RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLAST AND U937 MACROPHAGE/MONOCYTE CELL LINE INTERACTION IN CARTILAGE DEGRADATION

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Objective. To examine the interaction between synovial fibroblasts and macrophages in the context of cartilage degradation.

Methods. An in vitro model of human cartilage degradation was used, in which purified populations of fibroblasts and macrophages were added to a radio-labeled cartilage disc. Cartilage destruction was measured by the percentage of radiolabel release.

Results. Fibroblasts, obtained from either rheumatoid arthritis (RA) or osteoarthritis synovial tissue, could mediate cartilage degradation if cocultured with the U937 macrophage cell line. Skin and RA bone marrow fibroblasts had no degradative effect on cartilage. Fibroblast–macrophage contact was not required for cartilage degradation. Cartilage degradation by synovial fibroblasts was inhibited by antibodies to tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), and IL-6. Cartilage degradation was almost completely abrogated by a combination of antibodies to TNFα and IL-1β. Contact between fibroblasts and cartilage was shown to be essential. Antibodies to CD44, but not to intercellular adhesion molecule 1, markedly inhibited cartilage degradation.

Conclusion. TNFα, IL-1β, and IL-6 were involved in the activation of synovial fibroblasts to cause cartilage degradation. Cartilage degradation occurred only when fibroblasts were in contact with cartilage. CD44 was demonstrated to be involved in the fibroblast–cartilage interaction.

Rheumatoid arthritis (RA) is a disease in which immune system mediators evoke an inflammatory response within the joint resulting in cartilage and bone destruction. Evidence has been accumulated to indicate that the cartilage and bone destruction is mediated by synovial macrophages and fibroblasts as a consequence of metalloproteinase release (1–4). It has been suggested that fibroblasts and macrophages within the synovium orchestrate the destructive processes via a self-perpetuating autocrine/paracrine network (5). In support of this concept, fibroblasts and macrophages have an activated phenotype and secrete cytokines capable of paracrine interactions (i.e., interleukin-1β [IL-1β], tumor necrosis factor α [TNFα], IL-6, and granulocyte–macrophage colony-stimulating factor) (6–10). In addition, it has been recently demonstrated that anti-TNFα therapy is effective in the treatment of RA (11–13).

To study the pathobiologic process within the RA joint, we reduced the inflammatory response to the level of its constitutive cellular elements. Using purified populations of cells, we were able to determine, in vitro, the cellular interactions that lead to joint destruction. We demonstrated a key, contact-dependent role for the synovial fibroblast in the destruction of cartilage, in synergy with 1 or more macrophage-generated cytokines. We also demonstrated that cartilage degradation in our assay system could be inhibited with antibodies directed to macrophage- or fibroblast-generated cytokines (i.e., anti-TNFα, anti–IL-1β, and anti–IL-6) and with antibodies to adhesion molecule CD44. An additional inhibitory effect was demonstrated by using a combination of anti-TNFα and anti–IL-1β.

MATERIALS AND METHODS

Cell culture. Synovial fibroblast cell lines were generated from synovial tissue that was obtained at the time of joint
arthroplasty from 18 patients with RA (diagnosed according to the American College of Rheumatology [formerly, the American Rheumatism Association] criteria for RA [14]) and from 11 patients with osteoarthritis (OA). Synovial fibroblast cell lines were established by placing finely minced synovial tissue into a 25-cm² tissue culture flask with media. Minced synovial tissue was left in the culture flask for a period of 1 week to allow the fibroblasts to grow out of the tissue and on to the surface of the culture flask, at which point the tissue was removed. Although there was variation from line to line, in general, cells were passaged every 2 weeks and media were replenished twice weekly.

