

Removal of Hyaline Articular Cartilage Reduces Lymphocyte Infiltration and Activation in Rheumatoid Synovial Membrane

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ABSTRACT. Objective. To analyze the effect of removal of hyaline articular cartilage on synovial membrane pathology in chronic arthritis.

Methods. Synovial membrane samples were obtained from patients with rheumatoid arthritis or ankylosing spondylitis in association with total hip arthroplasty, either primary or revision surgery. Synovial membrane histopathology was assessed by immunochemical staining and morphometry.

Results. CD68 positive macrophages were common in revision synovial membranes. In contrast, T lymphocytes were much more common in primary rheumatoid synovial membranes ($p < 0.001$). Many T lymphocytes in primary synovial membrane were HLA-D/DR positive ($p < 0.001$) and interleukin 2 receptor (IL-2R) positive ($p < 0.001$) and contained interferon- γ (IFN- γ ; $p < 0.001$) and tumor necrosis factor- β (TNF- β ; $p < 0.001$). In contrast, revision synovial membranes from patients with chronic arthritis contained only a few HLA-D/DR positive T cells and practically no IL-2R, IFN- γ , or TNF- β positive activated T lymphocytes.

Conclusion. The components of hyaline articular cartilage may be the source of autoantigen responsible for perpetuation of chronic arthritides. (J Rheumatol 2001;28:2184-9)

Key Indexing Terms:

RHEUMATOID ARTHRITIS
ARTICULAR CARTILAGE

TOTAL HIP ARTHROPLASTY

SYNOVIAL MEMBRANE
T LYMPHOCYTES

Rheumatoid arthritis (RA) is considered an autoimmune disease. Rheumatoid synovial membrane contains many T lymphocytes, which are found in perivascular infiltrates¹⁻³. Plasma cells can produce the immunoglobulin with rheumatoid factor activity and they are mainly found around these infiltrates⁴. T lymphocyte activation in rheumatoid synovial membrane may be oligoclonal and antigen driven. Some

studies have found biased or preferential usage of V alpha and/or V beta gene segments in rheumatoid joints⁵⁻⁸. The usage of the T cell receptor may be oligoclonal^{9,10}. T lymphocyte involvement is further supported by the major genetic contribution to RA of HLA-DRB1*0401, HLA-DRB1*0404, and other alleles, which have a shared sequence motif in the third hypervariable region of the DR β chain¹¹. However, the antigen that drives rheumatoid synovitis is unknown, although type II collagen and human cartilage gp39 have been considered the prime candidates¹². It is supposed that HLA-D/DR positive antigen presenting cells present some unknown antigen to T cells, which are activated to express HLA-D/DR and T lymphokines.

Hyaline articular cartilage is avascular and non-neural. Thus some type of injury or insult to hyaline articular cartilage might reveal autoantigens that the immune system is not tolerant for and may respond to. As a result of its avascular nature, lymphocytes do not recirculate through the cartilagenous micromilieu. Injury to articular cartilage does not lead to hemorrhage. Therefore, local recruitment of inflammatory cells and the first event in the process finally leading to phagocytosis of injured and necrotic host tissue followed by healing is defective compared to vascularized tissue. Although this is one of the reasons for the poor or almost nonexistent inherent healing capacity of hyaline articular cartilage¹³⁻¹⁵, it may at the same time protect cartilage against immune attack. In RA, however, hyaline articular cartilage is subjected to extensive degradation and destruction, which to a large extent is mediated by matrix

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metalloproteinases in pannus tissue. Fibroblast-type collagenase was earlier thought to be mainly responsible^{16,17}, but lately emphasis has been on the mesenchymal form of “neutrophil” collagenase or collagenase-2 (MMP-8), collagenase-3 (MMP-13), and membrane-type matrix metalloproteinase type 1 (MT1-MMP or MMP-14), which are all interstitial collagenase and have been localized to rheumatoid pannus¹⁸⁻²¹.

