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Although biomechanical signals generated during joint mobilization are vital in maintaining integrity of inflamed cartilage, the molecular mechanisms of their actions are little understood. In an experimental model of arthritis, we demonstrate that biomechanical signals are potent anti-inflammatory signals that repress transcriptional activation of proinflammatory genes and augment expression of anti-inflammatory cytokine IL-10 to profoundly attenuate localized joint inflammation. The Journal of Immunology, 2006, 177: 8757–8766.

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The earliest histological sign of joint degeneration is fibrillation or disruption of the most superficial layers of the cartilage, resulting in roughened articular cartilage. Simultaneously, exuberant induction of proinflammatory genes and synthesis of their corresponding gene products results in excessive production of cytokines and other inflammatory mediators. IL-1β and TNF-α appear to be prominent mediators of cartilage destruction (1–3). Both can activate chondrocytes and synovial cells to produce IL-1β, TNF-α, MMPs, as well as COX-2 and NO. Up-regulation of COX-2 mediates pain and inflammation largely through synthesis of PGE_2. Its integral role in the pathogenesis of RA is demonstrated by the effectiveness of COX-2-specific inhibitors in reducing joint inflammation and preventing IL-1β-mediated proteoglycan turnover in cartilage (4–6). MMPs are collagenolytic enzymes primarily involved in collagen type II degradation in articular cartilage. Collectively, the molecular inflammation and associated cartilage destruction driven by chondrocytes and synovial cells, results in production of measurable molecular markers that can be used to assess the therapeutic efficacy of treatment regimens (4, 7).

The results of clinical and experimental studies demonstrate that signals generated by mobilization are vital in maintaining integrity of arthritic joints. Early mobilization of joints counteracts the deleterious effects of IMM and promotes healing of articular cartilage in rabbits and humans (8–10). Nevertheless, the role of mobilization vs IMM on the inflamed joints remains controversial. Presently, IMM and mobilization both are used as therapeutic measures to treat acutely and chronically inflamed joints with variable success (11–13). This is partly due to the fact that mechanisms by which these two differing treatments exert their effects remain unclear. Recent in vitro studies demonstrate that biomechanical signals are potent anti-inflammatory signals that attenuate proinflammatory gene induction in chondrocytes. These signals act on chondrocytes in a magnitude-dependent manner. At low magnitudes, biomechanical signals inhibit IL-1β- or TNF-α-induced transcriptional activation of COX-2, MMPs, IL-1β, and other inflammatory molecules in inflamed chondrocytes in response to biomechanical signals. A rabbit model of Ag-induced arthritis (AIA) that engenders massive induction of proinflammatory mediators in chondrocytes was used to examine the consequences of continuous passive motion (CPM) or immobilization (IMM) on the articular cartilage of knees during initial stages of inflammation. We show that exposure of inflamed knees to CPM or IMM results in strikingly diverse pathways and disease progression. IMM of AIA-affected knees causes a time-dependent increase in cell disorganization, matrix degradation, loss of stratification, and eventual cartilage destruction within 96 h. Contrarily, CPM rapidly attenuates progression of the inflammatory disease, demonstrating that signals generated by CPM act directly on chondrocytes to abrogate inflammation-induced transcriptional activation of IL-1β and synthesis of cyclooxygenase (COX)-2 and matrix metalloproteinase (MMP)-1. In parallel, biomechanical signals up-regulate induction of IL-10 to block the actions of proinflammatory cytokines and ameliorate inflammatory events. These novel findings reveal that biomechanical signals are potent anti-inflammatory signals that repress the manifestation of arthritic diseases and may have a profound role in attenuating localized joint inflammation in vivo.

RA is characterized by generalized inflammation of joints leading to progressive destruction of cartilage and subchondral bone. The earliest histological sign of joint degeneration is fibrillation or disruption of the most superficial layers of the cartilage, resulting in roughened articular cartilage. Simultaneously, exuberant induction of proinflammatory genes and synthesis of their corresponding gene products results in excessive production of cytokines and other inflammatory mediators. IL-1β and TNF-α appear to be prominent mediators of cartilage destruction (1–3). Both can activate chondrocytes and synovial cells to produce IL-1β, TNF-α, MMPs, as well as COX-2 and NO. Up-regulation of COX-2 mediates pain and inflammation largely through synthesis of PGE_2. Its integral role in the pathogenesis of RA is demonstrated by the effectiveness of COX-2-specific inhibitors in reducing joint inflammation and preventing IL-1β-mediated proteoglycan turnover in cartilage (4–6). MMPs are collagenolytic enzymes primarily involved in collagen type II degradation in articular cartilage. Collectively, the molecular inflammation and associated cartilage destruction driven by chondrocytes and synovial cells, results in production of measurable molecular markers that can be used to assess the therapeutic efficacy of treatment regimens (4, 7).

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proinflammatory molecules (14–18). These mediators are involved in cartilage destruction and by blocking their gene expression, biomechanical signals can potentially attenuate cartilage destruction. Additionally, biomechanical signals up-regulate expression of proteins associated with cartilage repair, such as aggrecans, collagen type II, and sulfated glycosaminoglycans. Not surprisingly, mechanical signals intercept the IL-1β-signaling pathway and inhibit nuclear translocation of NF-κB and subsequent transcriptional activation of genes under its control (17, 19). Because NF-κB transactivation occurs within a few minutes following activation of chondrocytes with IL-1β or TNF-α, the actions of biomechanical signals are rapid and can be observed within a few hours. Moreover, biomechanical signals act on cells in a sustained manner and can be observed in the presence of an inflammatory stimulus (17, 19). Clearly, mechanistic analysis of the actions of biomechanical signals in vivo is necessary to understand the biological basis of motion-based therapies that may contribute significantly to the healing of inflamed joints.

