ARTHRITIS & RHEUMATISM
Vol. 40, No. 3, March 1997, pp 490–498
© 1997, American College of Rheumatology

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

BOYD B. SCOTT, LISA M. WEISBROT, JANICE D. GREENWOOD, EARL R. BOGOCH, CHRISTOPHER J. PAIGE and EDWARD C. KEYSTONE

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 radiolabeled 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.**

Boyd B. Scott, PhD, Janice D. Greenwood, PhD, Earl R. Bogoch, MD, Christopher J. Paige, PhD, Edward C. Keystone, MD: Wellesley Hospital Research Institute, and University of Toronto, Toronto, Ontario, Canada; Lisa M. Weisbrot, MSc: Wellesley Hospital Research Institute.

Address reprint requests to Christopher J. Paige, PhD, Wellesley Hospital Research Institute, 160 Wellesley Street East, Toronto, Ontario M4Y 1J3, Canada.

Submitted for publication May 23, 1996; accepted in revised form September 18, 1996.

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 metallaproteinase 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-cm^2 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-cm^2 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 their unique ability to survive multiple passages in the absence of added growth factors in vitro were used to assign a lineage to these cells.

Skin fibroblast cell lines (n = 5) were obtained from the American Type Culture Collection (CCD-974, CCD-944, CCD-866, CCD-967, and CCD-976; Rockville, MD) and cultured in conditions identical to those of the synovial fibroblast cell lines. All cells were cultured in Opti-MEM (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (FCS; Gibco), antibiotic–antimycotic mix (penicillin G sodium [1,000 units]/streptomycin sulfate [1,000 units]/amphotericin B [2.5 μg/ml]; Gibco), 5.5×10^{-5}*M* β-mercaptoethanol (Sigma, St. Louis, MO), and 2.4 gm/liter sodium bicarbonate (Mallinckrodt, Point-Claire, Quebec, Canada).

The macrophage cell line U937 or U937-conditioned medium was used in all experiments, except for experiments in which a comparison between the U937 cell line and RA synovial fluid CD14+ cells was made, in which case CD14+ and CD14− cells isolated from synovial fluid were used. To prepare U937-conditioned media, U937 cells were grown to a concentration of 1×10^7 cells in 20 ml of Opti-MEM. The media were subsequently centrifuged (3,000 revolutions per minute for 15 minutes at 4°C) and filtered (0.2 μm millex-GV filter; Millipore, Bedford, MA) prior to use in the assay. If the conditioned medium was not used immediately, it was stored at −70°C. All experiments were conducted with fibroblasts (synovial, bone marrow, and skin) at passages 2, 3, or 4.

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 cell lines 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 ^{35}S-labeled Na_2SO_4 (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 m*M* EDTA · 4Na (Gibco).

Experiments were carried out using macrophage cell numbers between 2×10^3 and 2×10^5 and fibroblast cell numbers between 2.5×10^3 and 2.5×10^5 per 96-plate well to optimize the assay. For all experiments, 1×10^4 synovial fibroblasts and 1×10^5 U937 macrophages were used. They were cultured together for 7 days in 96-well Nunclon 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 ^{35}S released into the supernatant, calculated using the following equation:

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

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 ^{35}S 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 10^7 cells, and incubated for 15 minutes at 10°C. Cells were resuspended in 500 μl buffer (PBS, 5 m*M* 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