In total hip arthroplasty (THA), the femoral head and acetabulum are removed and replaced with a gliding pair, usually consisting of metal and ultrahigh molecular weight polyethylene (UHMWPE). It is quite common that synovitis reoccurs after chemical or surgical synovectomy after an initially successful operation. In contrast, our clinical observation has been that chronic synovitis rarely reoccurs in joints from which articular cartilage has been totally removed. Therefore, we decided to collect synovial membranes from primary THA performed for chronic arthritis. Such tissue specimens should exhibit a typical macrophage and T lymphocyte driven autoimmune inflammation. This was compared to RA or ankylosing spondylitis (AS) synovial membrane obtained from patients undergoing revision surgery due to aseptic loosening of THA. It is pertinent in this situation that all hyaline articular cartilage is removed in association with THA. Our hypothesis was that if the hyaline articular cartilage contains a sequestered autoantigen driving and perpetuating rheumatoid synovitis, then the typical “autoimmune” immunohistopathological features of chronic arthritis should not reoccur in the revision synovial membranes.

MATERIALS AND METHODS

Patients and samples. Ten synovial membrane samples were collected from patients with RA undergoing primary THA. Of these patients, 3 were men and 7 women, the mean age being 41.6 yrs (range 38–74). Pain, stiffness, and joint instability were the main complaints leading to THA. During the

period 1995–99, eight synovial membrane samples were obtained from patients with RA (n = 6) or AS (n = 2) undergoing revision THA due to aseptic loosening of femoral components of prostheses. Of these patients, 3 were men and 5 women, mean age 48.4 yrs (range 40–68). The original diseases that had led to the primary THA were RA or AS of the hip joints. Painful loosening of THA was the main reason for revision surgery. The mean interval from primary to revision THA was 9.8 yrs (range 6–15). All patients were evaluated by a certified rheumatologist and diagnosed according to the American College of Rheumatology criteria²². Treatment with disease modifying antirheumatic drugs (DMARD) may affect the composition of synovial infiltrates. However, the medications in the 2 study groups were similar (Table 1).

All tissue samples were snap-frozen in isopentane precooled in dry ice and stored at –70°C. In total, 9 serial cryostat sections (6 µm thick) were cut from each sample. The first and last sections were stained with hematoxylin. Sections number 2 to 7 were used for immunohistochemical staining with different antibodies, and section 8 was used for negative control staining.

Immunohistochemistry. The sections were fixed in cold acetone for 15 min at –20°C. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in absolute methanol for 30 min. The sections were incubated with the following reagents at room temperature: (1) Normal horse serum (Vector Laboratories, Burlingame, CA, USA), diluted 1:50 in Tris buffered saline (TBS) containing 0.1% bovine serum albumin (BSA) for 20 min. (2) The following monoclonal mouse anti-human antibodies for 60 min (Table 2): CD2 (total T cells), CD4 (inducer/helper T cells), CD8 (suppressor/cytotoxic T cells), CD68 (a macrophage marker), HLA-DR (labeling activated T cells and macrophages), interleukin 2 receptor (IL-2R; a marker of lymphocyte activation), and interferon-γ (IFN-γ) and tumor necrosis factor-β (TNF-β), the main lymphokines produced by activated lymphocytes. (3) Biotinylated horse anti-mouse IgG (Vector; diluted 1:100 in TBS containing 0.1% BSA) for 30 min. (4) Avidin-biotin-peroxidase complex (Vector; diluted 1:100 in TBS) for 30 min. (5) A combination of 0.023% diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA) and 0.006% hydrogen peroxide for 5 min. Between steps, the sections were washed in TBS for 3 × 5 min. Finally, the slides were counterstained with hematoxylin, dehydrated in a graded ethanol series, cleared in xylene, and mounted using Diatex. Monoclonal mouse IgG of the same subtype but with irrelevant specificity (*Aspergillus niger* glucose oxidase) was used at the same concentration as and instead of the primary antibodies as a negative staining control.

Semiquantitative microscopic assessment. Semiquantitative morphometric

Table 1. Therapeutic modalities.