In the current study, we have used the rabbit model of AIA to evaluate the molecular basis for the beneficial effects of motion-based therapies. The histopathogenesis of AIA has been well-characterized and the model demonstrates many features of human RA, including joint inflammation and damage to articular cartilage via both increased breakdown and decreased synthesis of the cartilaginous matrix (15, 20). Using this model, we document that CPM attenuates AIA-induced inflammation in the cartilage. By blocking transcriptional activation of IL-1β, COX-2, and MMP-1, signals generated by CPM inhibit cartilage destruction in a severe inflammatory environment. More importantly, CPM up-regulates IL-10 induction in chondrocytes, thus potentially creating an anti-inflammatory environment that diminishes the actions of proinflammatory cytokines produced during AIA. These novel observations provide the molecular basis for the beneficial effects of mobilization of inflamed joints as well as an opportunity for developing optimal therapeutic interventions for arthritic diseases.

Materials and Methods

Induction of AIA

All protocols were approved by the Institutional Animal Care and Use Committee at the University of Toronto and University of Pittsburgh. New Zealand White male rabbits (18–20 wk old) were sensitized with s.c. injections of 0.5 ml of 0.5% BSA in CFA at four dorsal sites. Twenty days postsensitization, hypersensitivity to BSA was examined by s.c. injection of 0.5 ml of 0.01% BSA in IFA. Five days later, hypersensitized rabbits were anesthetized, right knees were shaved, and 0.5 ml of 0.5% BSA in saline was injected intra-articularly to induce AIA (15, 20).

IMM or CPM treatment of joints

Following intra-articular injection, the right knees of the rabbits (n = 5/group/time point) were immediately subjected to IMM or CPM (provided by Orthomotion) (20). In the CPM group, the angle of flexion of the joint ranged between 40° and 110° at 0.022 Hz (9). In the immobilized group (n = 5/group/time point), right knees were immobilized in a neutral position with a plaster cast. In both groups, the left limbs of the rabbits were not subjected to any treatment. To examine the early and late molecular events induced by motion-based therapies, the rabbit knees were immobilized or exposed to CPM for 24, 48, 96 h, or 12 h per day for 2 wk. Thereafter, rabbits were sacrificed, synovial fluid (SF) was harvested, joints were excised, and skin and soft tissue were removed. Following AIA induction, one group of rabbits at each time point was also allowed free movement (FM) of knees in cages to assess basal inflammation in AIA-affected knees.

SF analysis

SF from all joints were harvested by injecting 1.0 ml of PBS containing 100 U of heparin (PBS-h) through the patellar ligament, cutting the synovial membrane, and carefully aspirating the SF. After assessing the volume of SF, an aliquot was removed for the cell smears and the remaining fluids were immediately frozen at −70°C. The quantitative assessment of BSA in SF was resolved with a BSA sandwich ELISA, using rabbit anti-BSA polyclonal IgG as the capture Ab (Santa Cruz Biotechnology) and chicken anti-BSA HRP-labeled IgG (BioSource International) as the detection Ab. Briefly, 96-well plates (Falcon) were coated with the anti-BSA Ab (100 ng/well) in PBS overnight at 4°C. The wells were blocked with 5% nonfat dry milk in PBS/0.05% Tween 20, and the diluted BSA standards and aliquots of SF (100 µl/well) were added to each plate in triplicate and incubated for 1 h at room temperature. The wells were washed thoroughly with PBS, and the bound Ab was detected with chicken anti-BSA-HRP Ab in PBS (1/3000 dilution) using SuperSignal ELISA Femto Maximum Sensitivity Substrate (Pierce). The chemiluminescence was detected using a Victor multilabel plate reader (PerkinElmer). The data was expressed as micrograms per milliliter of BSA derived from a BSA standard curve generated from a concentration range between 0.01 and 10 ng/ml. The concentration of IL-1β in SF was determined using IL-1β ELISA kits from BD Biosciences, according to the manufacturer’s recommended protocols.

For the analysis of cells in the SF, 2 µl of SF was diluted in an equal volume of PBS and smeared into a thin film on a glass slide, stained with H&E, and counted in four different areas of 500 µm². Polymorphonuclear cells (PMN) were identified by nuclear morphology and differential staining, monocytes by the presence of CD14 molecule (Invitrogen Life Technologies) and nuclear morphology, and lymphocytes by the presence of CD3 molecules (Santa Cruz Biotechnology).

Histology and immunohistochemical analysis

After harvesting, distal femoral condylar cartilage from control and experimental groups were cleaned and kept cold on ice. The severity of arthritic changes at macroscopic and histologic levels was categorized according to Pritzker et al. (21) on a scale of 0–6. For macroscopic examination, femurs were kept cold and moist after removal and immediately examined under stereomicroscope to record surface structures. For histological examination, the distal femoral cartilage was fixed in 10% buffered formalin, dehydrated, cut into 5-µm-thick sections through longitudinal median axis through intercondylar fossa separating the joint surface into medial and lateral condyles. Condylar pairs were aligned, embedded in paraffin, and sectioned in a plane parallel to the original cut and stained with H&E. This orientation enabled simultaneous visualization of cartilage from median and lateral sides of the joint. Using stereomicroscope to record surface structures. Histological examination, the distal femoral cartilage was fixed in 10% buffered formalin, dehydrated, cut into 5-µm-thick sections through longitudinal median axis through intercondylar fossa separating the joint surface into medial and lateral condyles. Condylar pairs were aligned, embedded in paraffin, and sectioned in a plane parallel to the original cut and stained with H&E. This orientation enabled simultaneous visualization of cartilage from median and lateral sides of the joint.

