killer cells). Endothelial cells and synovial cells in unimplanted menisci stained positively for human leukocyte antigen (HLA) class-I (HLA-ABC) and class-II (HLA-DR) major histocompatibility complex antigens, while meniscal fibrochondrocytes in unimplanted menisci stained positively only for class-I antigens. No meniscal cells stained with CD-11b, a marker for activated macrophages.

Meniscal allografts: Biopsy specimens from meniscal allografts had repopulation with cells that stained positively with CD-68 and 5B5. Cells throughout the biopsy sample stained with these markers. Overall, six of the ten meniscal specimens contained immunoreactive cells (B-lymphocytes or cytotoxic T-cells). There were occasional cells that stained with one of the B-cell markers (three of nine were positive for CD-19, and six of ten were

TABLE III
HISTOLOGICAL SCORING SYSTEM

Case	Procedure†	Histological Data*			
		Cellularity‡	Predominant Cell Type	Collagen Organization	Matrix Morphology
1	ACL, MM	Normal (increased)	Fibroblast	Well organized	Chondromucoid degeneration
2	LM	Decreased	Fibrochondrocytes	Well organized	Chondromucoid degeneration
3	ACL, LM	NA			
4	ACL, MM	Acellular	Acellular biopsy	Well organized	Normal (fibrous)
5	ACL, MM	Decreased	Mononuclear/ inflammatory	Disorganized	Chondromucoid degeneration
6	ACL, MM§, LM	Decreased	Mononuclear/ inflammatory	Disorganized	Chondromucoid degeneration
7	ACL, MM	Decreased	Fibrochondrocytes	Well organized	Chondromucoid degeneration
8	ACL, MM	Decreased	Fibroblast	Disorganized	Chondromucoid degeneration
9	ACL, MM	Normal (increased)	Fibroblast	Well organized	Normal (fibrous)
10 (medial transplant)	ACL, MM, LM	Decreased	Fibroblast	Disorganized	Normal (fibrous)
10 (lateral transplant)	ACL, MM, LM	Decreased	Fibrochondrocytes	Well organized	Normal (fibrous)
11 (medial transplant)	ACL, MM, LM	Acellular	Acellular biopsy	Well organized	Chondromucoid degeneration
11 (lateral transplant)	ACL, MM, LM	Decreased	Fibroblast	Well organized	Chondromucoid degeneration
12	ACL, MM	Decreased	Fibroblast	Well organized	Chondromucoid degeneration
13	LM	Decreased	Fibroblast	Disorganized	Normal (fibrous)
14	PTO, MM	Decreased	Fibroblast	Well organized	Chondromucoid degeneration
15	LM	NA			
16	ACL, MM	NA			
17 (medial transplant)	ACL, MM, LM	Normal (increased)	Fibrochondrocytes	Well organized	Chondromucoid degeneration
17 (lateral transplant)	ACL, MM, LM	Decreased	Fibrochondrocytes	Well organized	Normal (fibrous)
18	ACL, LM	Decreased	Fibroblast	Disorganized	Chondromucoid degeneration
19	ACL, MM§, LM	Decreased	Fibrochondrocytes	Well organized	Chondromucoid degeneration
20	ACL, MM	Decreased	Fibroblast	Disorganized	Normal (fibrous)
21	ACL, MM	Acellular	Acellular biopsy	Disorganized	Chondromucoid degeneration
22	ACL, LM	Normal (increased)	Fibrochondrocytes	Well organized	Chondromucoid degeneration
23	ACL, LM	Decreased	Mononuclear/ inflammatory	Disorganized	Chondromucoid degeneration
24	LM	Decreased	Fibroblast	Disorganized	Chondromucoid degeneration
25	MM	Decreased	Fibroblast	Disorganized	Chondromucoid degeneration
26	LM	Normal (increased)	Mononuclear/ inflammatory	Disorganized	Normal (fibrous)
27	LM	Normal (increased)	Mononuclear/ inflammatory	Well organized	Chondromucoid degeneration
28	LM	Decreased	Fibroblast	Disorganized	Chondromucoid degeneration

