

SPONTANEOUS PRODUCTION OF FIBROBLAST- ACTIVATING FACTOR(S) BY SYNOVIAL INFLAMMATORY CELLS

A Potential Mechanism for Enhanced Tissue Destruction

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Hyperplasia of synovial lining cells and fibroblasts is an early and characteristic feature of the synovitis associated with rheumatoid arthritis. The synovial fibroblast hyperplasia contributes to the excessive levels of collagenase and prostaglandins generated in synovitis (1), which likely mediate connective tissue destruction. The mechanisms underlying this hyperplastic response are not known, but are important to an understanding of the tissue injury in rheumatoid arthritis. Associated with this excessive proliferation of synovial fibroblasts is a marked influx of mononuclear cells, including lymphocytes and monocytes, into the sublining areas (2, 3). Based on recent *in vitro* evidence that human lymphocytes and monocytes can modulate fibroblast growth (4–9), it was of considerable interest to determine whether these cells might be involved in regulating expansion of the fibroblast population in chronic rheumatoid synovitis. T lymphocytes, which are rarely found in the normal human synovial tissue, are found in large numbers, in close apposition to macrophages, in the synovial membrane during chronic synovitis. These observations favor cell-mediated immune mechanisms in the pathogenesis of this condition. The proximity and concurrent presence of activated T lymphocytes and macrophages with proliferating synovial lining cells and fibroblasts also suggests a role for these mononuclear cells in inducing the hyperproliferative response of the synovial cells.

In this study, synovial fluid and/or synovial tissue were obtained from rheumatoid arthritis patients at knee arthroscopy. The synovial lymphocytes and monocytes were then isolated and the ability of these cells to generate mediators of fibroblast growth was analyzed. These inflammatory cells were found to spontaneously release fibroblast growth factors that are similar to those previously described as products of peripheral blood mononuclear cells after *in vitro* stimulation (4, 6). Synovial tissues obtained from patients with noninflammatory joint disease were not a source of fibroblast growth factors.

Materials and Methods

Synovial Tissue Collection and Immunohistopathology. Synovial tissue was obtained at arthroscopy from multiple sites in inflamed knees from 12 patients with confirmed definite

or classic rheumatoid arthritis (10). Synovial tissue was also obtained from four individuals with noninflammatory joint disease, including trauma and degenerative joint disease, who were undergoing corrective surgery. After removal from the joint, the tissue was washed in phosphate-buffered saline (PBS)¹ (pH 7.2, NIH Media Unit). Tissue aliquots were snap-frozen in a glycerol base embedding medium (Tissue-Tek II, O.C.T. compound; Miles Laboratories, Lab-Tek Div., Naperville, IL) by immersion in a mixture of dry ice and acetone, or fixed in 10% phosphate buffered formalin. Formalin-fixed tissue was embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

8- μ m sections were cut from the frozen blocks on a cryostat at -20°C (model CTI; International Equipment Company, Needham Heights, MA), placed on glass slides, and fixed in acetone (3). Cell surface markers were demonstrated in situ by immunoperoxidase staining (11, 12) (ABC Vecta Stain Kit; Vector Laboratories, Burlingame, CA) using the monoclonal antibodies Leu-1, a pan-T lymphocyte marker; Leu-2A, a "suppressor/cytotoxic" subset marker; Leu-3A, a "helper/inducer" subset marker; Leu-M1 and Leu-M3, monocyte/macrophage markers; anti-HLA-DR (Becton, Dickinson & Co., Sunnyvale, CA); and OKM1, a monocyte/macrophage, natural killer, and null cell marker (Ortho Diagnostic Systems, Inc., Westwood, MA). Sections were counterstained with 2% methyl green, dehydrated, and mounted in Permount (Fisher Scientific Co., Fair Lawn, NJ) under a glass coverslip. Control sections were treated with mouse ascites fluid or normal mouse IgG, or were stained in the absence of the primary antibody.

Synovial Tissue Culture and Mononuclear Cell Isolation. In addition to specimens for immunohistopathology, 250 mg of the synovial tissue were aseptically minced and resuspended in 10 ml Dulbecco's modified Eagle's medium (DMEM) (HEM Research Inc., Rockville, MD) containing 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, and 2 mM glutamine without serum. The cultures were incubated at 37°C for varying time periods and the supernatants collected and frozen at -20°C .

