Anchorage-independent Growth of Synoviocytes from Arthritic and Normal Joints

Stimulation by Exogenous Platelet-derived Growth Factor and Inhibition by Transforming Growth Factor-Beta and Retinoids

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Abstract

Exuberant tumor-like synovial cell proliferation with invasion of periarticular bone is a feature of rheumatoid arthritis in humans and of streptococcal cell wall (SCW)-induced arthritis in rats. These histologic observations prompted us to examine synoviocytes from arthritic joints for phenotypic characteristics of transformed cells. The capacity to grow in vitro under anchorage-independent conditions is a characteristic that correlates closely with potential in vivo tumorigenicity. In medium supplemented with 20% serum or in basal media supplemented with platelet-derived growth factor (PDGF), early passage synoviocytes from both SCW-induced and rheumatoid arthritic joints formed colonies in soft agarose. Epidermal growth factor (EGF), interleukin 1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), and transforming growth factor-beta (TGF-beta) did not support growth, although EGF enhanced PDGF-dependent growth. On the other hand, TGF-beta, as well as all-*trans*-retinoic acid, inhibited colony growth. Early passage normal rat and human synoviocytes also grew under the same conditions, but lung, skin, and late-gestation embryonic fibroblast-like cells did not. Considered in the context of other published data, our findings provide cogent evidence that synoviocytes, but not other types of fibroblast-like cells, readily acquire phenotypic characteristics commonly associated with transformed cells. Expression of the transformed phenotype in the inflammatory site is likely regulated by paracrine growth factors, such as PDGF and TGF-beta.

Introduction

An arthritic process that mimics many features of rheumatoid arthritis develops in female LEW/N rats after a single intraperitoneal injection of cell wall fragments (peptidoglycan-polysaccharide) from group A streptococci (SCW)[1] and several other bacteria (1). This animal model is characterized by an acute inflammatory phase that begins 2–3 d after injection and is clinically apparent for 1–2 wk. This phase appears to be complement dependent (2). It overlaps a chronic phase that becomes clinically apparent 2–6 wk after injection and results in proliferative and invasive destruction of the involved joints (1, 3). This phase appears to be thymic dependent since it does not develop in athymic nude LEW.rnu/rnu rats (1, 4), but does develop in athymic nude rats reconstituted with T cells (5). Moreover, it is markedly inhibited by treatment with cyclosporin A (6). Histologic examination of synovium during the chronic phase of the disease reveals marked hyperplasia of the spindle-shaped synovial cell population. Reminiscent of transformed cells, these cells are frequently characterized by large pale nuclei, prominent nucleoli, and invasion of periarticular bone (7). Mechanisms regulating this tumorlike synovial cell proliferation and invasion of bone are poorly understood.

Rheumatoid arthritis is similarly characterized by pronounced tumorlike expansion of the synovium primarily because of exuberant proliferation of synoviocytes (8, 9). Histologically, this proliferating tissue has many of the characteristics of a neoplasm. Transformed-appearing synovial cells with large pale nuclei and prominent nucleoli are abundant, particularly at sites of synovial invasion/erosion of surrounding periarticular bone. Although the disease process is commonly associated with intense synovial mononuclear cell infiltration, the mechanisms driving the massive tumorlike growth and invasive behavior of the fibroblast-like synovial cells are not understood.

The extent to which rheumatoid and SCW arthritic synoviocytes actually express phenotypic characteristics associated with cell transformation has not been fully investigated. Abnormal and variable cell morphology, and cell growth beyond a monolayer resulting in high saturation densities are generally correlated with transformed cell growth; however, the best in vitro assay of cell transformation is anchorage-independent growth of cells in soft agar or agarose (10–13). Anchorage-independent growth of cells and cell lines is closely associated with transformation and is highly correlated, particularly for cells of mesenchymal origin, with in vivo tumor growth in nude mice (11, 13).

We have recently reported that freshly explanted and early passage synovial fibroblast-like cells from rats with SCW-induced arthritis grow beyond a monolayer, form foci under anchorage-dependent conditions and grow under anchorage-independent conditions (7). In the present study, we have demonstrated that fresh explant and early passage synovial fibroblast-like cells (synoviocytes) from patients with rheumatoid arthritis, as well as LEW/N female rats with SCW-induced arthritis, also, express phenotypic characteristics generally associated with transformed cells. In contrast to late passage cells or early passage cells treated with retinoic acid, early passage

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1. *Abbreviations used in this paper:* EGF, epidermal growth factor; HS, human serum; SCW, streptococcal cell wall; TGF-beta, transforming growth factor-beta; TNF-alpha, tumor necrosis factor-alpha.