*A total histological score of 0 to 6 points was assigned according to a scale in which cellularity, predominant cell type, collagen organization, and matrix morphology were considered. For cellularity, 0 points = acellular, 1 point = incomplete cellular repopulation, and 2 points = normal cellularity. For predominant cell type, 0 points = clusters of mononuclear or inflammatory cells, 1 point = fibroblasts, and 2 points = fibrochondrocytes. For collagen organization, 0 points = disorganized, and 1 point = well organized. For matrix morphology, 0 points = chondromucoid degeneration, and 1 point = normal (fibrous).

†ACL = reconstruction of the anterior cruciate ligament, MM = medial meniscal transplant, LM = lateral meniscal transplant, and PTO = proximal tibial osteotomy.

‡NA = no tissue available.

§The histological data are for the medial meniscus.

positive for CD-37), and there were a few helper and cytotoxic T-cells (four of eight were positive for CD-3, four of eight were positive for CD-4, and two of nine were positive for CD-8) in the meniscal allografts (Fig. 4). Seven of nine meniscal allografts contained cells that stained for CD-11b, but there were no natural killer cells

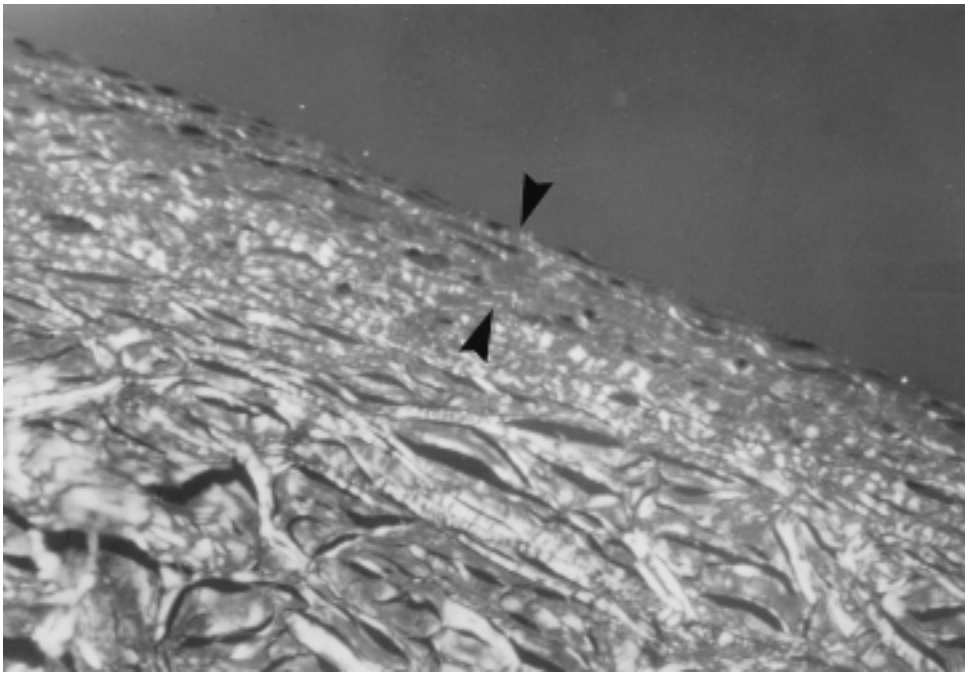

FIG. 3

Case 24. Polarized light microscopic view of the meniscal allograft biopsy specimen shown in Fig. 1-B. This view demonstrates coarse, thick collagen fibers in the central, acellular area of the tissue, in contrast to thin, fine collagen fibrils at the edge of the tissue, which is suggestive of newly synthesized matrix (between arrowheads) (hematoxylin and eosin, × 200). This newly synthesized matrix corresponds exactly to the area of cellular repopulation.