To isolate the mononuclear cells from the diseased synovial tissue, portions of minced synovial tissue were placed in a spinner flask containing collagenase (3.5 mg/ml; Worthington Biochemical Corp., Freehold, NJ) in DMEM and stirred for 20–30 min at 37°C . The cells in suspension were removed, the procedure repeated two to four times, and the remaining particulate tissue removed by passage through sterile gauze. The cell suspensions were pooled, centrifuged, and washed several times and resuspended in PBS. These cells were then centrifuged on Ficoll-Paque gradients (Pharmacia, Inc., Piscataway, NJ) to obtain the mononuclear cells. When this procedure was carried out on noninflamed synovial tissue, no mononuclear cells were obtained.

Synovial Fluid Collection and Isolation of Mononuclear Cells. Synovial fluid from clinically tender and swollen knees was aspirated under aseptic conditions with a 2-gauge needle into a sterile syringe. The fluid was maintained at room temperature and used within 3 h. The fluid was centrifuged and the cell pellet resuspended in PBS. These cells were centrifuged on Ficoll-Paque gradients and the mononuclear cells collected from the interface were washed and resuspended in DMEM. The remaining cell-free synovial fluid was frozen at -20°C until assayed for fibroblast-activating factor(s) (FAF).

Peripheral Blood Mononuclear Cells. 50 ml of venous peripheral blood were obtained from adult patients with confirmed definite or classic rheumatoid arthritis or from normal volunteers, diluted in PBS, and layered on Ficoll-Paque gradients. After centrifugation, the interface containing the mononuclear cells was collected, washed, and resuspended at 2×10^6 cells/ml in DMEM.

Identification of Cell Surface Antigens on Isolated Mononuclear Cells. Direct immunofluorescence studies on isolated single-cell suspensions used fluorescein-conjugated monoclonal antibodies directed to a common cell surface antigen on human peripheral blood T cells (OKT3), to the helper/inducer T cell subset (OKT4), to the suppressor/cytotoxic subset (OKT8), and to the HLA-DR antigen. OKM1 was used in indirect immunofluorescence studies with a goat F(ab')₂ anti-mouse IgG preparation conjugated with fluores-

¹ Abbreviations used in this paper: Con A, concanavalin A; DMEM, Dulbecco's modified Eagle's medium; FACS, fluorescence-activated cell sorter; FAF, fibroblast-activating factor; FCS, fetal calf serum; [³H]TdR, tritiated thymidine; LPS, lipopolysaccharide; PBS, phosphate-buffered saline.

cein isothiocyanate (Tago, Inc., Burlingame, CA). Control antibody directed against an irrelevant antigen (mouse Thy-1.1; Becton, Dickinson & Co.) was also used to stain the cells. The cells that were stained with experimental or control antibody were analyzed with a fluorescence-activated cell sorter (FACS II; B-D FACS Systems, Becton, Dickinson & Co.) for cell surface fluorescence as described (10).

Mononuclear Cell Culture. Mononuclear cells isolated from peripheral blood, synovial fluid or synovial tissue were suspended at 2×10^6 /ml in serum-free DMEM with antibiotics and glutamine. 1-ml aliquots were dispensed in TC24 culture dishes (Costar, Cambridge, MA). When adequate numbers of synovial fluid mononuclear cells were available, separation of monocytes and lymphocytes was achieved by adherence. After a 1–2 h incubation of the mononuclear cells, the nonadherent cells were removed, leaving OKM1⁺ adherent macrophages (1×10^6 /ml) in the culture wells. The nonadherent OKT3⁺ T lymphocytes were pooled, resuspended, and cultured at 1×10^6 /ml in TC24 culture wells. To some cultures, concanavalin A (Con A) (Calbiochem-Behring Corp., La Jolla, CA) or lipopolysaccharide (LPS) (*E. coli* 055:B5; Difco Laboratories, Inc., Detroit, MI) was added at the onset of culture. Culture plates were incubated at 37°C and the cell-free supernatants harvested at various time points up to 72 h. The supernatants were frozen at –20°C until assayed in the fibroblast proliferation assay.

Fibroblast Proliferation Assay. Primary fibroblast cultures established in DMEM containing antibiotics, 2 mM glutamine, and 10% fetal calf serum (FCS) (Gibco Laboratories, Grand Island, NY) were used in a system previously shown (4) to be regulated by human mononuclear cell products. For proliferation assays (4), trypsinized fibroblasts were plated at 5×10^4 cells per well in TC24 plates in 1 ml DMEM containing 10% FCS for 2–4 h, washed, and cultured overnight without serum. After removing the media, the synovial supernatants diluted in serum-free DMEM were added and cultures incubated 48 h before being pulsed for 4 h with 1 μ Ci/ml tritiated thymidine (³H]TdR) (sp act, 6 Ci/mM; Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, NY). Trypsinized cultures were harvested with an automated harvester and processed for determination of [³H]-TdR incorporation. Units of FAF in the supernatant were calculated by comparing the [³H]-TdR uptake with a standard FAF supernatant prepared from Con A-stimulated human mononuclear cells arbitrarily defined as 100 U/ml. FAF units were determined by the following formula: [(reciprocal titer of test supernatant at 50% maximum cpm of the standard) \times 100]/[reciprocal titer of the standard at 50% maximum cpm].