Deparaffinized, hydrated sections were treated with 0.1 M sodium citrate buffer (pH 6.0) at 70°C for Ag retrieval and used for immunohistochemical analysis. Sections blocked in Protein Blocking Agent (Thermoelectron) with 5% preimmune serum were reacted with primary Abs (1/400) and sectioned in a plane parallel to the original cut and stained with H&E. This orientation enabled simultaneous visualization of cartilage from median and lateral sides of the joint. Using stereomicroscope to record surface structures. Histological examination, the distal femoral cartilage was fixed in 10% buffered formalin, dehydrated, cut into 5-µm-thick sections through longitudinal median axis through intercondylar fossa separating the joint surface into medial and lateral condyles. Condylar pairs were aligned, embedded in paraffin, and sectioned in a plane parallel to the original cut and stained with H&E. This orientation enabled simultaneous visualization of cartilage from median and lateral sides of the joint.

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Cartilage subjected to IMM, CPM, or FM immediately following AIA induction (21).

degradation.

healthy matrix in the transitional and deep radial zones. (D) CPM for 24 h, demonstrating normal cartilage histology with columnar chondrocyte organization and exhibiting normal to (E) mild cellular disorganization. AIA-afflicted cartilage exposed to (F) control healthy knees articular injection of BSA and the knees were immediately subjected to CPM or IMM for 24, 48, 96 h, or 2 wk cartilage from (G) IMM and CPM regulate cartilage cell organization, stratification, and matrix integrity in AIA-afflicted knees. AIA was induced by intra-articular injection of BSA and the knees were immediately subjected to CPM or IMM for 24, 48, 96 h, or 2 wk cartilage from (A) control healthy knees exhibiting normal to (B) mild cellular disorganization (B). AIA-afflicted cartilage exposed to (C) IMM for 24 h, exhibiting cell and matrix disorganization in the deep radial and transitional zones, or (D) CPM for 24 h, demonstrating normal cartilage histology with columnar chondrocyte organization and healthy matrix in the transitional and deep radial zones. E, IMM for 48 h, demonstrating severe cell disorganization, loss of stratification, and matrix disruption in deep radial, transitional, and superficial zones. F, CPM for 48 h, exhibiting near normal histology, no cell disorganization, or matrix degradation. G, IMM for 96 h, exhibiting progressive cartilage destruction in the deep radial and transitional zones and loss of superficial zone, to (H) severe fissuring, loss of stratification, and loss of superficial zone. I, CPM for 96 h, showing mild arthritis with cellular and matrix disorganization, and some erosion of superficial layers. J, IMM for 2 wk, showing loss of matrix and cells in deeper and superficial zones, and massive loss of matrix, and cell disorganization in the transitional zone, or (K) severe fissuring in deeper and transitional zone, and erosion due to loss of superficial zone, or (L) CPM for 2 wk exhibiting near normal histology with columnar cell organization in transitional and deeper zones, to (M) minor matrix and cellular disorganization, and smooth superficial layers. N. Assessment of BSA in SFs of AIA-afflicted knees exposed to IMM or CPM for various time intervals. The data shown are representative sections from one of five rabbit knees at each time point under each treatment.

![Figure 1](https://example.com/figure1.png)

**FIGURE 1.** IMM and CPM regulate cartilage cell organization, stratification, and matrix integrity in AIA-afflicted knees. AIA was induced by intra-articular injection of BSA and the knees were immediately subjected to CPM or IMM for 24, 48, 96 h, or 2 wk cartilage from (A) control healthy knees exhibiting normal to (B) mild cellular disorganization (B). AIA-afflicted cartilage exposed to (C) IMM for 24 h, exhibiting cell and matrix disorganization in the deep radial and transitional zones, or (D) CPM for 24 h, demonstrating normal cartilage histology with columnar chondrocyte organization and healthy matrix in the transitional and deep radial zones. E, IMM for 48 h, demonstrating severe cell disorganization, loss of stratification, and matrix disruption in deep radial, transitional, and superficial zones. F, CPM for 48 h, exhibiting near normal histology, no cell disorganization, or matrix degradation. G, IMM for 96 h, exhibiting progressive cartilage destruction in the deep radial and transitional zones and loss of superficial zone, to (H) severe fissuring, loss of stratification, and loss of superficial zone. I, CPM for 96 h, showing mild arthritis with cellular and matrix disorganization, and some erosion of superficial layers. J, IMM for 2 wk, showing loss of matrix and cells in deeper and superficial zones, and massive loss of matrix, and cell disorganization in the transitional zone, or (K) severe fissuring in deeper and transitional zone, and erosion due to loss of superficial zone, or (L) CPM for 2 wk exhibiting near normal histology with columnar cell organization in transitional and deeper zones, to (M) minor matrix and cellular disorganization, and smooth superficial layers. N. Assessment of BSA in SFs of AIA-afflicted knees exposed to IMM or CPM for various time intervals. The data shown are representative sections from one of five rabbit knees at each time point under each treatment.

**Table I. Macroscopic and histopathologic grading of articular cartilage**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cartilage grading</th>
<th>Histological analysis grading</th>
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<tbody>
<tr>
<td>Time</td>
<td>IMM</td>
<td>CPM</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24 h</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>48 h</td>
<td>2–2.5</td>
<td>0–1</td>
</tr>
<tr>
<td>96 h</td>
<td>2–3</td>
<td>0–1</td>
</tr>
<tr>
<td>2 wk</td>
<td>3–4</td>
<td>0–1</td>
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*Macroscopic and histopathologic grading of articular cartilage of the femoral head subjected to IMM, CPM, or FM immediately following AIA induction (21).
molecules in rabbits exposed to IMM and CPM. Differences were regarded as statistically significant at values of $p < 0.01$.

**Results**

**Mobilization prevents cartilage degradation**

In these experiments, knees of AIA-afflicted rabbits were immediately subjected to CPM or IMM. Knees from untreated rabbits and AIA afflicted rabbits that were allowed FM in cages were used as negative and positive controls, respectively. Macroscopically, control articular cartilage from untreated rabbits exhibited normal smooth architecture with no obvious fissures or surface aberrations. Evidence of inflammation was not apparent, as assessed by swelling of the joint. Cartilage from knees exposed to CPM or IMM for 24 h, revealed smooth grade 0 surface morphology (21).