(CD-57). These immune cells were usually present in clusters of several cells or individually; there were no extensive infiltrates of these cells. The cells that repopulated the allograft stained positively for both class-I and class-II histocompatibility antigens, in contrast to no staining for class-II antigens in normal meniscal fibrochondrocytes. The frequency of class-II staining was higher than the frequency of immunoreactive cells, making it unlikely that only activated T-cells or B-cells in the meniscus were staining for class-II antigens.

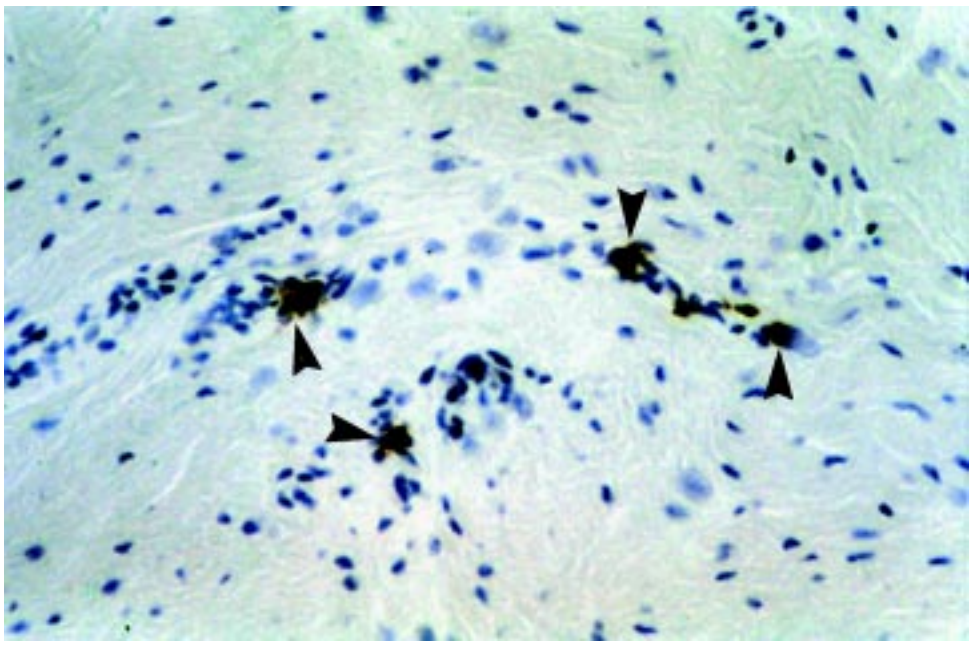

FIG. 4

Case 28. Immunohistochemical preparations of meniscal allograft tissue stained with antibody against cytotoxic T-lymphocytes (CD-8), demonstrating several positive cells (arrowheads) (hematoxylin counterstain, × 200). This meniscal specimen was taken twenty-one months after transplantation.