Sephacryl S-200 Gel Filtration. After dialysis against Tris buffer, pH 7.5, the synovial cell supernatants were lyophilized and reconstituted in PBS. 1-ml samples of concentrated fluid were applied to a 1.0 \times 45 cm column of Sephacryl S-200 (Pharmacia, Inc.) which had been equilibrated with 0.15 M NaCl, 0.02 M phosphate buffer, pH 7.2. The void volume was determined with blue dextran and the column was calibrated with cytochrome *c* and ovalbumin (Pharmacia, Inc.). The sample was eluted with PBS and the protein concentration of each fraction was determined by measuring the absorbance at 280 nm.

Results

Characterization of Inflammatory Cells Within the Synovial Tissue. Diseased synovial tissues from 12 rheumatoid arthritis patients with active synovitis were obtained at arthroscopy. Immunohistological evaluation of these diseased synovial tissues revealed hyperplasia of synovial lining cells and the presence of mononuclear inflammatory cells in the sublining layer (Fig. 1A). Phenotypic analysis revealed that many of these cells were Leu-1 (pan-T cell)-positive cells (Fig. 1B) and monocytes that stained with the OKM1 (Fig. 1C) and Leu-M1 and Leu-M3 monoclonal antibodies (not shown). Furthermore, HLA-DR antigens stained densely in synovial lining areas (Fig. 1D) and also stained mononuclear cells in the sublining layer (Fig. 1D). Mononuclear cells within the synovium from noninflamed tissues were insignificant (not shown).