![FIGURE 2. CPM inhibits IL-1β mRNA expression and synthesis in inflamed cartilage. A–J. Median longitudinal sections of femoral heads from knees exposed to IMM or CPM were immunostained with anti-IL-1β Abs. (A), Healthy control cartilage, (B) cartilage from 2 wk post-AIA induction, and (B') 2 wk post-AIA induction stained with second Ab alone. Chondrocytes in cartilage from AIA-afflicted knees immobilized for 24, 48, 96 h, or 2 wk (C, E, G, and I) exhibiting increasing levels of IL-1β-positive cells, or exposed to CPM for 24, 48, 96 h, or 2 wk (D, F, H, and J) exhibiting minimal presence of IL-1β in chondrocytes at all time points tested. K, IL-1β mRNA expression in cartilage exposed to CPM for 24, 48, 96 h, or 2 wk, as compared with that of IMM knees. L, Number of IL-1β-positive cells in superficial and transitional or deeper zones enumerated in $10^5 \mu m^2$ areas of cartilage. Each point represents mean and SEM of seven measurements, as described in Materials and Methods. *, $p < 0.05$. M, Monocyte; L, lymphocyte.](http://www.jimmunol.org/)

![Table II. Identification of cells (number/10 µl) in the SFs following CPM or IMM treatment of AIA-afflicted kneesa](http://www.jimmunol.org/)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CPM</th>
<th>IMM</th>
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<tbody>
<tr>
<td>Cells</td>
<td>PMN</td>
<td>M</td>
</tr>
<tr>
<td>24 h</td>
<td>1254 ± 229</td>
<td>25 ± 14</td>
</tr>
<tr>
<td>48 h</td>
<td>1897 ± 284</td>
<td>254 ± 76</td>
</tr>
<tr>
<td>96 h</td>
<td>1231 ± 233</td>
<td>212 ± 52</td>
</tr>
<tr>
<td>2 wk</td>
<td>586 ± 134</td>
<td>412 ± 124</td>
</tr>
</tbody>
</table>

a Data represents mean ± SEM. * indicates $p < 0.05$. M, Monocyte; L, lymphocyte.
However, joints exposed to IMM for 48 or 96 h exhibited a discontinuous surface morphology with surface abrasions typical of grade 2–2.5 features. Knees exposed to CPM showed smooth surface architecture and grade 0–1 surface without discontinuity or abrasions. More significant differences were apparent after subjecting the knees to CPM or IMM for 15 days. Immobilized knees exhibited grade 3 to grade 4 surface texture with erosion of the cartilage and minor vertical fissures. Knees subjected to CPM, showed areas of grade 1 (two of five) to grade 2 (three of five) surface morphology with smooth to minor surface abrasions (Table I). As expected, AIA-afflicted rabbits that were allowed FM in cages, the extent of cartilage damage differed widely from grades 1.5 to 3.5. Therefore, in the next experiments, knees subjected to CPM were compared with IMM knees, to more precisely assess the actions of CPM on inflamed knees.

**CPM protects matrix integrity in AIA-afflicted articular cartilage**

Four major characteristics of cartilage were taken in to consideration while grading the cartilage integrity, namely surface integrity, extracellular matrix preservation, stratification of matrix, and organization of cells in the matrix. Histological analysis demonstrated that untreated rabbit knees exhibited grade 0 histopathology ranging from intact uninvolved cartilage with columnar organization of chondrocytes to noncolumnar chondrocytes within middle and deeper layers of the matrix, without obvious signs of inflammation (Fig. 1, A and B). Knees exposed to CPM or IMM for 24–96 h after AIA induction, revealed pronounced differences in the histopathology of femoral cartilage (Fig. 1, C and D, Table I). Within 24 h, IMM caused extracellular matrix disruption and disorganization of chondrocytes characteristic of grade 1 histopathology (21). This subsequently led to an aggressive and progressive loss of stratification, severe disorganization of chondrocytes in middle and deeper zones of the hyaline matrix and surface erosion typical of grade 4 histopathology (Fig. 1, G and H). Finally, following 15 days of IMM, cartilage afflicted with AIA was either severely fissured (Fig. 1K), or exhibited severe loss of matrix, acellular areas in deeper zones, and loss of superficial zone (Fig. 1J).

Contrarily, femoral condyles from knees exposed to CPM for 24 h appeared to be normal with minimal changes in intact superficial, middle, and deeper zones, as compared with condyles from IMM-treated knees. The subsequent exposure to CPM for 48–96 h revealed normal to mild disorganization of matrix characteristic of grade 0–1 histopathology (Fig. 1F), to mild arthritis and cellular disorganization (Fig. 1I). Similarly, CPM for 15 days resulted in well-preserved cartilage surface and chondrocyte organization in the middle and deeper zones, to mildly inflammatory arthritis of grade 1 (Table I). More importantly, CPM significantly inhibited histopathological signs of AIA-induced inflammation in all animals, in each group, at all time points tested. FM knees exhibited

**FIGURE 3.** CPM attenuates COX-2 expression in inflamed cartilage. Femoral heads from AIA-afflicted knees exposed to IMM or CPM were sectioned through median longitudinal axis and immunostained with anti-COX-2 IgG. A, Healthy control cartilage. B, Cartilage from knees 2 wk post-AIA induction and (B') cartilage 2 wk post-AIA induction stained with second Ab alone. COX-2-positive cells in cartilage from AIA-afflicted knees, immobilized for 24, 48, 96 h, or 2 wk, exhibiting progressive up-regulation of COX-2 in chondrocytes (C, E, G, and J), or cartilage exposed to CPM for 24, 48, 96 h, or 2 wk exhibiting minimal presence of COX-2 in chondrocytes (D, F, H, and J). K, Enumeration of COX-2-positive cells in superficial and transitional zones or deeper zones in 10^4 μm^2 areas of cartilage, as described in Materials and Methods. Each point represents mean and SEM of seven measurements. *, p < 0.01, where cell numbers from deeper zones of IMM and CPM-treated knees were compared, or superficial and transitional zones of IMM and CPM were compared. L, Total COX-2-associated fluorescence density in 5 × 10^4 μm^2 areas of AIA-afflicted cartilage exposed to CPM or IMM for various time intervals, or FM for 15 days. Each point represents mean and SEM of six separate areas of 5 × 10^4 μm. *, p < 0.01. Each section in A–J is representative of one of five different rabbits at each time point.
compared with CPM-treated knees, or superficial and transitional zones of IMM were compared with CPM.