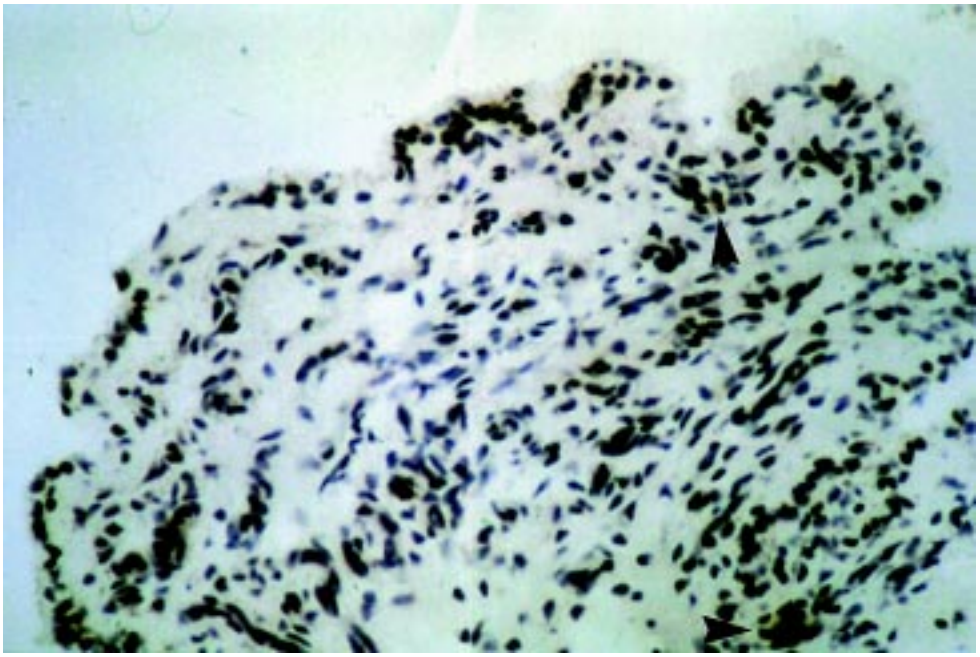

FIG. 5-A

Figs. 5-A and 5-B: Case 3. Immunohistochemical preparations of synovial tissue stained with antibody against cytotoxic T-lymphocytes (CD-8) (hematoxylin counterstain, × 200).
Fig. 5-A: Normal synovial tissue contains several cells that stain lightly (arrowheads).

Synovial specimens: Normal synovial tissue contained occasional T-lymphocytes (both helper and cytotoxic cells) and rare B-lymphocytes. There were cells that stained positively as activated macrophages (CD-11b), but there was no staining for natural killer cells in the normal synovial tissue. Overall, three of eight synovial biopsy specimens from allograft recipients stained more frequently for immunoreactive cells (B-lymphocytes and cytotoxic T-cells) than the normal synovial tissue did. The biopsy specimens from these three patients contained B-lymphocytes and T-lymphocytes (CD-3, CD-8, and CD-43), which appeared to be cytotoxic T-cells (CD-8) since there was no increase in CD-4 staining (Figs. 5-A and 5-B). The synovial biopsy specimens from the allograft recipients also contained some natural killer cells (CD-57), but there was no difference in the frequency of

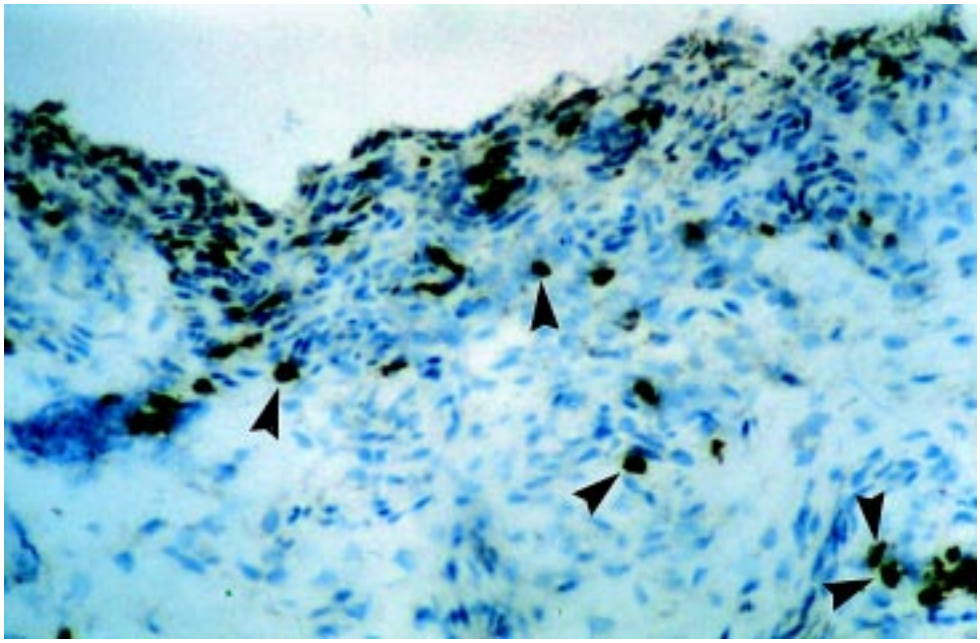

FIG. 5-B

There are positive cells (arrowheads) in this synovial biopsy specimen taken twenty-seven months after meniscal transplantation.

staining for activated macrophages compared with that in the normal synovial tissue.