In addition, fibroblast lines (n = 5) were generated from bone marrow obtained from the femoral heads of RA patients at the time of joint arthroplasty. After lysis of red blood cells, bone marrow cell lines were established by placing bone marrow into 25-cm² tissue culture flasks with media. From this point, generation and maintenance of bone marrow fibroblast cell lines was identical to that of synovial fibroblast cell lines. The distinct morphology of fibroblasts, along with CD14- cells isolated from synovial fluid were used. To prepare U937-conditioned media, U937 cells were grown to a density of 2X10⁶ cells in 20 ml of Opti-MEM. The media was subsequently centrifuged (3,000 revolutions per minute for 15 minutes at 4°C) and filtered (0.2-pm filter; Millipore, Bedford, MA) prior to use in the assay. If the conditioned medium was not used immediately, it was stored at −20°C prior to use.

Cartilage destruction assay. The cartilage degradation assay originally described by Steinberg et al (15), and modified by Janusz and Hare (16), was used with human cartilage and normal human cartilage. Briefly, measurement of degradation of cartilage was performed by culturing synovial fibroblasts and macrophages in the presence of radiolabeled human cartilage discs. Cartilage discs (4 mm × 1 mm) were prepared from normal human femoral cartilage using a 4-mm cork bore. Femoral cartilage was obtained from "normal appearing" cartilage at the time of joint arthroplasty in patients with OA. Discs were incubated overnight with Opti-MEM containing 35S-labeled Na₂SO₄ (10 μCi/ml; Amersham, Oakville, Ontario, Canada), which was incorporated into the glycosaminoglycan side chains of the proteoglycan within the cartilage. The discs were then washed 5 times with sterile phosphate buffered saline (PBS) to remove unincorporated radioisotopes. Discs were then freeze-thawed 5 times and heated at 65°C for 15 minutes to inactivate endogenous enzymes and cytokine activity. The discs were stored at −20°C prior to use. Incorporation of radionuclide was normally found to be between 50,000 and 100,000 disintegrations per minute per disc. Adherent fibroblasts to be cocultured were trypsinized from culture flasks with 0.05% trypsin/0.53 mM EDTA · 4Na (Gibco).

Experiments were carried out using macrophage cell numbers between 2 × 10⁵ and 2 × 10⁶ and fibroblast cell numbers between 2.5 × 10⁵ and 2.5 × 10⁶ per 96-plate well to optimize the assay. For all experiments, 1 × 10⁴ synovial fibroblasts and 1 × 10⁴ U937 macrophages were used. They were cultured together for 7 days in 96-well Nunc plates (Nunc, Roskilde, Denmark) in 200 μl of medium. On day 3, the original medium was removed and replaced with 200 μl of fresh medium. In some experiments, cells were cultured in transwell tissue culture inserts for 96-well tissue culture plates with a 0.2-μm anopore membrane (Nunc). On day 7, 200 μl of medium was removed and added to 3 ml of ready-safe scintillation fluid (Beckman Instruments, Fullerton, CA) and counted in a scintillation counter (LS1071; Beckman Instruments). The remaining incorporated isotope in the cartilage disc was measured by completely digesting the disc in 0.5 ml of tissue solubilizer (Beckman Instruments). Solubilized discs were counted in a scintillation counter using 3 ml of ready-organic scintillation fluid (Beckman Instruments).

Data were expressed as the percentage of 35S released into the supernatant, calculated using the following equation:

\[ \frac{\text{dpm in supernatant}}{\text{dpm in supernatant} + \text{disc}} \times 100 \]

In all experiments, culture of the radiolabeled disc alone was performed as a control. All experiments were carried out in quadruplicate and the percentage release of 35S was calculated for each replicate.