Materials and Methods.

Each point represents mean and SEM of seven measurements.

compared with CPM knees (Table II).

ulation was mainly driven by monocytic cells in immobilized knees as

itive monocytes and lymphocytes, suggesting that the inflamma-

neutrophils was accompanied by a parallel increase in CD14-pos-

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trophils significantly increased in response to 48 h of IMM of the

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Effect of CPM on synovial inflammation

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 Clearance of BSA from synovial cavity

The possibility existed that CPM may disseminate intra-articular

Effect of CPM on synovial inflammation

Because inflammatory cells in the synovium play an important role

varied histological characteristics that ranged between grade 1.5

and 3.5 arthritis (Table I).

Clearance of BSA from synovial cavity

The possibility existed that CPM may disseminate intra-articular

BSA faster than IMM of joints resulting in decreased BSA con-

centrations and thus lower induction of proinflammatory mediators

in joints subjected to CPM. Examination of the quantitative anal-

ysis of BSA by ELISA revealed that >80% of intra-articular BSA
disseminated within 8 h, and by 24 h >95% of BSA was depleted

from CPM- and IMM-treated knees. The differences in the levels

of BSA in the SFs at other time points were also not significant in

knees subjected to CPM or IMM (Fig. 1N).

Effect of CPM on synovial inflammation

Because inflammatory cells in the synovium play an important role

in exacerbating joint inflammation, we next enumerated inflam-
matory cells in the SF to examine whether CPM or IMM regulate

the influx to the synovium. The cellular smears of SFs revealed

a rapid influx of similar numbers of neutrophils during first 24 h in

both CPM- and IMM-treated knees. However, the number of neu-

trophils significantly increased in response to 48 h of IMM of the

knees. After 96 h of IMM, a consistent decline in the number

of neutrophils was accompanied by a parallel increase in CD14-pos-

itive monocytes and lymphocytes, suggesting that the inflamma-

tion was mainly driven by monocytic cells in immobilized knees as

compared with CPM knees (Table II).

CM down-regulates IL-1β mRNA expression and synthesis in inflamed chondrocytes

The major proinflammatory cytokine involved in cartilage destruc-
tion in arthritis is IL-1β. To better understand the mechanisms

underlying the actions of biomechanical signals, we investigated

the extent of IL-1β expression and synthesis in chondrocytes in

AIA-afflicted cartilage in response to CPM or IMM. Immunohis-
tostaining of tissue sections showed a lack of staining for IL-1β in

chondrocytes in the cartilage from control knees (Fig. 2A)

and subsequently in superficial layers 2–4 days post-IMM treat-

ment (Fig. 2G). The knees subjected to IMM also revealed a marked staining for IL-1β in

depth zones of cartilage during 48–96 h of IMM

(Fig. 2, E, G, and K). A progressive increase in the number of cells

expressing high levels of IL-1β was evident in the deeper layers,

and subsequently in superficial layers 2–4 days post-IMM treat-

ment (Fig. 2, E, G, and K). However, the abundance of IL-1β-positive cells was significantly reduced in IMM-treated knees likely due to fissuring, matrix degradation, and loss of chondro-

cytes in these specimens (Fig. 2, J and K). In parallel to histological

findings above, CPM treatment of AIA-afflicted knees resulted in

a strong and sustained suppression of IL-1β-positive cells in the

deeper zones, as evidenced by the minimal presence of IL-1β in

t chondrocytes at 24, 48, 96 h, and 2 wk (Fig. 2, D, F, H, and K).

FIGURE 4. CPM suppresses AIA-induced MMP-1 expression. A–K. Sections obtained from femoral heads of knees exposed to IMM or CPM were immunostained with anti-MMP-1 IgG. Cross-sections from (A) healthy control cartilage, (B), cartilage 2 wk post-AIA induction, and (B’) cartilage 2 wk post-AIA induction stained with second Ab alone. The cross-sections of cartilage subjected to IMM for CPM for 24, 48, 96 h, or 2 wk exhibiting progressive accumulation of MMP-1 in chondrocytes and matrix (C, E, G, I, and J) or CPM for 24, 48, 96 h, or 2 wk exhibiting minimal expression of MMP-1 (D, F, H, and K). L. Enumeration of MMP-1-positive cells in superficial and transitional zones, or deeper zones in 105 μm2 areas of cartilage, as described in Materials and Methods. Each point represents mean and SEM of seven measurements. * p < 0.01, where cell numbers from deeper zones of IMM were compared with CPM-treated knees, or superficial and transitional zones of IMM were compared with CPM. M. Mean fluorescence density of 5 × 104 μm2 areas of AIA-afflicted cartilage exposed to CPM or IMM for various time intervals, or FM for 15 days. Each point represents mean and SEM of six separate areas of 5 × 105 μm. * p < 0.01. Each section in A–K is representative of one of five different rabbits at each time point.

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Importantly, in CPM-treated knees, IL-1β expression was significantly lower in the deeper zones of cartilage suggesting a role of CPM in preventing cartilage degradation in deeper zones (Fig. 2K).

Because IL-1β is secreted in the milieu, the total relative fluorescence of cells and matrix in the articular cartilage was also examined. Fig. 2L demonstrates relative fluorescence of IL-1β in cartilage treated with IMM or CPM. The decrease in IL-1β-associated relative fluorescence density observed in CPM-treated cartilage paralleled the number of cells positive for IL-1β, confirming CPM-mediated inhibition of IL-1β induction by CPM as compared with IMM, at all time points examined.