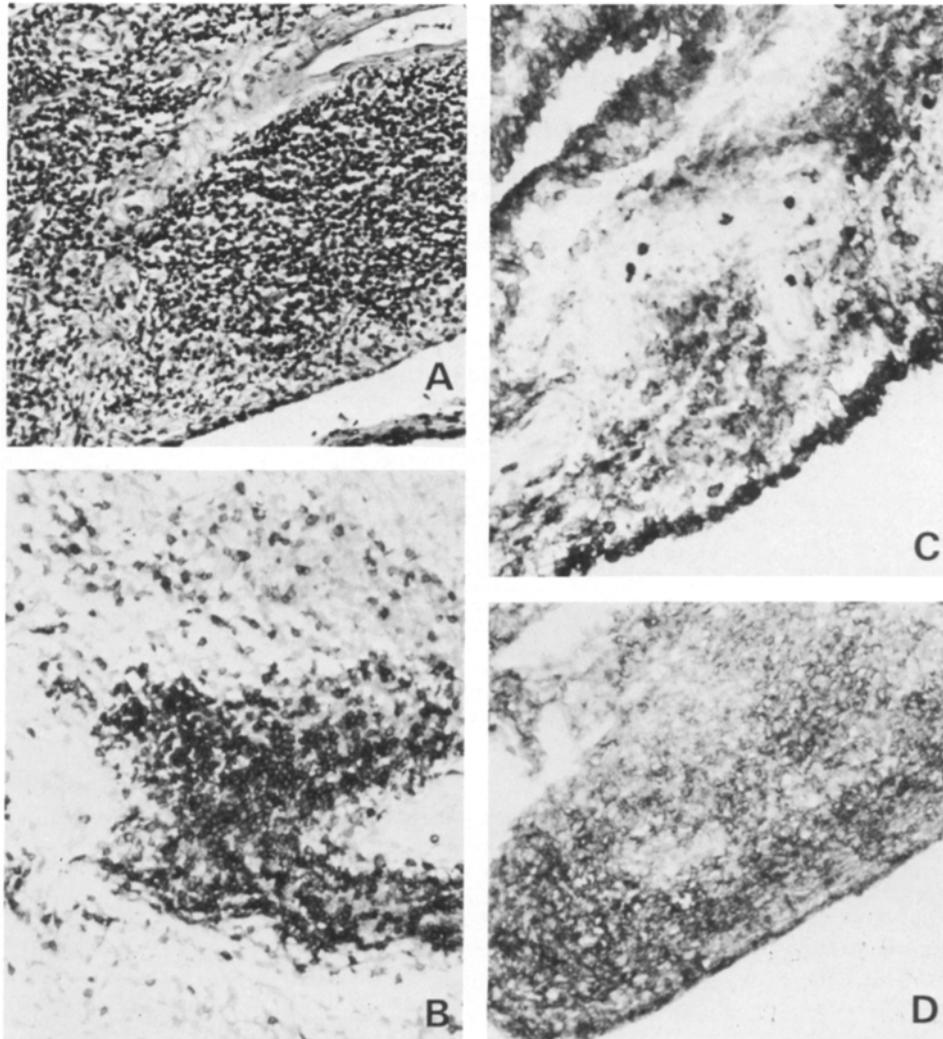


FIGURE 1. Immunohistologic identification of synovial tissue mononuclear cells. Synovial tissue was fixed in formalin, sectioned, and stained with hematoxylin and eosin (A) or frozen, fixed in acetone, and immunoperoxidase-stained using Leu-1 (B), OKM1 (C), or HLA-DR (D) monoclonal antibodies.

Release of FAF by Cultured Synovial Tissue and Mononuclear Cells. To determine whether the inflammatory cells within the synovial tissue produced FAF-like activity that might account for the hyperplasia of the synovial fibroblasts, 250 mg of minced synovial tissue from the rheumatoid arthritis patients described above were cultured. Supernatants were harvested from the cultures at varying time periods and assayed for their ability to induce fibroblast growth. Supernatants from 10 of 12 synovial tissue cultures were found to contain significant FAF (activity >10 U/ml) (Fig. 2A). No exogenous stimuli were added to the synovial cultures; FAF-like activity was detected in the supernatants within 24 h

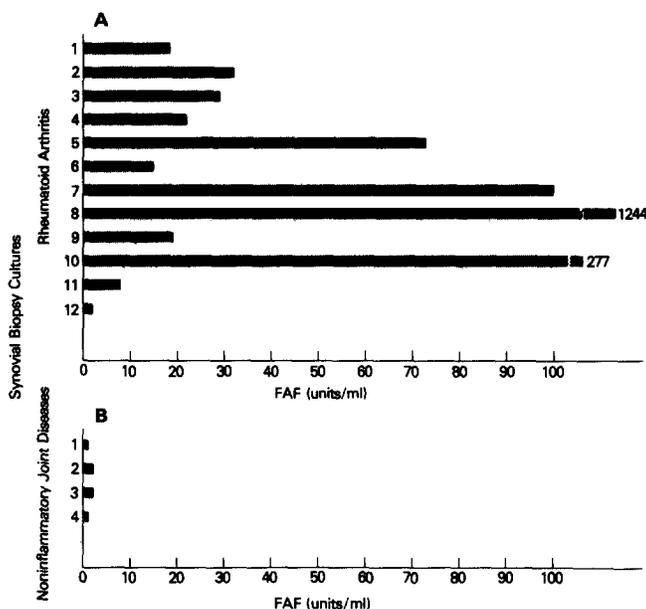


FIGURE 2. FAF activity in synovial biopsy cultures. ~250 mg of finely minced synovial tissue from joints with synovial inflammation (A) or noninflammatory disease (B) were cultured in 10 ml serum-free medium for 48 h. Supernatants were diluted and assayed for their ability to induce fibroblast [^3H]TdR incorporation. [^3H]TdR cpm were transformed into units of FAF by comparison with a standard FAF preparation containing 100 U/ml.

and increased and plateaued by 72 h. In contrast, when synovial tissues obtained from individuals with noninflammatory joint disease were processed in a similar manner, the supernatants from these cultures did not contain significant levels of FAF (Fig. 2B).

Moreover, the mononuclear cells were isolated from the diseased rheumatoid synovial tissue, cultured at 2×10^6 cells/ml, and the 48-h supernatants tested for FAF activity. Since these cells spontaneously generated substantial levels of FAF activity (Table I), the mononuclear cells were clearly a source of the FAF activity. With no in vitro stimulation, synovial tissue mononuclear cell supernatants contained FAF activity capable of enhancing fibroblast proliferation 50-fold above the levels of [^3H]TdR incorporated in unstimulated fibroblast cultures. The production of this activity could not be attributed to activation of the mononuclear cells by the collagenase or contamination of the collagenase with agents such as endotoxin, since the activity was also generated by inflamed synovium before enzymatic digestion. The addition of LPS or Con A to these cultures did not enhance the maximum levels of FAF activity already being produced, even when tested at lower dilutions. Thus, these cells appear to have been activated in vivo and continued to secrete mediators of fibroblast growth in vitro. Enzymatic digestion of synovial tissue from individuals with noninflammatory joint disease yielded insufficient mononuclear cells for culture, and no FAF activity could be measured.