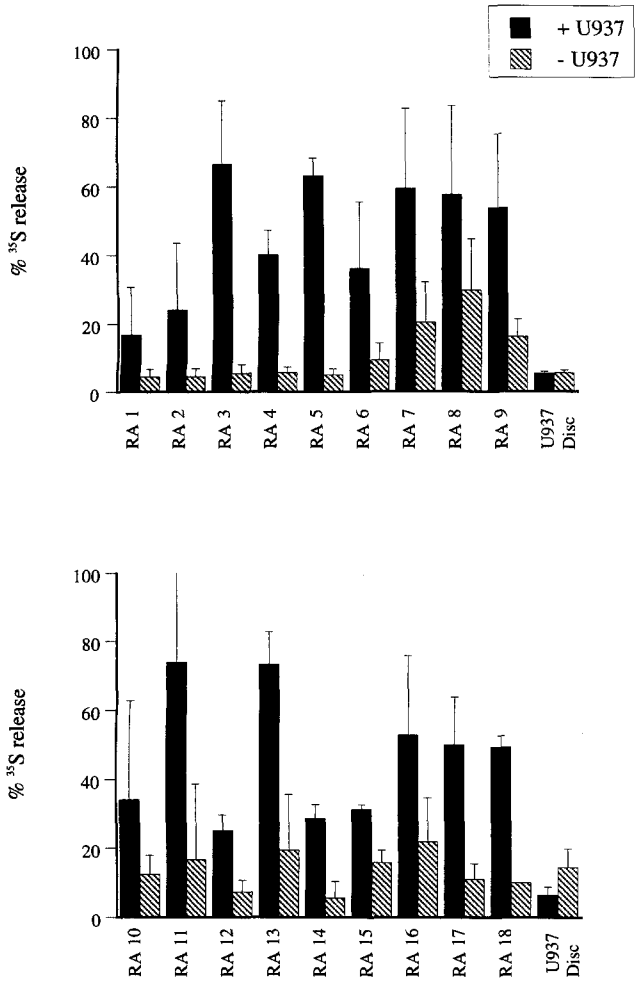

Figure 1. Degradation of cartilage caused by rheumatoid arthritis (RA) fibroblasts and U937 macrophage lines. Bars show the mean and SD percentage of ^{35}S release at day 7 in 4 replicate cultures of RA synovial fibroblast lines (n = 18) in the presence (+U937) or absence (−U937) of the U937 macrophage 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.

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 IgG1κ), anti-human IL-6 (chimeric IgG1κ), anti-human TNFα (human/murine chimeric IgG1κ), antihepatoma (murine IgG1κ), and antihepatoma (human/murine chimeric IgG1κ) 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 IgG1κ) anti–interferon-γ (IFNγ) (murine IgG1κ) from R & D Systems (Minneapolis, MN); anti-CD44 (murine IgG1κ) from Sigma; and anti–intercellular adhesion molecule 1 (ICAM-1) (murine IgG1κ) 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.

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

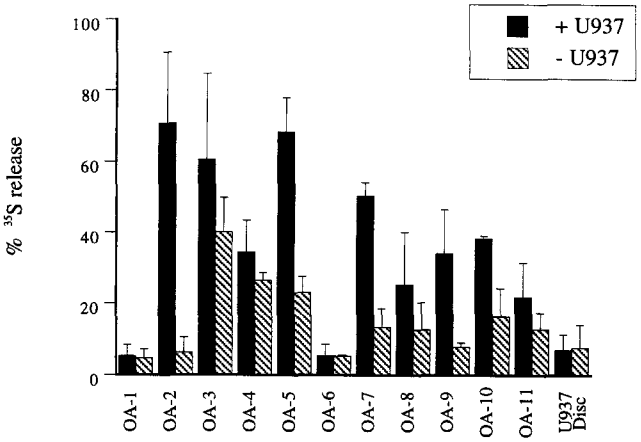

Figure 2. Degradation of cartilage caused by osteoarthritis (OA) fibroblasts and U937 macrophage lines. Bars show the mean and SD percentage of ^{35}S release at day 7 in 4 replicate cultures of OA synovial fibroblast lines (n = 11) in the presence (+U937) or absence (−U937) of the U937 macrophage cell 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.