Enrichment of CD14+ cells from synovial fluid. CD14+ cells were isolated from synovial fluid using magnetic cell sorting (MACS) analysis. Synovial fluid was first treated with 1 mg/ml hyaluronidase (Sigma) for 30 minutes at 37°C to reduce the viscosity of the fluid. The fluid was then strained through a steel mesh prior to isolation of leukocytes by Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden). Isolated leukocytes were labeled with CD14 microbeads (Miltenyi Biotec, Sunnyvale, CA), 20 μl MACS CD14 microbeads per 10⁷ cells, and incubated for 15 minutes at 10°C. Cells were resuspended in 500 μl buffer (PBS, 5 mM EDTA, and 0.5% bovine serum albumin), and CD14+ cells were recovered by passing the cell suspension through a washed MACS ferromagnetic matrix A2 column (Miltenyi Biotec) while in the magnetic cell separator. The effluent was collected as the CD14− fraction. The column was then removed from the magnetic cell separator, and the effluent from the column was collected as the CD14+ fraction. Both CD14+ and CD14− fractions were analyzed by fluorescence-activated cell sorter (FACS) to determine the purity of the populations.

FACS analysis of isolated CD14 cells. CD14+ and CD14− fractions separated by MACS were analyzed for purity
Figure 1. Degradation of cartilage caused by rheumatoid arthritis (RA) fibroblasts and U937 macrophage lines. Bars show the mean and SD percentage of $^{35}$S release at day 7 in 4 replicate cultures of RA synovial fibroblast lines ($n = 18$) in the presence (+U937) or absence (-U937) of the U937 macrophage cell line on a radiolabeled cartilage disc. Controls were cultures of U937 with the cartilage disc and cultures of the disc alone. $P < 0.05$ for comparisons of $^{35}$S release between the following cultures: fibroblast with U937 macrophage versus U937 macrophage alone with cartilage disc for all synovial fibroblast lines except for RA-1 and RA-10, fibroblast with U937 macrophage versus fibroblast alone with cartilage disc for all synovial fibroblast lines except for RA-1, RA-8, and RA-10, and fibroblast alone versus U937 macrophage alone, for 5 synovial fibroblast lines (RA-7, RA-8, RA-9, RA-15, and RA-16). All differences were determined by t-test.

Figure 2. Degradation of cartilage caused by osteoarthritis (OA) fibroblasts and U937 macrophage lines. Bars show the mean and SD percentage of $^{35}$S release at day 7 in 4 replicate cultures of OA synovial fibroblast lines ($n = 11$) in the presence (+U937) or absence (-U937) of the U937 macrophage cell line on a radiolabeled cartilage disc. Controls were cultures of U937 with the cartilage disc and cultures of the disc alone. $P < 0.05$ for comparisons of $^{35}$S release between the following cultures: fibroblast with U937 macrophage versus U937 macrophage alone with cartilage disc for all synovial fibroblast lines except for OA-1, OA-6, OA-8, OA-9, and OA-11, fibroblast with U937 macrophage versus fibroblast alone with cartilage disc in 2 synovial fibroblast lines (OA-2 and OA-5), and fibroblast alone versus U937 macrophage alone for 3 synovial fibroblast lines (OA-3, OA-4, and OA-5). No statistical differences were observed among the skin fibroblasts, as determined by t-test.

RESULTS

Degradation of cartilage caused by fibroblasts and U937 macrophages. To test whether purified populations of synovial fibroblasts from RA and OA tissue, as well as RA bone marrow fibroblasts and normal skin fibroblasts, could cause cartilage degradation, we studied the effect of culturing fibroblasts from these sources by FACS staining. Each population was stained with anti-CD3 fluorescein isothiocyanate (FITC), anti-CD19 FITC, and anti-mouse IgG FITC (to stain for mouse anti-CD14 microbeads already on the cells) (Becton Dickinson, San Jose, CA). Each cell fraction was stained with the 3 antibodies for 30 minutes at 4°C and then washed with PBS with 2% FCS prior to analysis by FACS (FACscan; Becton Dickinson).