Characterization of Synovial Mononuclear Cell-derived Factors Responsible for

TABLE I
*Stimulation of Fibroblast Proliferation by Synovial Tissue
 Mononuclear Cell Supernatants*

Stimulant	Fibroblast proliferation ($[^3\text{H}]\text{TdR}$ incorporation)
	<i>cpm</i>
None*	11,040 \pm 691 [†]
LPS	11,161 \pm 251
Con A	11,601 \pm 808
Control	246 \pm 24

* 2×10^6 /ml mononuclear cells obtained from synovial tissue were cultured without stimuli (*none*) or with 30 $\mu\text{g}/\text{ml}$ LPS or 5 $\mu\text{g}/\text{ml}$ Con A for 48 h, and the cell-free supernatants collected.

[†] 5×10^4 fibroblasts were cultured with a 1:10 dilution of the mononuclear cell supernatants for 48 h. The cultures were pulsed with 1 $\mu\text{Ci}/\text{ml}$ $[^3\text{H}]\text{TdR}$ for 4 h and processed for the determination of $[^3\text{H}]\text{TdR}$ incorporation. Data represent the mean \pm SE of triplicate cultures from a representative experiment. Control represents fibroblasts cultured in medium only.

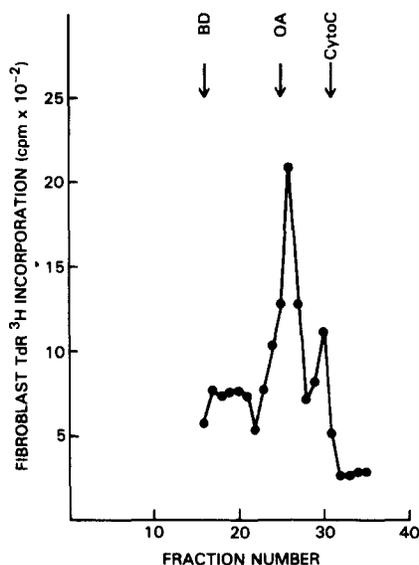


FIGURE 3. Sephacryl S-200 chromatography of synovial mononuclear cell supernatants. Concentrated (10 \times) supernatants were applied to a calibrated Sephacryl S-200 column and eluted in PBS. Fractions were assayed for their ability to induce fibroblast $[^3\text{H}]\text{TdR}$ incorporation.

Stimulating Fibroblast Growth. To determine whether the mediators of fibroplasia spontaneously produced by the synovial mononuclear cells were similar to those previously identified as peripheral blood T cell and monocyte products *in vitro*, preliminary characterization of the supernatant factors was initiated. Two peaks of FAF activity were eluted from a Sephacryl S-200 column (Fig. 3). The first peak, eluting near 40,000 mol wt, was consistent with the product generated by peripheral blood T lymphocytes (4). A second, smaller peak of activity, in the

15,000 mol wt range, may represent the monocyte product that has been reported (6) to have both FAF and interleukin 1 activity, or another fibroblast growth factor. The evidence suggests that the inflammatory lymphocytes and monocytes found in the diseased synovial tissue can generate mediators of fibroblast growth that have been shown to be produced by lymphocytes and monocytes stimulated in vitro. Studies are in progress to obtain sufficient quantities of these mediators for more definitive analysis.

Identification of Fibroblast Growth-promoting Activity in Synovial Fluid. Synovial fluid obtained from adult patients with definite or classic rheumatoid arthritis was centrifuged and the cell-free fluid was diluted and assayed for its effects on fibroblast proliferation. As is evident in Fig. 4, which is representative of synovial fluid from three patients, maximal activity was seen in diluted joint fluid, with inhibition at higher concentrations. Although the fibroblast growth-promoting activity titers were variable, 30 of 30 synovial fluids tested were found to contain significant levels of activity (>10 U/ml) with some of these fluids containing activity >600 U/ml. In preliminary experiments, we have identified similar activity in the synovial fluid of patients with psoriatic arthritis and inflammatory osteoarthritis, suggesting that the presence of fibroblast growth activity is not specific for rheumatoid arthritis but rather is associated with inflammation. Since it is difficult to dissociate the contribution of serum proteins and other factors in the synovial fluid, we focused on the isolation of inflammatory cells from the synovial fluid.