To examine whether the suppression of IL-1β expression by mechanical signals is controlled at transcriptional levels, the levels of IL-1β mRNA were examined in cartilage. As shown in Fig. 2M, CPM markedly inhibited IL-1β mRNA expression in chondrocytes as compared with IMM knees. The suppression of IL-1β mRNA expression by CPM was rapid and sustained for the entire 2 wk.

To further characterize the effects of CPM and IMM on inflammation of the joints, we analyzed the levels of IL-1 in the SFs. CPM and IMM treatments resulted in similar levels of IL-1β induction in the SFs during the initial 24 h. Nonetheless, subjecting knees to CPM for 48 h or longer caused a significant suppression of IL-1 in AIA-affected knees as compared with those subjected to IMM (Fig. 2N).

**CPM suppresses COX-2 synthesis in inflamed cartilage**

Biomechanical signals at physiologic levels are anti-inflammatory and inhibit COX-2 induction in the presence of IL-1β in chondrocytes in vitro (17, 19). Therefore, we next determined whether mechanical signals also exert anti-inflammatory effects in vivo, via inhibition of COX-2 induction. Control untreated knees did not exhibit COX-2 in chondrocytes (Fig. 3A). The expression of COX-2 was markedly up-regulated in chondrocytes from immobilized joint within 24 h. This up-regulation of COX-2 was sustained over the entire period of IMM (Fig. 3, C, E, G, and I). In contrast, CPM significantly prevented (p < 0.01) the expression of COX-2 in a significant and sustained manner by chondrocytes during the entire length of CPM treatment, from days 2 to 15 (Fig. 3, D, F, H, and J).

Further enumeration of COX-2-positive cells in the cartilage matrix revealed markedly greater expression of COX-2 in all deeper, transitional, and superficial zones of IMM-treated cartilage. For example, a 4.6-, 8.1-, 5.2-, and 3.8-fold lower expression of COX-2 was observed in the deeper zone of the CPM-treated knees as compared with IMM-treated knees at 24, 48, 96 h, and 2 wk, respectively (Fig. 3K). For example, CPM group showed a 2.5-fold fewer at 24 h, 4.8-fold fewer at 48 h, 2.9-fold fewer at 96 h, and 1.8-fold fewer COX-2-positive chondrocytes at 2 wk, as compared with cartilage from IMM knees (Fig. 3K).

We also examined the COX-2 associated relative fluorescence in cells and matrix of the cartilage exposed to IMM or CPM (Fig. 3L). As evident, not only the number of COX-2-positive chondrocytes, but also COX-2-dependent relative fluorescence per cell was significantly lower in cartilage of the knees treated with CPM as compared with IMM (Fig. 3L).

**CPM suppresses MMP-1 synthesis in inflamed chondrocytes**

AIA-affected joints exhibit significant collagen and proteoglycan degradation and their fragments in the SFs (24). To learn more about the status of matrix degrading enzymes in AIA-affected joints following CPM or IMM, the cartilage was examined for the presence of MMP-1, an enzyme known for its collagenolytic activity. In control animals, MMP-1 was similar to background levels, however, AIA-affected cartilage showed marked increase in MMP-1-positive cells (Fig. 4, A, B, and B'). Similar to other mediators, MMP-1 expression was apparent in immobilized knees within 24 h, which progressively increased following 48, 96 h, and 2 wk antigenic challenge with BSA and IMM (Fig. 4, C, E, G, I, and J). MMP-1-positive cells were present in the superficial and deeper zones of the cartilage after IMM. In contrast, the knees...
subjected to CPM revealed a strikingly lower fluorescence in chondrocytes and matrix at all time points tested (Fig. 4, D, F, H, and K).

The quantitative analysis revealed that MMP-1-positive chondrocytes in the superficial and middle zones of cartilage subjected to CPM was 3.9-fold lower when compared with the immobilized group at 24 h, 4.2-fold lower at 48 h, 5.6-fold lower at 96 h, and 3.8-fold lower at 2 wk as compared with knees subjected to IMM (Fig. 4L). Moreover, the MMP-1-positive cells were found predominantly in the superficial layers of the cartilage subjected to CPM. In contrast, chondrocytes in deeper zones of IMM cartilage exhibited a significantly greater number of MMP-1-positive chondrocytes as compared with those subjected to CPM (Fig. 4, C–K).

Next, analysis of the MMP-1-associated relative fluorescence density in the cartilage matrix and cells in knees subjected to CPM and IMM revealed that CPM suppressed AIA-induced MMP-1 production significantly and in a sustained manner as compared with knees subjected to IMM, at all time points tested (Fig. 4M).

**CPM up-regulates IL-10 synthesis in inflamed cartilage**

To understand the mechanisms by which CPM may mediate its anti-inflammatory effects, the presence of IL-10 was determined in the articular cartilage subjected to CPM or IMM (25, 26). Minimal levels of IL-10 were observed in the superficial and deeper zones of cartilage from untreated control knees (Fig. 5, A and B). Immunohistostaining with anti-IL-10 Abs revealed that expression of IL-10 was significantly lower in AIA-affected knees to both IMM or CPM gradually up-regulated IL-10 during the first 48 h. Following this period CPM induced significantly higher levels of IL-10 production as compared with IMM (Fig. 5, C–F and K). More importantly, chondrocytes from immobilized knees exhibited significantly lower expression of IL-10 in the deeper zones of the cartilage as compared with IMM (Fig. 5K).

Examination of total IL-10-specific fluorescence in cartilage exposed to CPM or IMM revealed that CPM induced significantly higher levels of IL-10, as compared with IMM knees. The relative amount of IL-10 expressed by the chondrocytes in cartilage subjected to CPM was 2.7-, 2.7-, 3.6-, and 1.5-fold higher at 24, 48, 96 h, and 2 wk, respectively (Fig. 5L).