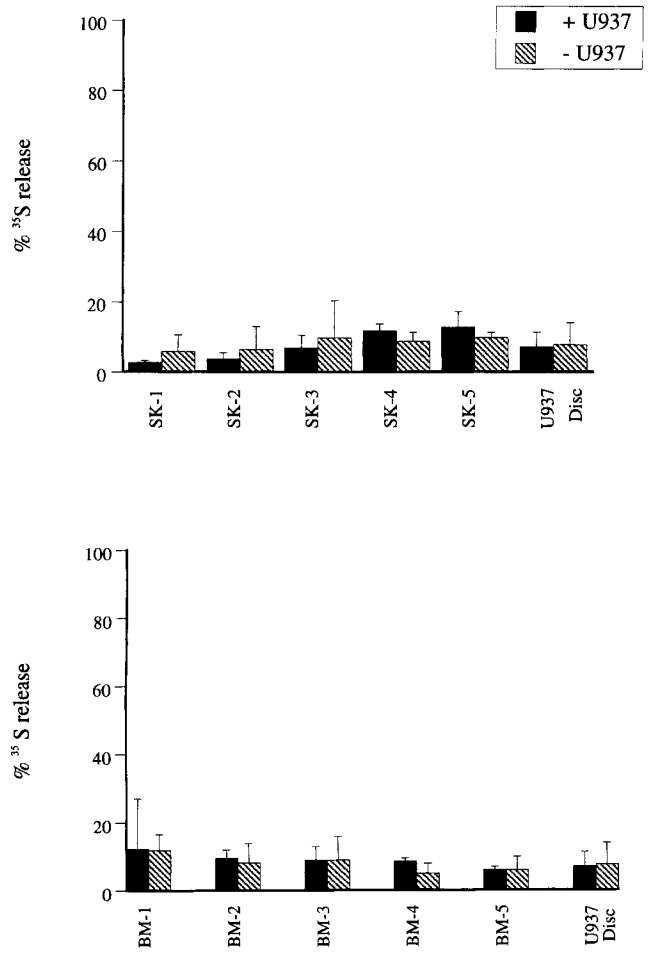

Figure 3. Degradation of cartilage caused by rheumatoid arthritis (RA) bone marrow (BM) (n = 5) or normal skin (SK) (n = 5) fibroblasts and U937 macrophage lines. Bars show the mean and SD percentage of ^{35}S release at day 7 in 4 replicate cultures of fibroblast lines in the presence (+U937) or absence (−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. No statistical differences were observed among the RA bone marrow fibroblasts or among the skin fibroblasts.

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 cocultured 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

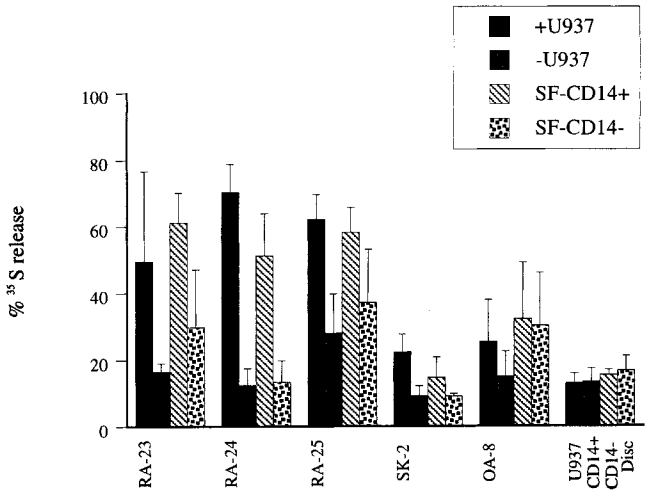

Figure 4. Degradation of cartilage caused by fibroblasts (3 rheumatoid arthritis [RA], 1 osteoarithitis [OA], and 1 skin [SK]) and either U937 macrophage lines, RA synovial fluid CD14+ cells, or RA synovial fluid CD14− cells. Bars show the mean and SD percentage of ^{35}S release at day 7 in 4 replicate cultures of fibroblast lines in the presence (+U937) or absence (−U937) of the U937 macrophage cell line, RA synovial fluid CD14+ cells (SF-CD14+), RA synovial fluid CD14− cells (SF-CD14−), or the fibroblasts alone, on a radiolabeled cartilage disc. Controls were cultures of U937, RA synovial fluid CD14+ cells, or RA synovial fluid CD14− cells with the cartilage disc and cultures of the disc alone.