Production of FAF by Synovial Mononuclear Cells. Mononuclear cells were isolated from synovial fluid to determine directly whether these cells were producing FAF and contributing to the fibroblast growth activity found in the synovial fluid. In addition, the isolated synovial mononuclear cells were stained

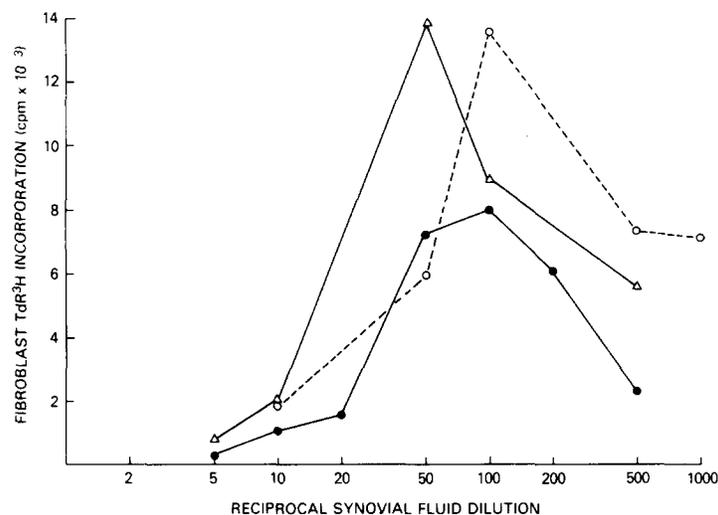


FIGURE 4. Stimulation of fibroblast proliferation by synovial fluids. Synovial fluids obtained from three patients at arthrocentesis were centrifuged, diluted in DMEM at the indicated concentrations, and assayed for their ability to induce fibroblast [³H]TdR incorporation. Fibroblasts cultured in medium only incorporated $<1,000$ cpm.

directly with fluorescein-conjugated, cell-specific monoclonal antibodies or indirectly with the monoclonal antibodies and fluorescein-labeled secondary antibodies, and analyzed by flow microfluorometry (FACS). OKT3⁺ T lymphocytes were the predominant cell isolated from some patients; OKM1⁺ monocytes were the majority in others, and equivalent numbers of OKT3⁺ and OKM1⁺ monocytes were also observed. Four patients are represented in Table II, which demonstrates the heterogeneity of these mononuclear cell isolates. Many of the cells were found to be HLA-DR-positive (Table II), emphasizing their activated state in the inflammatory site. The mononuclear cells were cultured serum-free at 2×10^6 cells/ml for 48 h with or without exogenous stimuli. The supernatants were then assayed for the presence of FAF activity. As is evident in Table III and Fig. 5, supernatants from synovial fluid mononuclear cells contained significant FAF activity even in the absence of in vitro stimulation. Limited enhancement of activity was detected if the synovial mononuclear cells were further stimulated in vitro with LPS or Con A (Table III).

TABLE II
Phenotypes of Mononuclear Cell Populations in the Synovial Fluid of Rheumatoid Arthritis Patients

Antibody	Synovial fluid mononuclear cells* from patient:			
	1	2	3	4
OKT3	24.5	61.2	40.0	56.9
OKT4	13.9	40.3	35.8	32.7
OKT8	8.5	23.7	14.3	29.2
HLA-DR	68.1	42.7	47.7	44.7
OKM1	71.2	24.1	47.2	39.8

* Aliquots of mononuclear cells isolated from synovial fluid of four patients were stained with the indicated antibodies and analyzed by flow microfluorometry for cell surface fluorescence. Data represent the mean percent positive staining.

TABLE III
Stimulation of Fibroblast Proliferation by Synovial Fluid Mononuclear Cell Supernatants

Stimulant	Fibroblast proliferation (³ H]TdR incorporation)
	<i>cpm</i>
None*	4,612 ± 577 [‡]
LPS	6,386 ± 551
Con A	8,773 ± 1,155
Control	246 ± 24

* 2×10^6 /ml mononuclear cells obtained from synovial fluid were cultured without stimuli (*none*) or with 30 μ g/ml LPS or 5 μ g/ml Con A for 48 h and the cell-free supernatants collected.

[‡] 5×10^4 fibroblasts were cultured with a 1:10 dilution of the mononuclear cell supernatants for 48 h. The cultures were pulsed with 1 μ Ci/ml [³H]TdR for 4 h and processed for the determination of [³H]TdR incorporation. Data represent the mean \pm SE of triplicate cultures from a representative experiment. Control represents fibroblasts cultured in medium only.