Correlations with Clinical Data

There was no significant difference in the overall histological score between successful and failed transplants ($p = 0.88$, Mann-Whitney rank-sum test). There was no correlation between the overall histological score and the duration between the meniscal transplantation and the biopsy ($r^2 = 0.29$ and $p = 0.14$, Spearman rank-order correlation test). The average histological score was significantly better for meniscal transplants without attached bone plugs (3.5 points) than it was for transplants with bone plugs (2.3 points) ($p = 0.004$). There was no significant difference in the histological score between the patients who underwent concomitant bone-patellar tendon-bone allograft transplantation for reconstruction of the anterior cruciate ligament and those who did not ($p = 0.39$).

There was no significant difference in clinical outcome (successful versus failed transplants) between the nine patients who had evidence of an immune response (B-lymphocytes or cytotoxic T-cells) in the meniscal or synovial biopsy specimen and the three who did not. However, the histological score was significantly better for the transplants with no evidence of an immune response compared with the transplants with evidence of an immune response ($p = 0.015$, Mann-Whitney rank-sum test). The presence of a concomitant anterior cruciate ligament reconstruction or bone plugs attached to the meniscal transplant did not correlate with histological evidence of an immune response.

Discussion

To our knowledge, the present study is the first to use cell-specific markers to examine the phenotype of the cells that repopulate a human meniscal allograft transplant. It is important to note the limitations of biopsy in patients. Only a small piece of the meniscus could be obtained from most patients, and there was no way to be certain that the biopsy specimen was representative of the entire graft. Some patients had symptoms referable to the knee, while others were asymptomatic and underwent arthroscopic inspection as part of a planned, routine evaluation of this new procedure. The results of our study indicate that the cells that repopulate the transplant are probably derived from the synovial membrane. These results support those of animal studies that demonstrated synovial-cell repopulation of frozen meniscal transplants[1]. It cannot be stated with certainty that the repopulating cells are of synovial origin, since there is no single marker that is specific only for synovial cells. It is possible that the repopulating cells are marrow-derived monocytes that assumed a synovial-cell phenotype; however, support for a synovial-cell origin of the repopulating cells is provided by CD-68-positive staining, staining for class-II histocompatibility antigens (which are expressed on synovial cells but not on meniscal fibrochondrocytes), and some biopsy specimens in which there were cells at the periphery but not in the central core of the tissue. The menisci in our patients were transplanted without viable cells, as all were fresh-frozen. It is likely that, even with cryopreserved tissue (with viable cells at the time of transplantation), the cells that eventually repopulate the transplant are derived from host synovial tissue. Jackson et al.[8] reported that no donor DNA remained four weeks after fresh meniscal transplants were used in a goat model, and de Boer and Koudstaal[4] found no staining with cell proliferation markers in an analysis of three failed human cryopreserved meniscal transplants.

Normal meniscal cells demonstrate both fibroblastic and fibrochondrocytic features, which vary in different regions of the meniscus, while the cells that repopulate the allograft appear more fibroblastic than fibrochondrocytic. Noyes[13] also reported that the cells in biopsy specimens from human meniscal allografts were generally fibroblastic rather than fibrochondrocytic. Noyes examined only biopsy specimens from failed meniscal transplants, while we were able to examine small biopsy specimens from intact transplants. It is important to note that there is no unique cell-specific marker for meniscal cells. Identifying the exact phenotype of the repopulating cells requires analysis of the RNA produced by these cells in comparison with the RNA produced by normal meniscal fibrochondrocytes.