Antibodies. Anti-human IL-1β (murine IgG1k), anti-human IL-6 (chimeric IgG1k), anti-human TNFα (human/murine chimeric IgG1k), antitumor (murine IgG1k), and antitumor (human/murine chimeric IgG1k) were all a kind gift from Dr. J. N. Woody (Centocor, Malvern, PA). Anti-human TGFβ1-3 (murine IgG1) and anti-human IL-2 (polyclonal rabbit IgG) were obtained from Genzyme (Cambridge, MA); anti-human IL-10 (murine IgG1k) anti-interferon-γ (IFNγ) (murine IgG1k) from R & D Systems (Minneapolis, MN); anti-CD44 (murine IgG1k) from Sigma; and anti-intercellular adhesion molecule 1 (ICAM-1) (murine IgG1k) from Pharmingen (San Diego, CA). Antibodies were used at concentrations of 100 µg/ml, 10 µg/ml, and 1 µg/ml, and were added at day 0 and at day 3.
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100 ~
80 ~
60 ~
40 ~

Fibroblast lines cultured with the U937 cell line led to increased cartilage degradation, which was significant in 6 of the 9 lines (P < 0.05) (Figure 2). In the remaining 2 OA synovial fibroblast lines, no degradation of the cartilage occurred, with or without the U937 cell line. Three OA synovial fibroblast lines caused a significant degree of cartilage degradation in the absence of U937 macrophages.

No degradation of cartilage occurred when the 5 RA bone marrow or 5 skin fibroblast lines were cultured with cartilage discs in the presence or absence of U937 (Figure 3). Degradation of cartilage by the U937 cell line alone was never observed.

Analysis, by Student’s t-test, of the degradative effect of RA fibroblasts (n = 18) versus that of OA fibroblasts (n = 11) revealed no significant difference between these 2 populations.

Replacement of U937 macrophages with CD14+ cells in the cartilage degradation assay. Our initial experiments utilized the U937 macrophage cell line as a substitute for synovial macrophages. To determine the validity of this approach, we isolated CD14+ synovial fluid cells and tested their ability to act in synergy with fibroblasts in the cartilage degradation assay. Five fibroblast lines (3 RA, 1 OA, 1 skin) were cultured with either

...in the presence of a radiolabeled cartilage disc with or without the U937 macrophage cell line. The results showed that all 18 RA synovial fibroblast lines caused cartilage degradation when cocultured with the U937 macrophage cell line (Figure 1). The increase in degradation ranged from 10% to 60% above the degradation caused by the macrophage alone. This was significant in 16 of 18 lines (P < 0.05, by paired t-test). In 5 cases, in the absence of U937 macrophages, the fibroblast lines still caused cartilage degradation.

Nine of 11 OA synovial fibroblast lines cocul-

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Role of fibroblast-macrophage contact in cartilage degradation. Bars show the mean and SD percentage of \(^{35}\)S release at day 7 in 4 replicate cultures under various conditions designed to evaluate the effect of contact between the cells in the degradation. 1 = synovial fibroblast and U937 macrophage cell lines cocultured with cartilage disc; 2 = synovial fibroblasts cultured with cartilage disc; 3 = macrophages cultured in fibroblast-conditioned media with disc; 4 = synovial fibroblasts cultured in macrophage-conditioned media with disc; 5 = synovial fibroblasts cultured with macrophages and disc physically separated in a transwell; 6 = synovial fibroblasts cultured with discs and macrophages physically separated in a transwell; 7 = macrophages cultured with disc and fibroblasts physically separated in a transwell. Controls were cultures of U937 with the cartilage disc and cultures of the disc alone.

Figure 5. Role of fibroblast-macrophage contact in cartilage degradation. The results demonstrated that CD14+ synovial fluid cells were able to replace U937 macrophages in this assay (Figure 4). The coculture of RA fibroblast lines with CD14− synovial fluid cells resulted in significantly reduced levels of cartilage degradation than that mediated by the fibroblast lines cultured with CD14+ cells. In most cases, the coculture of fibroblasts with the CD14− cells resulted in slightly higher levels of degradation than with the fibroblast alone. This may reflect contamination of the CD14− fraction with CD14+ cells. Culture of the U937 cell line, the CD14+, or the CD14− populations with the cartilage disc alone resulted in little or no degradation. The OA line that resulted in little degradation in coculture with U937 gave a similar result when cocultured with CD14+ cells.