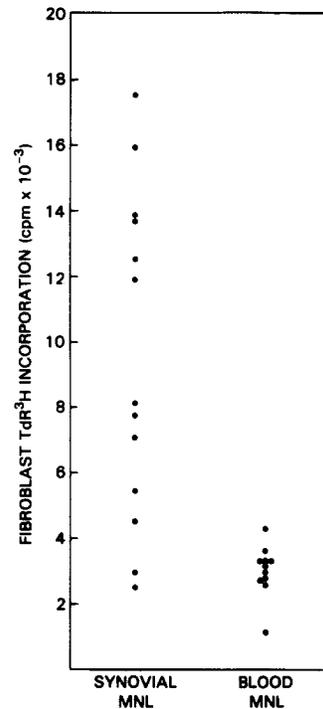


FIGURE 5. Spontaneous production of FAF activity by synovial but not peripheral blood mononuclear cells. 2×10^6 mononuclear cells/ml obtained from joints with active inflammation or from peripheral blood were cultured for 48 h in the absence of serum or exogenous stimuli, and the supernatants tested for their ability to induce fibroblast [^3H]TdR incorporation (cpm).

Mononuclear cells were found in very limited numbers or not at all in the synovial fluid of patients with noninflammatory joint disease or trauma, making it impossible to either characterize them or to culture them for FAF production. Thus, to compare the mononuclear cells from the inflamed synovium of arthritis patients with a noninflammatory mononuclear cell population, we compared mononuclear cells from the synovial fluid of patients with arthritis with mononuclear cells obtained from peripheral blood, for the production of FAF activity. Little or no FAF activity was detected in the supernatants of the peripheral blood mononuclear cells ($2 \times 10^6/\text{ml}$) in the absence of *in vitro* stimulation, whereas substantial levels of FAF were seen in similar cultures of synovial mononuclear cells (Fig. 5). FAF activity could, however, be generated after stimulation of the peripheral blood cells with LPS or Con A (4). Thus, in contrast to circulating peripheral blood lymphocytes and monocytes, cells isolated from inflamed synovium spontaneously produced significant quantities of this mediator(s). To determine whether the monocytes or the lymphocytes were the source of this fibroblast growth activity, synovial fluid cells were separated into adherent OKM1⁺ monocyte monolayers and nonadherent OKT3⁺ cells. Supernatants were collected from these cells and, as demonstrated in Fig. 6, both cell populations released activity that could stimulate fibroplasia into their supernatants.

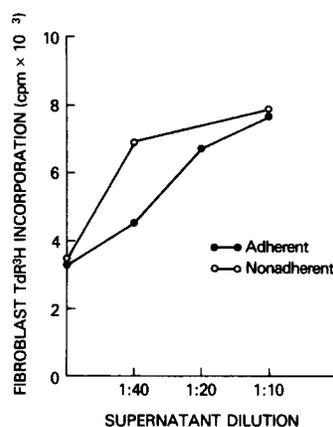


FIGURE 6. Spontaneous production of FAF activity by adherent and nonadherent synovial mononuclear cells. 1×10^6 OKM1⁺ or 1×10^6 nonadherent OKT3⁺ cells obtained from synovial fluid of joints with active inflammation were cultured for 48 h in the absence of serum or exogenous stimuli, and the supernatants tested for their ability to induce fibroblast [³H]TdR incorporation (cpm).

Discussion

The increased proliferation of synovial lining cells occurring in association with the appearance and persistence of lymphocytes and macrophages in the subsynovium is a well-recognized histological feature of both early and advanced rheumatoid arthritis. However, the immune reactions taking place in the synovial tissue in rheumatoid arthritis are poorly understood.

By immunohistologic analysis, the mononuclear inflammatory cells within the inflamed tissue were identified as Leu-1⁺/OKT3⁺ T lymphocytes and OKM1⁺, Leu-M1⁺ macrophage-like cells. Consistent with previous studies (13–15), Leu-3⁺/OKT4⁺ lymphocytes predominated among the lymphocytes in these tissues, particularly in nodular lymphocytic areas. The relatively high intensity anti-HLA-DR staining suggested that both the lymphocytes and macrophages were activated within the inflammatory locus (2, 16). These observations suggest that mechanisms similar to those operating in delayed-type hypersensitivity reactions are involved in the pathogenesis of rheumatoid arthritis (17). When these diseased synovial tissues were cultured *in vitro* and the supernatants assayed for their ability to induce fibroblast growth, significant FAF activity was measurable in the culture supernatants. In the studies described here, FAF activity refers only to the initiation of growth in quiescent fibroblast monolayers. In 10 of 12 supernatant preparations, >10 U of FAF/ml were released within the first 24 h of culture. Similar preparations of synovial tissue obtained during corrective surgical procedures for noninflammatory joint disease did not release significant FAF activity into the culture supernatants, emphasizing the inflammatory cell dependence of the generation of this mediator of fibroplasia.