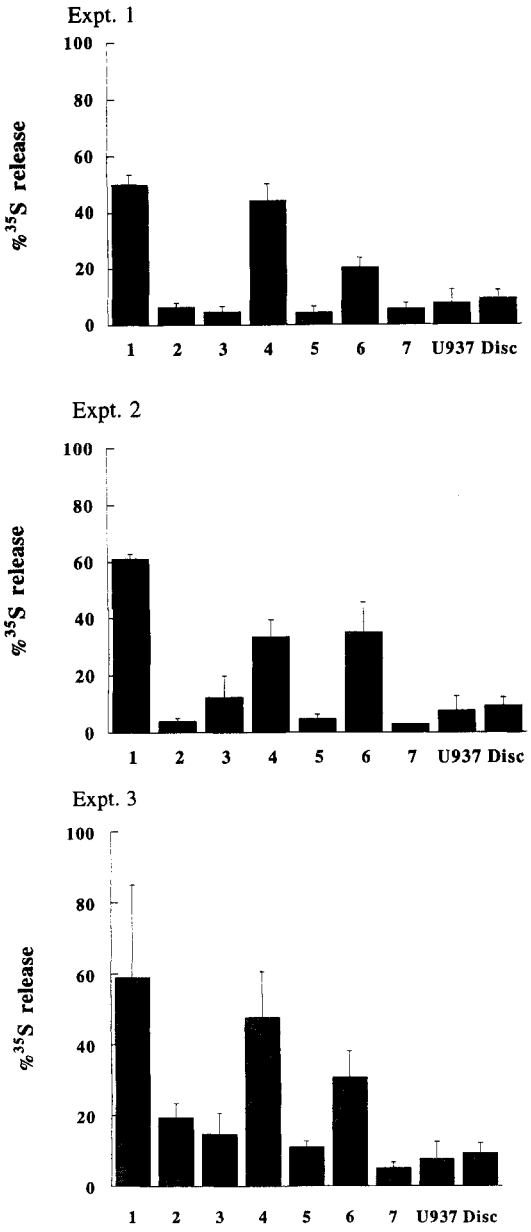

Figure 5. Role of fibroblast–macrophage contact in cartilage degradation. Bars show the mean and SD percentage of ^{35}S release at day 7 in 4 replicate cultures under various conditions designed to evaluate the effect of contact between the cells in the degradation. 1 = synovial fibroblast and U937 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.

the U937 cell line, allogeneic RA synovial fluid CD14+ cells, or RA synovial fluid CD14− cells. The OA line used (OA-8) was a synovial fibroblast which, in coculture with U937, did not cause cartilage degradation (Figure 2). This line was used to observe whether a synovial fibroblast that did not lead to cartilage degradation in coculture with U937 could be activated to degrade cartilage when in coculture with CD14+ synovial fluid cells. The CD14+ enriched fraction, as determined by FACS analysis, contained 89% CD14+ cells. The CD14− population contained 13% CD14+ cells and 75% lymphocytes.