Patients with Primary THA	Medications	Patients with Revision THA	Medications
RA-1	NSAID, HCQ	RA-1	NSAID, prednisone, HCQ
RA-2	NSAID, SSZ, alendronate, aurothiomalate	RA-2	NSAID
RA-3	NSAID, aurothiomalate	RA-3	NSAID, MTX
RA-4	NSAID	RA-4	NSAID, azathioprine, methylprednisone
RA-5	NSAID, prednisone, SSZ	RA-5	NSAID
RA-6	NSAID, prednisone, SSZ, MTX	RA-6	NSAID
RA-7	NSAID, HCQ, MTX, SSZ	AS-1	NSAID
RA-8	NSAID, chloroquine	AS-2	NSAID, SSZ
RA-9	Cyclosporine A, SSZ, azathioprine, MTX, prednisone		
RA-10	NSAID, cyclosporine A, MTX, HCQ		

RA: rheumatoid arthritis, AS: ankylosing spondylitis, NSAID: nonsteroidal antiinflammatory drug, HCQ: hydroxychloroquine; SSZ: sulfasalazine; MTX: methotrexate.

Table 2. Mouse monoclonal antibodies (Mab) used in this study.

Mab	Subtype	Concentration, µg/mg	Producer
CD68	IgG1	3.90	Dako
CD2	IgG1	1.37	Dako
CD4	IgG1	1.00	Dako
CD8	IgG1	2.50	Dako
HLA-DR	IgG1	2.03	Dako
IL-2R	IgG1	2.00	Dako
IFN-γ	IgG1	4.00	PharMingen
TNF-β	IgG2b	1.00	Bender Medsystem

IL-2R: interleukin 2 receptor; IFN-γ: interferon-γ; TNF-β: tumor necrosis factor-β.

assessment was done with a low light-charge screen mounted with a 12 bit PC digital image camera (SensiCam, Kelheim, Germany) on a Leitz Diaplan light microscope (Wetzlar, Germany). The camera was further linked to a semiautomatic Analysis Pro 3.0 image analysis and processing system (Soft Analysis System GmbH, Münster, Germany). The images were visualized under ×200 magnification and recorded in digitized form in a computer. After parameters were set for the detection of positive cells, they were fixed and used during the following analysis procedure. Cell counting was carried out blinded. The whole section areas were used for analysis. The total number of cells for each sample was recorded as the average number of cells in the first and last sections stained with hematoxylin. The positive cells labeled by different antibodies were counted in the sections without hematoxylin counterstaining. The final result for each section was reported as the average number of positive cells out of 1000 hematoxylin stained cells. T tests were used to analyze differences between the rheumatoid synovial membranes obtained at the primary or revision THA operations.

RESULTS

Histological features of synovial membrane samples from revision THA. Tissue structure and cellularity were examined after hematoxylin staining. Revision RA and AS synovial membranes were characterized by a foreign body reaction, with histiocytosis and fibrosis. A synovial lining was found in all samples. Macrophages were the main cells in the revision RA and AS synovial membrane samples. Although lymphocytes were invariably found, they never formed aggregates, as seen in the primary RA synovial membranes. The vascularity in revision synovial samples was greatly reduced compared to that in primary RA samples. Wear particles from THA components were present in the revision RA and AS synovial membranes. Metal debris appeared as small and black particles. Polymethylmethacrylate bone cement particles could only be observed indirectly in the form of large, irregular empty spaces, because they are soluble in the conventional solvents used for sample processing. Wear particles of UHMWPE acetabular components were usually not visible under regular light microscopy, but were clearly seen and strongly birefringent under polarized light.

Immunochemical study results. CD68 positive cells were detected throughout the revision RA and AS synovial membranes, especially in the lining layers and sublining

areas. Macrophage accumulations were also present in the stroma of these samples. The numbers of CD2, CD4, and CD8 positive cells were low in revision synovial membrane samples compared to the primary RA samples. Most of the HLA-DR positive cells were present in the sublining area and in the deep stroma, but such cells were rarely found in the lining layers of the revision RA or AS samples. The numbers of HLA-DR positive cells in the revision RA or AS synovial membranes were lower than in the primary RA membranes (Figure 1). There were no IL-2R positive or IFN-γ or TNF-β containing cells in the revision RA or AS synovial membranes. In contrast, IL-2R positive and IFN-γ and TNF-β containing cells were invariably found in primary RA synovial membrane, although their numbers were quite low (Figure 2, Table 3).