Since the diseased synovial tissue was a major source of FAF-like activity, experiments were subsequently initiated to determine which of the cells in the inflamed tissue were the source of this activity. After enzymatic digestion of the tissue and isolation of the mononuclear cells that were characterized as OKT3⁺

T lymphocytes and OKM1⁺ monocytes, cultures were established for mediator production. Supernatants from unstimulated cultures contained significant amounts of FAF-like activity. Attempts to enhance production of this mediator with Con A and/or LPS were ineffective, suggesting that the mononuclear cells were already maximally activated in vivo. The activity detected in the synovial cell supernatants shares the biological and physicochemical characteristics of the factor that is released by activated peripheral blood T cells, with a molecular weight near 40,000 (4), and also may represent interleukin 1 (15,000 mol wt), produced by activated monocytes in vitro (6). In additional experiments, the synovial mononuclear cells were separated into T lymphocyte-enriched (OKT3⁺) and adherent monocyte (OKM1⁺) populations. Both populations were the source of fibroblast growth activity. Thus, both T cells and monocytes obtained from this inflammatory lesion are activated in vivo, resulting in the secretion of mediators that can promote fibroblast growth.

Further evidence that the synovial lymphocytes are activated in vivo has been previously demonstrated by the enhanced production of migration inhibition factor (18) and of lymphocyte blastogenic factor and immunoglobulins (19) in the synovial tissue of patients with rheumatoid arthritis. Mononuclear cells eluted from synovium also spontaneously secrete leukocyte migration inhibitor factor (LIF) (20). In addition to FAF, other mediators can be found within the synovial fluid of arthritis patients (21). The monocyte-derived factor, interleukin 1, has also recently been identified in synovial effusions (22, 23). Although the signals leading to activation of the mononuclear cells are unknown, it appears that these cells are triggered in vivo to generate mediators associated with delayed-type hypersensitivity.

As to the potential role of these mediators in the pathogenesis of rheumatoid arthritis, it is likely that the infiltrating populations of T lymphocytes and monocytes found in the rheumatoid synovium, activated by some yet unidentified, but probably persistent stimulus, begin secreting lymphokines and monokines, including those that can modulate fibroplasia. The enhanced fibroblast numbers can, in turn, generate elevated levels of inflammatory mediators and tissue-destructive enzymes. In addition to stimulating fibroblast growth, mononuclear cell factors (MCF, IL-1) can also regulate the production of prostaglandins and collagenase by synovial cells and fibroblasts (24-27). Thus, the hyperplasia of the synovial tissue, as well as the production and release of degradative enzymes that contribute to the perpetuation and tissue destruction characteristic of rheumatoid arthritis, may all be modulated by mononuclear cell signals.

Summary

A characteristic feature of rheumatoid arthritis is hyperplasia of the synovial lining cells and fibroblasts, the source of tissue-degrading mediators, in association with the appearance and persistence of lymphocytes in affected joints. Diseased synovial tissue obtained at arthroscopy from 10 of 12 rheumatoid arthritis patients was found to release a factor(s) that could stimulate quiescent fibroblasts to proliferate in vitro. Mononuclear cells isolated from this synovial tissue and from the synovial fluid spontaneously produced fibroblast-activating factor(s) (FAF). In contrast, synovial tissue from patients with noninflammatory

joint disease did not release FAF. By gel filtration, FAF was detected in two peaks (40,000 and 15,000 mol wt) that were consistent with the previously described peripheral blood T lymphocyte- and monocyte-derived factors with identical activity. The mononuclear cells were predominantly OKT3⁺/Leu-1⁺ T lymphocytes and OKM1⁺ cells of monocyte/macrophage lineage that expressed HLA-DR antigens, suggesting prior activation of these cells. Mononuclear cells isolated from the peripheral blood of these patients did not spontaneously secrete FAF. Lymphocytes and monocytes from the site of synovial inflammation appear to be activated in situ to produce factors that may contribute to the hyperplasia and overgrowth of the synovial membrane in rheumatoid arthritis.

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