Our histological analysis did not conclusively demonstrate that the cells in the transplant were viable. Special staining techniques are required to prove cell viability. However, there was evidence of new matrix synthesis around the repopulating cells, suggesting that these cells were viable. Also, it is known that the nonviable cells that are present in the tissue at the time of transplantation lyse and disappear after transplantation. Since the earliest biopsy specimen was obtained four months after transplantation, it is quite unlikely that any (dead) donor cells still existed in the tissue at that time. Furthermore, the pattern of cellularity in the biopsy specimens differed sufficiently from that in a normal meniscus to suggest that these were the cells that repopulated the meniscus.

Satisfactory function of a tissue transplant requires the presence of viable cells that can synthesize and remodel the extracellular matrix and repair microscopic damage. Our histological analysis demonstrated ongoing matrix remodeling by the repopulating cells. There was prominent staining with an antibody directed against prolyl 4-hydroxylase, an enzyme involved in collagen synthesis. Newly synthesized fine collagen fibrils were seen in areas with repopulating cells. Similarly, Wada et al.[25], who transplanted deep-frozen meniscal allografts into rabbit knees, found active collagen remodeling by the repopulating cells as indicated by the expression of type-III

procollagen messenger RNA and an increased content of the reducible collagen cross-link dihydroxylysinonorleucine. Arnoczky et al.[1] also reported collagen remodeling associated with cellular repopulation, in frozen meniscal transplants in a dog model.

Although there was no evidence of frank immunological rejection, histological analysis demonstrated the possibility of a low-level immune response directed against the transplanted meniscus. Several meniscal biopsy specimens contained a few B-lymphocytes, cytotoxic T-cells, and activated macrophages, which were not present in the control menisci. Similarly, three of eight synovial biopsy specimens demonstrated B-lymphocytes, cytotoxic T-cells, and natural killer cells. Although some foreign-body giant cells were seen, these may just have been a response to suture. We found that frozen menisci at the time of the transplantation expressed both class-I and class-II histocompatibility antigens, as reported previously by Khoury et al.[9]. The presence of these histocompatibility antigens at the time of transplantation indicates the potential for an immune response, since class-II antigens act as antigen-presenting cells and class-I antigens activate T-lymphocytes[20]. At the time of the operation, this human-leukocyte-antigen-containing meniscal graft is attached to the host synovial membrane, which contains abundant immunocompetent cells. Furthermore, most meniscal allografts are currently transplanted with attached bone plugs, which also increases the antigenic load since frozen bone allografts elicit an immune response[2]. Of note, we found that the histological score was significantly better for meniscal transplants without attached bone plugs than for transplants with bone plugs ($p = 0.004$). However, we found better clinical results when the meniscal allograft had been transplanted with bone plugs at the horn attachments, probably because of better fixation to the tibia. Definitive identification of a cellular immune response would require assaying a mixed lymphocyte culture, and identification of humoral immunity would require a complement-dependent lymphocytotoxicity test.

Previous studies support the possibility of an immune response to meniscal transplants. Van Arkel et al.[23] demonstrated sensitization to class-I and class-II human leukocyte antigens in eleven of eighteen recipients of cryopreserved, non-tissue-antigen-matched meniscal allografts. There was no clinical evidence of rejection in these patients. Ochi et al.[14] studied the cellular and humoral immune response to fresh meniscal allograft transplants into a subcutaneous location in mice and concluded that there was no significant immune response. However, they reported elevated antibody titers against allogenic major histocompatibility antigen in two mice and postulated that this was due to so-called contamination of the synovial tissue at the edge of the meniscus. Hamlet et al.[7] reported a case of presumed acute rejection of a cryopreserved, non-tissue-antigen-matched meniscal allograft. De Boer and Koudstaal[3] reported hyperplasia and a mild inflammatory response with T-lymphocytes in a synovial biopsy specimen from a patient who had undergone previous transplantation of a cryopreserved, non-tissue-antigen-matched meniscal allograft. This patient had a good clinical result one year postoperatively. Antibody to donor human leukocyte antigens has been demonstrated in serum in human patients following reconstruction of the anterior cruciate ligament with a patellar tendon allograft[18,21] and in synovial fluid following insertion of frozen anterior cruciate ligament allografts and osteochondral allografts in dogs[19,24].