**Discussion**

In this study, we used AIA as an experimental model of RA, in which the onset of disease is quite rapid. The use of this model provided a unique opportunity to examine the early events of the disease development and progression in predictable stages. The arthritic rabbits exhibited the major characteristics of RA that are reminiscent of human disease. Nevertheless, the striking differences from human disease were its early onset (1 day), the rapid cartilage degradation, and the acute rather than chronic nature of the disease. We used this model of RA to understand the biochemical basis for the effects of biomechanical signals in resolving arthritic diseases. Therefore, rabbit knees were subjected to either CPM or IMM immediately after inception of arthritis and were examined 1, 2, 4, or 15 days later. The finding that the majority of BSA is disseminated from the joints within 24 h in knees subjected to CPM and IMM suggests that 1) IMM does not completely inhibit the fluid circulation from the synovial cavity, and 2) that the suppression of inflammation in knees subjected to CPM may not be due to decreased concentration of intraarticular BSA, but is likely due to mechanical forces imparted on the cartilage by CPM.

To exclude the possibility that a decrease in the influx of inflammatory cells in the synovium may have reduced the inflammatory responses in the knees exposed to CPM, we enumerated the number of inflammatory cells in the SFs of joints exposed to CPM and IMM. The acute inflammatory response during the initial 48 h failed to show clear differences in the number of inflammatory cells in SFs of joints exposed to CPM or IMM. Nevertheless, a significant increase in the number of monocytes and lymphocytes in SFs after IMM for 96 h or 2 wk indicates that prolonged IMM may sustain chronic inflammation by either decreasing the clearance or increasing the influx of monocytic cells in the synovium.

The relevance of mechanical signaling in cartilage repair has been well-established because the initial studies, where long-term motion-based therapies were shown to be beneficial to joints afflicted with RA or OA in experimental arthritic models and in humans (9, 20). Nevertheless, to date, the benefits of IMM and mobilization are still controversial due to a lack of clear understanding of the biologic basis of these therapies. Histological analysis demonstrated that IMM of inflamed joints is deleterious and leads to progressive fissuring, loss of stratification, and matrix degradation especially in the deeper zones of cartilage. The degradation of superficial and middle zones of cartilage is a delayed event and parallels the inflammatory process. Nevertheless, some matrix synthesis in the deeper zones is observed after 15 days of IMM, suggesting a degree of cartilage adaptation to IMM.

The most striking effects of CPM are the attenuation of cartilage degradation in a severe proinflammatory environment. Mobilization prevents matrix degradation, cell disorganization, and loss of stratification in cartilage. The actions of mechanical signals generated by CPM are rapid, persistent, and result in the preservation of cartilage integrity during the entire course of CPM treatment. More importantly, biomechanical signals generated by CPM attenuate AIA-induced bone destruction in both the deeper and superficial zones of cartilage. Protection from inflammation in knees exposed to AIA was likely due to the inhibition of proinflammatory gene induction by CPM. Although CPM effectively protected the structural integrity of the articular cartilage, a complete reversal of the effects of AIA was not attained by CPM regimens used in this study. Whether this was due to the inability of CPM to suppress the effects of a large bolus of intra-articular BSA used to induce AIA, or the phenomenon of fatigue as a result of persistent mobilization over a period of 2 wk is as yet not clear. Nevertheless, results from CPM-treated knees clearly showed that CPM plays a significant role in preserving cartilage integrity and has remarkable potential for therapeutic use in suppressing cartilage inflammation.

To understand the molecular basis of the beneficial effects of joint mobilization, the possible regulation of proinflammatory molecules by CPM or IMM was examined. Mechanistically, herein we provide direct evidence that static forces imposed on the tissue by IMM induce cartilage destruction by up-regulation of IL-1β in chondrocytes. As little as 24 h of IMM of inflamed knees is sufficient to augment transcriptional activation of IL-1β, and it continues increases over the entire period of IMM. The up-regulation of IL-1β, first apparent in the deeper zones of cartilage, is likely due to the direct effects of static forces on the deeper zones of the cartilage or due to the proximity of deeper zones of cartilage to the vascular region of the bone. IL-1β is well-documented for its potent proinflammatory effects on cartilage and has been the target of multiple pharmacologic therapies (3, 27). Greater IL-1β induction in chondrocytes and its abundant presence in cartilage exposed to IMM substantiate the deleterious effects of IMM on the arthritic knees.

Contrary to IMM, knees subjected to CPM exhibited a significantly lower expression of IL-1β in chondrocytes especially in the deeper zones of the cartilage. This decrease in IL-1β was due to inhibition of IL-1β mRNA expression as well as its synthesis. In view of these observations, CPM appears to inhibit inflammation via down-regulation of the IL-1β gene expression at the transcriptional level. This is in turn, reflected in the lower number of cells
expressing IL-1β, as well as the extent of overall IL-1β expression in the cartilage.

The fact that there were minimal differences in the concentrations of IL-1β in the SF until 96 h post-IMM or CPM exposure, and that the suppression of inflammation was obvious within 24 h of CPM treatment, further suggests that mechanical signals generated by CPM may act directly on the cells of cartilage to repress expression of proinflammatory genes. In fact, a significant amount of recent in vitro and ex vivo data has demonstrated that chondrocytes are mechanosensitive cells and compressive and tensile strain at low or physiological levels inhibit proinflammatory gene transcription induced by IL-1β and TNF-α (17, 19, 28, 29). Present findings confirm these in vitro and ex vivo data and demonstrate that the actions of mechanical signals are rapid and sustained and thus effectively protect cartilage destruction during inflammation. Nevertheless, mobilization is a dynamic process and its effects on the clearance of inflammatory exudates and proinflammatory mediators from the synovium cannot be underestimated.