Role of fibroblast-macrophage contact in cartilage degradation. We next investigated the nature of the interaction between the synovial fibroblast and the U937 macrophage cell line which led to cartilage degradation. These experiments were conducted using 3 RA synovial fibroblast cell lines (RA-3, RA-5, and RA-7), which we previously found to be among the most proficient in terms of cartilage degradation.

We first determined whether cartilage degradation required contact between fibroblasts and macrophages. In these experiments, we physically separated the fibroblasts or macrophages in the coculture system using transwell tissue culture inserts. The results (Figure 5) indicated that contact between the 2 cell types was not necessary, since degradation of cartilage still occurred when the U937 cells were separated from RA fibroblasts in the culture system. This result suggested that macrophage-derived soluble factors were sufficient to induce fibroblast-mediated cartilage degradation. This interpretation was strengthened by experiments in which U937 macrophage-conditioned medium was shown to be as effective as U937 macrophages in the induction of fibroblast-mediated cartilage degradation. Parallel experiments were performed in which the U937 cells in
contact with cartilage were cultured with fibroblast-conditioned media (Figure 5). Cartilage degradation did not occur under these conditions. These experiments also showed that contact between the fibroblast and the cartilage was essential for cartilage degradation. Thus, no cartilage degradation occurred when the disc and fibroblasts were physically separated (Figure 5).

Inhibition of fibroblast–macrophage interaction with anticytokine antibodies. To define the macrophage-generated factors responsible for fibroblast-mediated cartilage destruction, we added antibodies directed against cytokines to cultures containing fibroblasts, U937-conditioned medium, and the cartilage disc. Our results demonstrated that antibodies (100 μg/ml) to TNFα, IL-1β, and IL-6 all had a marked inhibitory effect on the degradation of cartilage (Figure 6A). The inhibitory effect was dose dependent. Antibodies to the cytokines IFNγ, IL-2, IL-10, and TGFβ had little or no inhibitory effect on cartilage degradation. Two antihepatoma antibodies used in the experiment as isotype controls had no inhibitory effect.

The observation that no single anticytokine antibody caused a complete inhibition of cartilage degradation implied that more than 1 cytokine must be involved in the activation of the fibroblast. To test this possibility, pairs of anticytokine antibodies were tested. As shown in Figure 6B, more than 90% inhibition of cartilage degradation was achieved with the combination of anti-TNFα and anti-IL-1β

Inhibition of fibroblast–cartilage interaction with anti-adhesion molecule antibodies. In the transwell experiments, it was observed that no cartilage degradation occurred when the cartilage disc and the fibroblast were physically separated in culture (Figure 5, columns 5 and 7). Consequently, in the next series of experiments, we examined cell surface molecules that might play a role in this fibroblast–cartilage interaction. To investigate the interaction between fibroblasts and cartilage, we added anti-adhesion molecule antibodies in the assay, to cultures containing fibroblasts, U937-conditioned medium, and the cartilage disc. The results demonstrated that antibodies to CD44 caused marked inhibition of