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

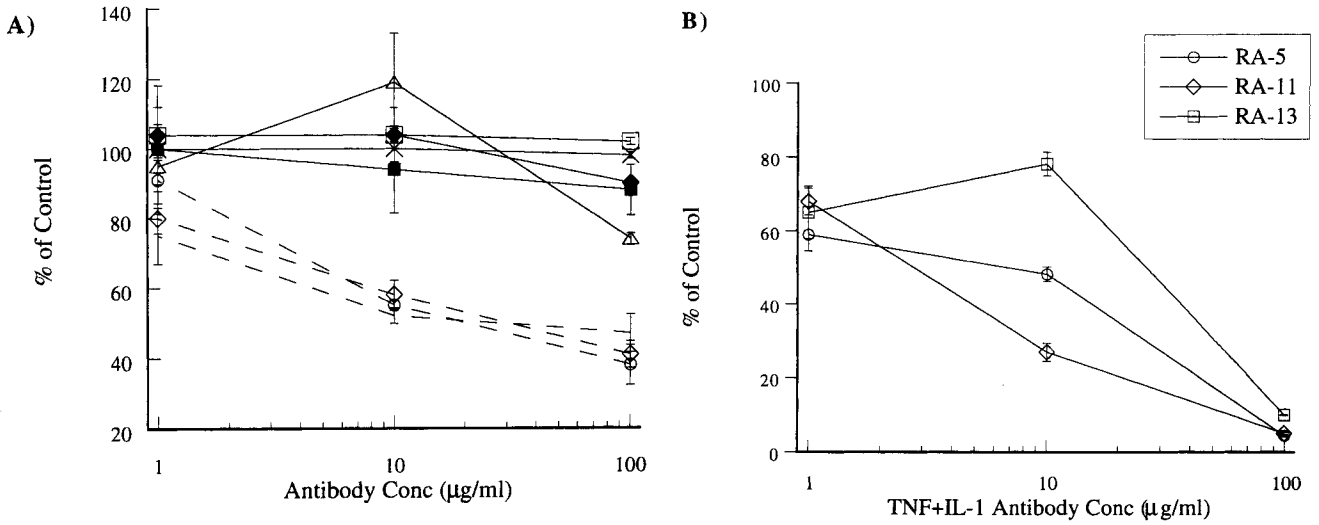

Figure 6. Inhibition of the fibroblast–macrophage interaction with anticytokine antibodies. **A**, Effect of 7 different anticytokine antibodies on cartilage degradation. Antibodies used were anti–tumor necrosis factor α (anti-TNFα) (○), anti–interleukin-1β (anti–IL-1β) (----), anti–IL-6 (◇), anti–IL-2 (□), 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 ^{35}S release for the control culture, which was the same culture system without the addition of antibodies to adhesion molecules (mean ± SD percentage of ^{35}S 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 ^{35}S release for the control culture (mean ± SD percentage of ^{35}S 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%).

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

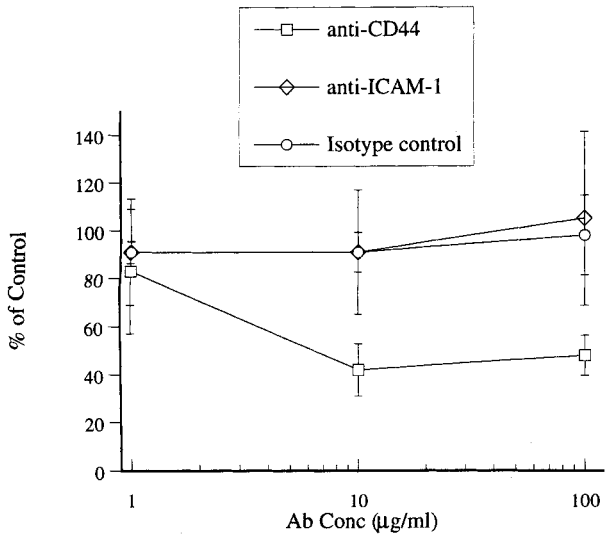

Figure 7. Inhibition of rheumatoid arthritis (RA) fibroblast–cartilage interaction with the anti–adhesion molecule antibodies (Ab) anti-CD44, anti–intercellular adhesion molecule 1 (anti–ICAM-1), and an isotype control. Each anti–adhesion molecule antibody was added to the RA synovial fibroblast line/U937 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 ^{35}S release for the control culture, which was the same culture system without the addition of antibody to adhesion molecules (mean ± SD percentage of ^{35}S release at day 7 in 4 replicate control cultures was 55.9 ± 7.98%). Graph shows 1 of 3 RA synovial fibroblast lines tested (all 3 gave similar results). Significant ($P < 0.05$) differences in inhibition of cartilage degradation was observed between anti-CD44 and either anti–ICAM-1 or isotype control at 100 μg/ml and 10 μg/ml, as determined by *t*-test. The isotype control antihepatoma antibody had no effect. Other controls included cultures of the fibroblast with the cartilage disc in normal medium (14.2 ± 10.55%), U937 macrophage–conditioned media with the cartilage disc (5.2 ± 3.90%), and the disc cultured alone (1.6 ± 0.49%).

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-

lage 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, Chomorat and coworkers have recently demonstrated that coculture 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 1 cytokine. Addition of both anti–IL-1β and anti-TNFα resulted in almost complete inhibition of cartilage degradation, confirming that more than 1 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, selectively 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.