Although we found histological evidence of an immune response against the meniscal transplant, the clinical importance of this finding is unclear. Neither we nor other investigators[4,13,17,23] observed clinical signs of rejection. Immunological rejection is associated with extensive cellular infiltration, which was not seen in any of our patients. With the numbers available in our study, the overall clinical outcome did not differ between the patients with and those without immunoreactive cells in their meniscal or synovial biopsy specimens. The presence of a concomitant patellar tendon allograft for reconstruction of the anterior cruciate ligament (which may increase the antigenic load) did not affect the overall clinical or histological outcome. It is likely that multiple factors determine the outcome following meniscal transplantation, including the degree of arthrosis, other concomitant pathology, and alignment and stability of the knee. Since most patients in this series had at least moderate chondral degeneration in the involved tibiofemoral compartment, it is likely that biomechanical factors have a major influence on the clinical outcome. Despite the absence of frank immunological rejection, observations such as delayed graft-healing, horn detachment, persistent effusions in the knee, graft shrinkage, and graft degeneration may be due, at least in part, to a subtle immune response directed against the graft. Such an immune reaction may modulate healing, incorporation, and revascularization of the graft.

Future studies with synovial-fluid analysis for anti-donor human leukocyte antigens are needed to document an immune response.

In conclusion, our histological analysis of biopsy specimens from meniscal allografts and the adjacent synovial tissue demonstrated repopulation with cells that appeared to be derived from the synovial tissue. These cells had some phenotypic dissimilarities compared with normal meniscal fibrochondrocytes. The repopulating cells actively remodeled the extracellular matrix. It is possible that the structural remodeling associated with cellular repopulation may render the meniscus more susceptible to injury. There was histological evidence of an immune response directed against the allograft; however, such an immune response did not appear to affect the clinical outcome in our patients.