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synoviocytes from both arthritic rats and humans grow to high saturation densities, form foci, and are able to grow under anchorage-independent conditions. Moreover, we have analyzed the effects of several cytokines and growth factors known to be present in the inflammatory extracellular fluid of the rheumatoid joint on anchorage-independent growth of rat and human synovial cells. We show that for both rat and human synovial cells platelet-derived growth factor (PDGF), but not interleukin 1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), transforming growth factor-beta (TGF-beta), epidermal growth factor (EGF), or interferon-gamma (IFN-gamma), is a potent stimulator of anchorage-independent synoviocyte growth. Further, we show that TGF-beta partially inhibits growth. Of further significance, we show that nonrheumatoid human and normal rat synoviocytes also grow under anchorage-independent conditions, but, in sharp contrast, lung, dermal, and late-gestation embryonic fibroblasts and late-passage synoviocytes fail to form colonies under the same conditions.

Methods

Reagents and cytokines. Mouse EGF, human platelet PDGF, insulin, ITS+ 100× (insulin 0.625 mg/ml, transferrin 0.625 mg/ml, selenium 0.625 mg/ml, BSA 0.125 g/ml, and linoleic acid 0.535 mg/ml) and goat IgG anti-human PDGF were purchased from Collaborative Research, Inc., Bedford, MA. Recombinant human TNF-alpha, and recombinant rat and human IFN-gamma were purchased from Amgen Biologicals, Thousand Oaks, CA. Recombinant human IL-1 alpha and beta were obtained from Genzyme Corporation, Boston, MA. Goat IgG was purchased from Jackson Immunoresearch Laboratories, Inc., West Grove, PA. The purification of TGF-beta has been previously described (14). All-*trans*-retinoic acid (Sigma Chemical Co., St. Louis, MO) was dissolved in dimethyl sulfoxide at a concentration of 0.1 M immediately before use and diluted in culture medium as needed. All procedures with retinoic acid were carried out in subdued light. Fetal bovine serum (FBS) was obtained from Gibco Laboratories, Grand Island, NY. Human serum, AB+ (HS) was obtained from Whittaker M. A. Bioproducts, Walkersville, MD.

Synovial cell isolation. Synovial tissues were dissected from the joints of 100–150-g normal LEW/N female rats and from LEW/N female rats with SCW-induced arthritis. SCW-arthritis was induced by intraperitoneal injection of an aqueous solution of cell wall fragments as previously described in detail (15). Normal synovium, lung, and dermal tissues were obtained from normal LEW/N female rats. In all cases, the tissue (~ 1 g) was minced and digested in collagenase (4 mg/ml type III; Worthington Biochemical Corp., Freehold, NJ) in Dulbecco's modified Eagle's medium (DME) at 37°C for 4–5 h. After digestion, the dissociated cells were collected by centrifugation at 500 *g* for 10 min, resuspended in complete medium (DME supplemented with penicillin 100 U/ml, streptomycin 100 μg/ml, and 10% FBS). The cell suspension was then cultured in a six-well cluster dish (35-mm diam wells; Costar, Cambridge, MA) at 37°C in 5% carbon dioxide (CO_2). The adherent synoviocytes were uniformly confluent in 7–10 d and were used without further passage for experiments on primary cell cultures. At the time of initial culture 20–40% of the cells were macrophages; however, by the time of confluence, < 0.1% of the cells were macrophages as determined by the ingestion of latex beads and nonspecific esterase staining. For later passage synoviocytes, primary cultures were passaged at 1:3 dilution approximately once per week. Synovial cells from arthritic rats grew indefinitely under these conditions (> 9 mo) without senescence, although the growth rate slowed after several passages.