DISCUSSION

In this study 10 samples from primary THA operations performed for RA were compared to 8 revision synovial membrane samples also obtained from patients with chronic arthritis of the hip, either RA or AS, but undergoing revision operation for aseptic loosening of THA prosthetic devices. As expected, the primary RA synovial membrane samples revealed typical features of rheumatoid synovitis in the form of large perivascular CD2 positive T lymphocyte infiltrates. Many of the perivascular T lymphocytes were HLA-D/DR positive. In addition, a small but significant proportion of all local T lymphocytes in such samples were obviously activated, as shown by their IL-2R expression and IFN-γ and TNF-β content. This is in accord with previous reports²³⁻²⁶. It was unfortunate that we did not get samples from any AS patients undergoing primary THA operations. It should be emphasized, however, that clinically, coxitis in RA and AS are similar during the chronic inflammatory phase and that the immunohistopathology of chronic peripheral synovitis in AS is reminiscent of that seen in RA^{27,28}.

Revision operations are not very common at our unit, but during 1995-99 we managed to collect 8 synovial membrane samples from patients who had had a chronic arthritis of the hip joint as the primary indication for their THA operation and who were later reoperated for aseptic loosening of the femoral component. It would have been better to collect the synovial membrane samples first during the primary THA operations and then later from the same patient undergoing revision operation and to compare these 2 samples. However, the time interval between the primary THA operation and aseptic loosening/revision THA operation is usually quite long, 9.8 years (mean) in our patient sample. Thus it was not possible to follow individual patients from the primary to the revision THA. Instead, separate groups were used. It should be emphasized that all patients, including those in the revision group, had active and systemic disease. The medications were similar, although slightly less aggressive in the revision group, in part due to

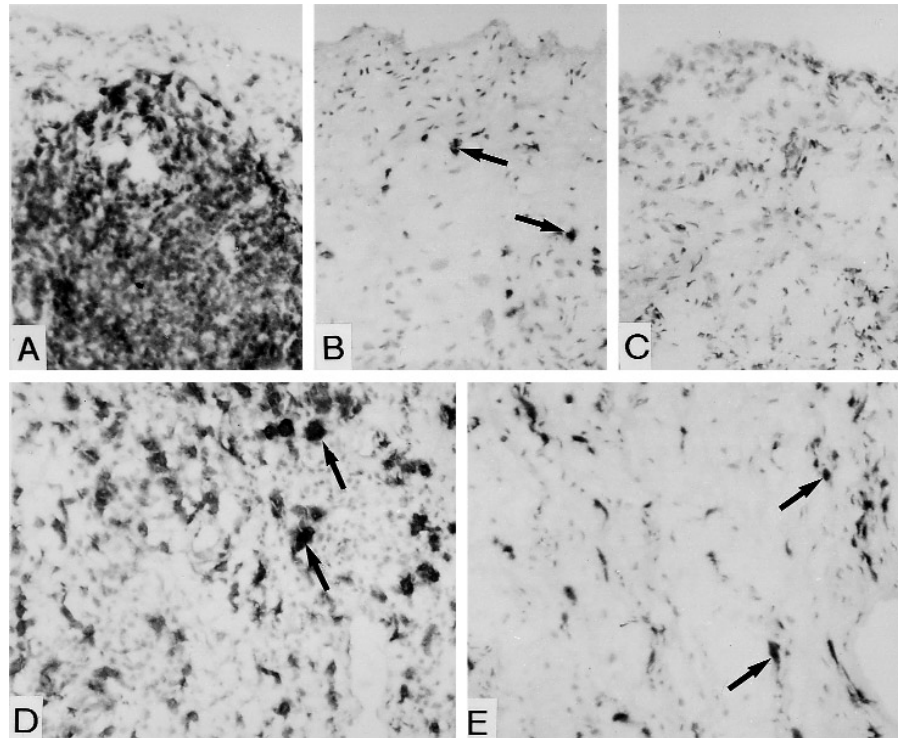


Figure 1. A comparison of immunochemical staining of CD2 and HLA-D/DR in rheumatoid synovial membrane obtained from either primary or revision THA. (A) The perivascular inflammatory cell infiltrate containing CD2 positive cells in RA synovial membrane obtained from primary THA surgery. (B) Compared to such rheumatoid synovitis samples, the CD2 positive cells (arrows) were sparse in RA synovial membrane obtained from revision THA. (C) Negative staining control confirmed the specificity of the method. (D) Similarly, HLA-DR positive cells (arrows) were quite frequent in the sublining stroma in RA synovial membrane obtained from primary THA surgeries compared to panel E, HLA-DR positive cells (arrows) in RA synovial membrane obtained from revision THA (slight hematoxylin counterstain, original magnification $\times 250$).