**Figure 6.** Inhibition of the fibroblast–macrophage interaction with anticytokine antibodies. A, Effect of 7 different anticytokine antibodies on cartilage degradation. Antibodies used were anti-tumor necrosis factor α (anti-TNFα) (○), anti-interleukin-1β (anti-IL-1β) (----), anti-IL-6 (●), anti-IL-2 (□), anti-IL-10 (×), anti-transforming growth factor β (△), anti-interferon-γ (◆), and an isotype control (■). Each anticytokine antibody was added to the rheumatoid arthritis (RA) synovial fibroblast line/U937 macrophage–conditioned media culture system at concentrations (Conc) of 100 μg/ml, 10 μg/ml, and 1 μg/ml. Bars show the mean ± SD percentage of 35S release for the control culture, which was the same culture system without the addition of antibodies to adhesion molecules (mean ± SD percentage of 35S release at day 7 in 4 replicate control cultures was 42.4 ± 3.74%). Graph shows 1 of 3 RA synovial fibroblast lines tested (all 3 gave similar results). The isotype control antihepatoma antibody had no effect. Other controls included cultures of the fibroblast with the cartilage disc in normal medium (3.6 ± 0.57%), U937 macrophage–conditioned media with the cartilage disc (4.1 ± 0.31%), and the disc cultured alone (5.0 ± 1.45%). B, Effect of the combination of anti-TNFα and anti-IL-1β antibodies on cartilage degradation. Graph shows the 3 RA synovial fibroblast lines tested. Bars show the mean ± SD percentage of 35S release for the control culture (mean ± SD percentage of 35S release at day 7 in 4 replicate control cultures was 37.7 ± 6.12% for RA-5, 40.1 ± 1.19% for RA-11, and 37.6 ± 16.2% for RA-13). Other controls included cultures of the fibroblast with the cartilage disc in normal medium (2.5 ± 0.84% for RA-5, 11.5 ± 2.8% for RA-11, and 3.0 ± 1.55% for RA-13), U937 macrophage–conditioned media with the cartilage disc (2.6 ± 1.09%), and the disc cultured alone (5.5 ± 0.87%).
cartilage degradation, whereas anti-ICAM-1 and the isotype control had no effect on cartilage degradation (Figure 7).

**DISCUSSION**

Synovial cartilage and bone destruction are the hallmarks of chronic RA synovitis. Understanding the underlying mechanisms of this destruction within the RA joint may help us to develop effective therapies to treat this relatively common and severely debilitating disease. Although it has been well demonstrated that synovial macrophages and fibroblasts are among the major cell populations within the joint that are responsible for the destructive processes in RA, it has been largely accepted that T cells are regulating this process (17,18).

We have examined an in vitro model of human cartilage degradation to investigate the destructive effect of the synovial fibroblast when cocultured with a macrophage cell line. Our results demonstrated the degradative role of synovial fibroblasts in RA. It is unlikely that T cells are present in the coculture assay system, due to the long-term culture of synovial fibroblasts prior to use in the assay. We have been unable to identify any T cells within the fibroblast population by FACS staining (data not shown). These results support the contention that cartilage degradation caused by synovial fibroblasts and macrophages can occur in the absence of T cells, as has been previously suggested (5). Several synovial fibroblast lines mediated cartilage degradation (significant in 5 RA and 3 OA fibroblast lines) in the absence of U937 macrophages. Nevertheless, in every case, the addition of macrophages augmented degradative activity.

To test the physiologic relevance of using the U937 cell line as the macrophage in the coculture, we substituted the U937 cell line with an enriched population of CD14+ synovial fluid macrophages. This comparison revealed that the U937 cell line caused levels of cartilage degradation similar to those caused by CD14+ synovial fluid macrophages in coculture with the fibroblasts. This degradation did not occur when the CD14− population was used in coculture with the fibroblasts. Neither the CD14+ synovial fluid cells nor the CD14− synovial fluid cells mediated cartilage degradation when cultured alone with the cartilage. From these findings, we conclude that the U937 cell line is a suitable replacement for freshly derived synovial macrophages in this assay.

We have also demonstrated that the ability to degrade cartilage was restricted to synovial fibroblasts. Bone marrow fibroblasts from RA patients and skin fibroblasts were inactive in this assay system. Surprisingly, OA fibroblasts had degradative activity similar to that of RA fibroblasts in this assay. In previous studies of fibroblast activity, OA fibroblasts generally differed from RA fibroblasts. Our results suggest that OA fibroblasts have the same degradative potential as RA fibroblasts and that different properties attributed to these 2 types of fibroblasts may be irrelevant to disease progression. The data suggest that cells or soluble factors interacting with synovial fibroblasts determine the destructive nature of the synovial lining.