Overexpression of COX-2 and PGE2 is a common feature in inflammatory arthritic diseases, and specific inhibitors of COX-2 have demonstrated therapeutic effects in RA and OA (30, 31). IMM exacerbates cartilage destruction via up-regulation of COX-2 induction. The findings that COX-2 is up-regulated in the deeper zones first and then in the middle and superficial zones parallel the IL-1β induction and point out that IMM may act via IL-1β induction in chondrocytes. Our results are consistent with earlier studies demonstrating the presence of higher concentration of COX-2 in joints immobilized via contractures (32). More notable is the fact that a significant suppression of COX-2 expression is observed in cartilage exposed to CPM, further documenting that biomechanical signals may act as anti-inflammatory signals via inhibiting expression of COX-2 in the inflammatory cascade. This is not surprising, because in vitro studies show a significant down-regulation of COX-2 gene transcription in response to cyclic tension (17, 19). Interestingly, patients with inflamed joints subjected to motion have reported reduction in pain after mobilization of joints (10). Our findings that joints subjected to CPM exhibit lower COX-2 expression may explain the basis of lesser pain perception following exercise in inflamed joints.

In arthritic diseases, cartilage destruction correlates with the expression of MMPs and aggrecanases, implicating these enzymes as key mediators in matrix destruction (28, 33–35). In our studies, the rapid fissuring of the deeper zones followed by the massive disorganization of matrix and its subsequent loss are paralleled by the induction of MMP-1. The fact that exposure to joints of CPM resulted in the inhibition of AIA-induced MMP-1 expression further supports that biomechanical signals generated by CPM are potent suppressors of proinflammatory gene induction and thus may be critical in protecting the cartilage integrity (33). In fact, in vitro application of dynamic strain inhibits NF-κB transactivation in chondrocytes to prevent a plethora of proinflammatory gene transcriptions (3, 17, 19). Therefore, in vivo CPM likely suppresses induction of MMP-1 along with IL-1β and COX-2 by inhibiting their expression at transcriptional levels.

IL-10, an anti-inflammatory cytokine, is known for its chondro-protective actions (25, 26, 36). As a result, intra-articular injections of IL-10 are used in arthritic joints to reduce inflammation (25, 26, 37). We, in this study, demonstrated that biomechanical signals generated by CPM also render anti-inflammatory effects via induction of IL-10, a likely mechanism by which attenuation of proinflammatory genes is achieved during inflammation. By regulating induction of IL-10, mechanical signals may additionally prolong the anti-inflammatory effects of CPM. Our data show that IMM also induces IL-10 in chondrocytes. This observation is in agreement with other studies indicating that IL-1 and TNF-α both induce IL-10 as a counterreaction to inflammation (38, 39). Nevertheless, the extent of IL-10 synthesized in the cartilage subjected to IMM was far lesser than those exposed to CPM. This lower production of IL-10 is likely due to the cellular loss accompanying cartilage damage as well as its synthesis. Recently, IL-1 alone or in conjunction with compressive forces is shown to act as an anti-inflammatory signal to inhibit IL-1β-induced nitrite release, in vitro. These findings further demonstrate the fact that mechanical signals act on cartilage as potent anti-inflammatory signals via elaboration of anti-inflammatory mediators (40, 41). Further studies may uncover the causative relationship between CPM and IL-10/IL-4 induction in the regulation of anti-inflammatory cytokines in vivo.

Present findings demonstrate that signals generated by CPM can elicit anti-inflammatory effects, even in the presence of acute inflammation. We have used CPM at a rotation of motion and frequency, shown earlier to be beneficial to arthritic joints (9, 10, 20). However, studies directed at defining optimal efficacy of CPM at other magnitudes and frequencies are necessary to fully understand its potential in cartilage repair. Recent findings demonstrate that the effects of mechanical signals are sustained and exposure of chondrocytes to cyclic tensile forces for short durations is sufficient to suppress proinflammatory gene induction for several hours (42). This temporal regulation of the actions of mechanical signals suggests that for optimal therapeutic efficacy, long-term continuous exposure of motion may not be necessary for the inhibition of proinflammatory gene induction in inflamed joints. By determining a threshold of time, beyond which mechanical forces are not necessary for their protective effects, it is conceivable to achieve optimal anti-inflammatory actions of motion-based therapies. This may result in motion-based therapies that could be used in conjunction with or to replace the anti-inflammatory medications that are used to suppress inflammation (9, 10, 20). Joint mobilization by CPM is suggested to disseminate the inflammatory exudates, whereas IMM is suggested to create a stagnant environment in the synovium, leading to increased inflammation and cartilage damage. Although these effects of mobilization and IMM cannot be excluded, the fact that CPM directly inhibited the proinflammatory gene induction in chondrocytes suggests that mechanical signals regulate inflammation at the intracellular level. In fact, significant recent in vitro and ex vivo data demonstrates that chondrocytes are mechanosensitive cells and respond to mechanical signals in a magnitude-dependent manner (15–19, 28, 29). For example, cyclic tensile or compressive forces in the presence of inflammatory signals are shown to induce matrix synthesis in chondrocytes and fibrochondrocytes (15–19, 28, 29). Present findings confirm these in vitro and ex vivo data and demonstrate in vivo that mechanical signals act directly on chondrocytes to prevent cartilage destruction due to inflammation.

In summary, these studies are the first to reveal the potential intracellular mechanisms of actions for the beneficial effects of motion-based therapies, in vivo. Appropriate mechanical signals generated during such therapies play a profound role in attenuating localized joint inflammation in vivo. These signals act on inflamed chondrocytes by two equally important mechanisms. First, these signals are potent inhibitors of proinflammatory gene induction and inhibit expression of catabolic mediators, e.g., IL-1β, COX-2, and MMP-1. Second, these signals induce expression of the anti-inflammatory cytokine, IL-10. Together, the results demonstrate that biomechanical signals have profound effects in minimizing cartilage destruction in a severe proinflammatory environment and
provide continuing evidence that motion-based therapies are more efficacious than perceived earlier.

Disclosures

The authors have no financial conflict of interest.

References