Table 3. The number of positive cells per 1000 hematoxylin stained cells (%).

Sample	CD68	HLA-DR	CD2	CD4	CD8	IL-2R	IFN- γ	TNF- β
Primary RA-1	494	367	169	108	57	5	4	4
RA-2	413	370	205	131	63	6	4	3
RA-3	574	382	186	128	64	7	4	4
RA-4	509	406	228	152	79	5	3	2
RA-5	423	384	186	124	60	4	4	2
RA-6	427	395	185	122	67	5	3	2
RA-7	564	403	184	126	61	5	5	3
RA-8	388	336	130	87	40	4	4	2
RA-9	528	494	140	90	48	4	4	2
RA-10	592	426	169	110	57	5	4	1
Mean \pm SD	474.0 \pm 71.4	393.0 \pm 43.5	179.2 \pm 30.1	112.5 \pm 27.5	59.9 \pm 11.1	5.0 \pm 1.0	3.9 \pm 0.6	2.7 \pm 0.9
Revision RA-1	715	258	102	69	36	0	0	0
RA-2	576	365	37	23	12	0	0	0
RA-3	699	306	49	36	17	0	0	0
RA-4	711	344	51	34	19	0	0	0
RA-5	736	360	72	50	24	0	0	0
RA-6	689	367	115	71	37	0	0	0
Revision AS-1	659	314	84	52	26	0	0	0
AS-2	695	339	74	45	23	0	0	0
Mean \pm SD	683.6 \pm 53.1	330.6 \pm 40.2	72.0 \pm 29.9	44.8 \pm 18.9	24.4 \pm 9.4			
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Primary RA: synovial membrane samples obtained from patients with RA undergoing primary THA. Revision RA: synovial samples obtained from patients with RA undergoing revision THA. Revision AS: synovial samples obtained from patients with AS undergoing revision THA.