References

1. **Arnoczky, S. P.; DiCarlo, E. F.; O'Brien, S. J.;** and **Warren, R. F.:** Cellular repopulation of deep-frozen meniscal autografts: an experimental study in the dog. *Arthroscopy,* 8: 428-436, 1992.
2. **Bos, G. D.; Goldberg, V. M.; Zika, J. M.; Heiple, K. G.;** and **Powell, A. E.:** Immune responses of rats to frozen bone allografts. *J. Bone and Joint Surg.,* 65-A: 239-246, Feb. 1983.
3. **de Boer, H. H.,** and **Koudstaal, J.:** The fate of meniscus cartilage after transplantation of cryopreserved nontissue-antigen-matched allograft. A case report. *Clin. Orthop.,* 266: 145-151, 1991.
4. **de Boer, H. H.,** and **Koudstaal, J.:** Failed meniscus transplantation. A report of three cases. *Clin. Orthop.,* 306: 155-162, 1994.
5. **Friedlaender, G. E.; Strong, D. M.;** and **Sell, K. W.:** Studies on the antigenicity of bone. I. Freeze-dried and deep-frozen bone allografts in rabbits. *J. Bone and Joint Surg.,* 58-A: 854-858, Sept. 1976.
6. **Garrett, J. C.; Steensen, R. N.;** and **Steensen, R. N.:** Meniscal transplantation in the human knee: a preliminary report. *Arthroscopy,* 7: 57-62, 1991.
7. **Hamlet, W.; Liu, S. H.;** and **Yang, R.:** Case report: destruction of a cryopreserved meniscal allograft: a case for acute rejection. *Arthroscopy,* 13: 517-521, 1997.
8. **Jackson, D. W.; Whelan, J.;** and **Simon, T. M.:** Cell survival after transplantation of fresh meniscal allografts. DNA probe analysis in a goat model. *Am. J. Sports Med.,* 21: 540-550, 1993.
9. **Khoury, M. A.; Goldberg, V. M.;** and **Stevenson, S.:** Demonstration of HLA and ABH antigens in fresh and frozen human menisci by immunohistochemistry. *J. Orthop. Res.,* 12: 751-757, 1994.
10. **Kurosawa, H.; Fukubayashi, T.;** and **Nakajima, H.:** Load-bearing mode of the knee joint: physical behavior of the knee joint with or without menisci. *Clin. Orthop.,* 149: 283-290, 1980.
11. **McGinty, J. B.; Geuss, L. F.;** and **Marvin, R. A.:** Partial or total meniscectomy: a comparative analysis. *J. Bone and Joint Surg.,* 59-A: 763-766, Sept. 1977.
12. **Milachowski, K. A.; Weismeier, K.;** and **Wirth, C. J.:** Homologous meniscus transplantation. Experimental and clinical results. *Internat. Orthop.,* 13: 1-11, 1989.
13. **Noyes, F. R.:** A histological study of failed human meniscal allografts. Presented at Arthroscopy Association of North America Specialty Day, Orlando, Florida, Feb. 19, 1995.
14. **Ochi, M.; Ikuta, Y.; Ishida, O.;** and **Akiyama, M.:** Cellular and humoral immune responses after fresh meniscal allografts in mice. A preliminary report. *Arch. Orthop. and Trauma Surg.,* 112: 163-166, 1993.
15. **Outerbridge, R. E.:** The etiology of chondromalacia patellae. *J. Bone and Joint Surg.,* 43-B(4): 752-757, 1961.
16. **Potter, H. G.; Rodeo, S. A.; Wickiewicz, T. L.;** and **Warren, R. F.:** MR imaging of meniscal allografts: correlation with clinical and arthroscopic outcomes. *Radiology,* 198: 509-514, 1996.
17. **Rodeo, S.; Potter, H.; Berkowitz, M.; Sultan, P.; Wickiewicz, T.;** and **Warren, R.:** A clinical and objective evaluation of meniscal allograft transplantation: minimum two-year follow-up. *Orthop. Trans.,* 22: 74, 1998-1999.
18. **Rodrigo, J. J.; Jackson, D. W.; Simon, T. M.;** and **Muto, K. N.:** The immune response to freeze-dried bone-tendon-bone ACL allografts in humans. *Am. J. Knee Surg.,* 6: 47-53, 1993.
19. **Stevenson, S.; Dannucci, G. A.; Sharkey, N. A.;** and **Pool, R. R.:** The fate of articular cartilage after transplantation of fresh and cryopreserved tissue-antigen-matched and mismatched osteochondral allografts in dogs. *J. Bone and Joint Surg.,* 71-A: 1297-1307, Oct. 1989.
20. **Stevenson, S.,** and **Horowitz, M.:** Current concepts review. The response to bone allografts. *J. Bone and Joint Surg.,* 74-A: 939-950, July 1992.
21. **Thompson, W. O.; Harner, C. D.; Jamison, J. P.; Saidman, S. L.;** and **Whiteside-Nimick, T. E.:** The immunologic response to fresh frozen bone-patellar tendon-bone allograft ACL reconstruction. *Trans. Orthop. Res. Soc.,* 19: 624, 1994.
22. **van Arkel, E. R. A.,** and **De Boer, H. H.:** Human meniscal transplantation. Preliminary results at 2 to 5-year follow-up. *J. Bone and Joint Surg.,* 77-B(4): 589-595, 1995.
23. **van Arkel, E. R.; van den Berg-Loonen, E. M.; van Wersch, J. W.;** and **de Boer, H. H.:** Human leukocyte antigen sensitization after cryopreserved human meniscal transplantations. *Transplantation,* 64: 531-533, 1997.
24. **Vasseur, P. B.; Rodrigo, J. J.; Stevenson, S.; Clark, G.;** and **Sharkey, N.:** Replacement of the anterior cruciate ligament with a bone-ligament-bone anterior cruciate ligament allograft in dogs. *Clin. Orthop.,* 219: 268-277, 1987.
25. **Wada, Y.; Amiel, M.; Harwood, F.; Moriya, H.;** and **Amiel, D.:** Architectural remodeling in deep frozen meniscal allografts after total meniscectomy. *Arthroscopy,* 14: 250-257, 1998.