REFERENCES

1. McCachren SS, Haynes BF, Niedel JE: Localization of collagenase mRNA in rheumatoid arthritis synovium by in situ hybridization histochemistry. J Clin Immunol 10:19–27, 1990
2. Unemori EN, Hibbs MS, Amento EP: Constitutive expression of a 92-kd gelatinase (type V collagenase) by rheumatoid synovial fibroblasts and its induction in normal human fibroblasts by inflammatory cytokines. J Clin Invest 88:1652–1662, 1991
3. Woolley DE, Crossley MJ, Evanson JM: Collagenase at sites of cartilage erosion in the rheumatoid joint. Arthritis Rheum 20: 1231–1239, 1977
4. Trabandt A, Gay RE, Fassbender H-G, Gay S: Cathepsin B in synovial cells at the site of joint destruction in rheumatoid arthritis. Arthritis Rheum 34:1444–1451, 1991
5. Firestein GS, Zvaifler NJ: How important are T cells in chronic rheumatoid synovitis? Arthritis Rheum 33:768–773, 1990
6. Miossec P, Dinarello CA, Ziff M: Interleukin-1 lymphocyte chemotactic activity in rheumatoid arthritis synovial fluid. Arthritis Rheum 29:461–470, 1986
7. Macnaul KL, Hutchinson NI, Parsons JN, Bayne EK, Tocci MJ:

Analysis of IL-1 and TNF-α gene expression in human rheumatoid synoviocytes and normal monocytes by in situ hybridization. J Immunol 145:4154–4166, 1990
8. Guerne P-A, Zuraw BL, Vaughan JH, Carson DA, Lotz M: Synovium as a source of interleukin 6 in vitro: contribution to local and systemic manifestations of arthritis. J Clin Invest 83:585–592, 1989
9. Xu WD, Firestein GS, Taetle R, Kaushansky K, Zvaifler NJ: Cytokines in chronic inflammatory arthritis. II. Granulocyte-macrophage colony-stimulating factor in rheumatoid synovial effusions. J Clin Invest 83:876–882, 1989
10. Bucala R, Ritchlin C, Winchester R, Cerami A: Constitutive production of inflammatory and mitogenic cytokines by rheumatoid synovial fibroblasts. J Exp Med 173:569–574, 1991
11. Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, Leeb B, Breedveld FC, MacFarlane JD, Bijl H, Woody JN: Randomized double blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet 344:1105–1110, 1994
12. Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Bijl H, Woody JN: Repeated therapy with monoclonal antibody to tumour necrosis factor alpha (cA2) in patients with rheumatoid arthritis. Lancet 344:1125–1127, 1994
13. Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Katsikis P, Brennan FM, Walker J, Bijl H, Ghrayeb J, Woody JN: Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor α. Arthritis Rheum 36:1681–1690, 1993
14. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA Jr, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31:315–324, 1988
15. Steinberg J, Tsukamoto S, Sledge CB: A tissue culture model of cartilage breakdown in rheumatoid arthritis. III. Effects of antirheumatic drugs. Arthritis Rheum 22:877–885, 1979
16. Janusz MJ, Hare M: Cartilage degradation by cocultures of transformed macrophage and fibroblast cell lines. J Immunol 150:1922–1931, 1993
17. Harris ED: Rheumatoid arthritis: pathophysiology and implications for therapy. N Engl J Med 322:1277–1289, 1990
18. Panayi GS, Lanchbury JS, Kingsley GH: The importance of the T cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. Arthritis Rheum 35:729–735, 1992
19. Firestein GS, Alvaro-Gracia JM, Maki R: Quantitative analysis of cytokine gene expression in rheumatoid arthritis. J Immunol 144:3347–3353, 1990
20. Chomorat P, Rissoan MC, Pin JJ, Banchereau J, Miossec P: Contribution of IL-1, CD14 and CD13 in the increased IL-6 production induced by in vitro monocyte-synoviocyte interactions. J Immunol 155:3645–3652, 1995
21. Koshase M, Henriksen-DeStefano D, May LT, Vilcek J, Sehgal PB: Induction of B2-interferon by tumor necrosis factor: aq homeostatic mechanism in the control of cell proliferation. Cell 45:659–666, 1986
22. Kaushansky K, Lin N, Adamson JW: Interleukin 1 stimulates fibroblasts to synthesize granulocyte-macrophage and granulocyte colony-stimulating factors. J Clin Invest 81:92–97, 1988
23. Eastgate JA, Symons JA, Wood NC, Grinlinton FM, di Giovine FS, Duff GW: Correlation of plasma interleukin 1 levels with disease activity in rheumatoid arthritis. Lancet 2:706–709, 1988
24. Dayer J-M, de Rochemonteix B, Burrus B, Demczuk S, Dinarello CA: Human recombinant interleukin 1 stimulates collagenase and prostaglandin E2 production by synovial cells. J Clin Invest 77:645–648, 1986
25. Dayer J, Beutler B, Cerami A: Cachetin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. J Exp Med 162:2163–2168, 1985
26. Bergsteinsdottir K, Kingston A, Mirsky R, Jessen KR: Rat Schwann cells produce interleukin-1. J Neuroimmunol 34:15–23, 1991
27. Lorre K, Kasran A, van Vaeck F, de Boer M, Ceuppens JL: Interleukin-1 and B7/CD28 interaction regulate interleukin-6 production by human T cells. Clin Immunol Immunopathol 70:81–90, 1994
28. Lovett D, Kozan B, Hadam M, Resch K, Gemsa D: Macrophage cytotoxicity: interleukin 1 as a mediator of tumor cytostasis. J Immunol 136:340–347, 1986
29. Navarro S, Debili N, Bernaudin JF, Vainchenker W, Doly J: Regulation of the expression of IL-6 in human monocytes. J Immunol 142:4339–4345, 1989
30. Pujol-Borrel R, Todd I, Doshi M, Bottazzo GF, Sutton R, Gray D, Adolf GR, Feldmann M: HLA class II induction in human islet cells by interferon-gamma plus tumour necrosis factor of lymphotoxin. Nature 326:304–306, 1987
31. Sengupta TK, Chen A, Zhong Z, Darnell JE, Ivashkiv LB: Activation of monocyte effector genes and STAT family transcription factors by inflammatory synovial fluid is independent of interferon γ. J Exp Med 181:1015–1025, 1995
32. Haynes BF, Hale LP, Patton KL, Martin ME, McCallum RM: Measurement of an adhesion molecule as an indicator of inflammatory disease activity: up-regulation of the receptor for hyaluronate (CD44) in rheumatoid arthritis. Arthritis Rheum 34:1434–1443, 1991
33. Johnson BA, Haines GK, Harlow LA, Koch AE: Adhesion molecule expression in human synovial tissue. Arthritis Rheum 36:137–146, 1993
34. Chin JE, Winterrowd GE, Krzesicki RF, Sanders ME: Role of cytokines in inflammatory synovitis: the coordinate regulation of intercellular adhesion molecule 1 and HLA class I and class II antigens in rheumatoid synovial fibroblasts. Arthritis Rheum 33:1776–1786, 1990
35. Postigo AA, Garcia-Vicuna R, Laffon A, Sanchez-Madrid F: The role of adhesion molecules in the pathogenesis of rheumatoid arthritis. Autoimmunity 16:69–76, 1993
36. Kriegsmann J, Keyszer GM, Geiler T, Brauer R, Gay RE, Gay S: Expression of vascular cell adhesion molecule-1 mRNA and protein in rheumatoid synovium demonstrated by in situ hybridization and immunochemistry. Lab Invest 72:209–214, 1995
37. Blue M-L, Conrad P, Webb DL, Sarr T, Macaro M: Interacting monocytes and synoviocytes induce adhesion molecules by a cytokine-regulated process. Lymphokine Cytokine Res 12:213–218, 1993
38. Bombara MP, Webb DL, Conrad P, Marlor CW, Sarr T, Ranges GE, Aune TM, Greve JM, Blue M-L: Cell contact between T cells and synovial fibroblasts causes induction of adhesion molecules and cytokines. J Leukoc Biol 54:399–406, 1993
39. Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH: Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. J Cell Biol 109:877–889, 1989
40. Mikecz K, Brennan FR, Kim JH, Glant TT: Anti-CD44 treatment abrogates tissue oedema and leukocyte infiltration in murine arthritis. Nature Med 1:558–563, 1995