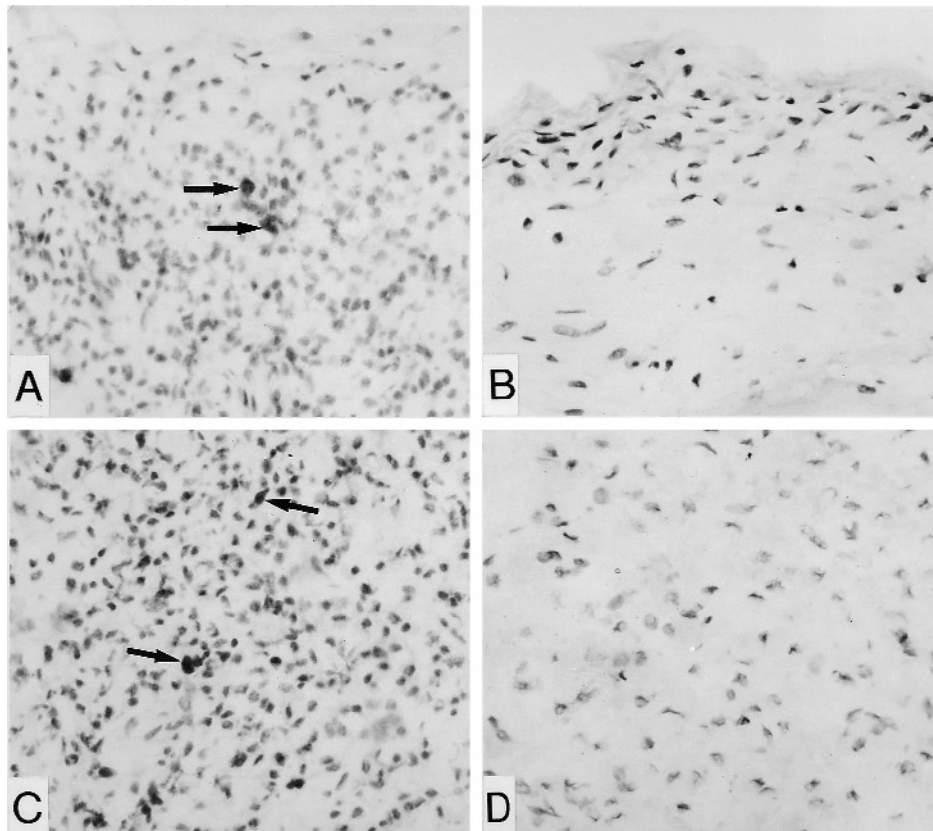


Figure 2. A comparison of IL-2R and IFN- γ immunoreactivity in rheumatoid synovial membrane obtained from either primary THA (A and C) or revision THA surgery (B and D). (A) IL-2R positive cells (arrows) could always be found in RA synovial membrane samples obtained from primary THA, although their numbers were quite low, as shown in panel A. (B) In contrast, no IL-2R positive cells were found in RA synovial membrane obtained from revision THA. (C) As well, there were always some IFN- γ positive cells (arrows) in RA synovial membranes from primary THA, but as shown in panel D, no IFN- γ positive cells were found in RA synovial membranes obtained from revision THA (hematoxylin counterstain, original magnification $\times 313$).

drug side effects. At least it is clear that the more indolent inflammation in the revision THR group is not easily explained as a result of a more aggressive DMARD.

The proportions of CD2, CD4, and CD8 positive T cells were significantly lower in the revision RA or AS synovial membrane samples than in the primary rheumatoid THA samples. In spite of the presence of many CD68 positive macrophages, very few T cells in revision synovial samples were HLA-D/DR positive. More significantly, practically no IL-2R positive cells containing T lymphokines IFN- γ or TNF- β were found in these revision RA or AS synovial membrane samples. This was in spite of the fact that these samples also contained cells potentially capable of processing and presenting antigens to T lymphocytes, which were also present *in situ* in the revision RA and AS synovial membrane samples. Further, CD68 positive macrophages were often seen, even in the revision RA and AS synovial membrane samples, in close contact with CD2 positive T lymphocytes. This finding suggests that in revision RA/AS synovial membrane the antigen presenting cells did not have any antigens to present to the local T lymphocytes.

Chemical synovectomy with osmic acid²⁹, radiation synovectomy with yttrium-90³⁰ or dysprosium 165-ferric hydroxide macroaggregates³¹, open surgical synovectomy, and arthroscopic synovectomy³² are all used in the treatment of refractory synovitis, usually of the knee joint. All are complicated by relapses, as described in the reports cited above. This is compatible with the systemic and polyarticular nature of RA and AS. Lymphocytes can be recruited to synovectomized joints as a result of chemokines and lymphocyte-endothelial cell adhesion events (the homing hypothesis) or they can increase in numbers as a result of local proliferation. In contrast to relapses after synovectomies, it is our clinical observation that if all articular cartilage is removed (in total joint replacement), chronic synovitis rarely relapses in the operated joint. This is sometimes seen after total knee replacement, but in such cases the patellofemoral joint has not been replaced with a patellar implant, and therefore part of the hyaline articular cartilage has remained *in situ*. The low frequency of relapses in totally replaced joints is fortunate, because joint implants form a relative contraindication to intraarticular glucocorti-

costeroid injections. This study is in accord with our clinical observations in confirming lack of T cell activation and involvement in revision RA or AS synovial membranes, as if there would be no antigen there to be processed and presented by the antigen presenting cells to T lymphocytes, which would lead to lymphocyte activation and local immune inflammation.

Our findings are in agreement with our working hypothesis. They indicate that the avascular hyaline articular cartilage may contain autoantigens that may perpetuate the autoimmune synovitis in RA and in other chronic arthritides. Therefore, although various forms of arthritides may have different etiologies and initiating factors, they may also share similar perpetuating pathomechanisms.

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