We ascertained from our experiments that contact between the synovial fibroblasts and macrophages was not a prerequisite for degradation of cartilage, since synovial fibroblasts could be activated to degrade carti-
lager by macrophage-conditioned media. Soluble factors released by the macrophage appear to be, at least in part, responsible for the fibroblast activation.

Experiments with anticytokines anti-TNFα, anti-IL-1β, and anti-IL-6 resulted in a profound reduction in cartilage degradation. It is notable that high levels of these cytokines have been detected in the RA joint (19). In contrast, antibodies to a number of other cytokines involved in inflammation (IFNγ, IL-2, IL-10, and TGFβ) resulted in little or no inhibition. These results support the presence of a paracrine interaction between synovial fibroblasts and macrophages.

It was unexpected that anti-IL-6 would cause such a marked reduction in fibroblast-mediated cartilage degradation. Although IL-6 is found at elevated levels within the joint and has been demonstrated to be involved in multiple inflammatory responses (8), its effect on fibroblasts is unclear. In addition, Chomarat and coworkers have recently demonstrated that culture of unstimulated blood monocytes and synoviocytes resulted in a synergistic release of IL-6, which was further enhanced by the addition of IL-1β and TNFα (20). How increased levels of IL-6 may lead to increased fibroblast-mediated cartilage degradation is unclear; however, evidence suggesting that IL-6 may be involved in fibroblast proliferation may present us with a possible mechanism (21).

These results are consistent with current concepts regarding the pathogenesis of RA, which have demonstrated the involvement of TNFα, IL-1β, and IL-6 in the disease process (8,22–31). The lack of effect with IFNγ and IL-2 was not unexpected, since no T cells were present in the culture system. The lack of effect with IL-10 and TGFβ, which exert mainly immunosuppressive effects on macrophages, was not surprising, since U937-conditioned media were used to activate the fibroblast. Thus, negative feedback was not possible in this in vitro model.

Since several anticytokine antibodies caused inhibition of the cartilage degradation, it is likely that the activation pathway may be invoked by more than one cytokine. Addition of both anti-IL-1β and anti-TNFα resulted in almost complete inhibition of cartilage degradation, confirming that more than one cytokine is involved in the synergy between macrophages and fibroblasts. Moreover, the data suggest that prevention of cartilage degradation may not be achieved by inhibition with 1 cytokine alone, but rather by a combination of anticytokine antibodies.

Of significance was the observation that contact between fibroblast and cartilage was a prerequisite for cartilage degradation. In experiments in which fibroblasts and macrophages were cocultured, but the fibroblast was physically separated from the cartilage, degradation did not occur. This observation indicates that contact between the fibroblasts and cartilage is necessary for cartilage destruction. Recent studies have reported the augmented expression of a number of adhesion molecules on synovial fibroblasts, including ICAM-1 and CD44 (32–36). In experiments in which anti-adhesion molecules were added to the culture system, our results demonstrated that contact-dependent degradation of cartilage, at least in part, involved adhesion molecule CD44. These findings suggest that the effect of binding of synovial fibroblasts to epitopes within cartilage is mediated by CD44, which may induce the secretion of enzymes contributing to cartilage degradation.

Adhesion molecules can be induced by cytokines found at high levels within the joint (37,38). The interaction of adhesion molecules with their ligands induces intracellular signals, which cause cells to proliferate, selectivly express certain genes, and secrete certain cytokines. Engagement of VLA-5 and αβ3 integrins, for example, cause the production of several metalloproteinases (39). It was surprising that CD44 was shown to be involved in this synovial fibroblast interaction with cartilage, since previous studies have suggested that a beneficial effect of antibodies to CD44, in animal models of arthritis, is due to the prevention of lymphocyte migration into the joint (40).

Further studies that address the interaction between fibroblast and cartilage are under way and may identify potential therapeutic target molecules for treatment of RA.

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