Embryonic mesenchymal limb bud cells were isolated from day 14–17 gestation LEW/N rats. Limb buds were dissected from whole embryos, rinsed in PBS, minced, and digested in 0.25% trypsin, 0.02% EDTA for 10 min at 37°C. After digestion, the cells were pelleted by centrifugation, resuspended in complete medium, and grown to confluence before assay of their anchorage-independent growth potential. Synovial tissues from patients with rheumatoid arthritis were obtained by arthroscopic biopsy in accordance with a research protocol approved by a research subpanel of the National Institutes of Health. Synovial tissues were also obtained by biopsy from the infrapatellar region of patients requiring arthroscopic surgery for joint trauma. In addition, osteoarthritic synovial tissue was obtained from patients undergoing joint replacement. These tissues were processed and cultured in the same manner as the rat tissues with the exception that the initial tissue explants were cultured in a slightly different culture media. Preliminary experiments using different basal media (DME vs. CMRL Medium 1066), with various combinations of human vs. FBS showed that cell growth and viability was optimal in DME supplemented with penicillin 100 U/ml, streptomycin 100 μg/ml, glutamine 2 mM, 10% FBS, and 10% HS. This complete medium was used for propagation of all human cell cultures.

Assays of saturation density. The saturation density of human synoviocytes was determined by culture of first passage, late passage, and all *trans* retinoic acid (10^{-6} M) treated first passage synoviocytes from patients with rheumatoid arthritis. Cells were plated into 96-well plates at 1,000 cells/well and cultured in complete medium (containing 10% FBS and 10% HS). Every 2 d the cells from two wells were washed with PBS, trypsinized 15 min at 37°C, and counted using a hemacytometer. The experiments were continued until cell growth had plateaued.

Assays of anchorage-independent cell growth. Agarose (Seaplaque agarose; FMC Corp., Rockland, ME) solutions (1% and 1.6%) were prepared in distilled water and autoclaved. DME (1.67×) was prepared by dissolving premixed 1× DME salts plus sodium bicarbonate (Sigma Chemical Co.) in 0.6 vol of distilled water. Typically, sufficient DME salts for 1 liter were dissolved in 550 ml distilled water; 49.3 grams sodium bicarbonate were added; the pH was adjusted to 7.2; the final volume was adjusted to 600 ml with distilled water; and the solution filter sterilized.

Soft agarose growth assays were carried out in 24-well cluster dishes. Wells were first coated with 0.5 ml mixture containing 0.64% agarose in basal media supplemented with serum at the desired concentration for the particular growth assay. For example, in experiments carried out in 20% FBS, this solution was prepared by mixing 5 ml FBS, 10 ml 1.67× DME and 10 ml 1.6% agarose, all preheated to 40°C before mixing and coating the wells. The agarose was allowed to solidify at room temperature. Next, the agarose layers that contained cells were prepared. These mixtures were prepared in the wells of a separate 24-well cluster dish and then transferred to the precoated wells, i.e., each well representing a different experiment. First, 1.67× DME plus serum was added to each well to give a final volume of 1.2 ml. The volume of serum added was determined by the desired serum concentration in a volume of 2 ml, i.e., the final volume of the cell containing agarose layer. The growth factor(s) or reagent to be tested was then added, and the concentrated media mixtures were allowed to equilibrate at 37°C in an incubator. While the media mixtures were equilibrating, the cells to be used in the assay were harvested by trypsinization, pelleted and resuspended in DME containing 10% FBS at the desired concentration, usually 10^6 cells/ml. These cells were passed through a 25-gauge needle to remove clumps and produce a single cell suspension. 10 μl of the cell suspension were then added to each of the wells of warmed media mixtures. Next, 0.8 ml of 1% agarose, prewarmed to 40°C, were added to each well, and this mixture was then layered onto the previously prepared dishes coated with 0.64% agarose. The final concentration of agarose in the upper cell-containing soft agarose layer was, therefore, 0.4%; the concentration of cells was optimally 10^4 cells/well or 5×10^3 cells/ml.

Analysis of anchorage-independent colony growth. To reproducibly quantitate colony growth in soft agarose, analysis was carried out using an image analyzer (model 982B; Artek Systems Corp., subsidiary of Dynatech Corp., Farmingdale, NY) interfaced to an IBM PC/XT computer, using software provided by the supplier. Counting was carried out through an inverted microscope with total magnification of

40. The analyzer was used in two programmed modes. The first mode measured colony size and number; using this mode, only colonies of 60 μm diam or greater were counted. The second mode of operation allowed for a finer discrimination of colony growth by measuring the area of all the colonies imaged in the analyzed field and then calculating fraction of this area in the total imaged field. This mode of operation was used for analysis of experiments in which growth factors were added to serum containing medium, which supported colony growth even without additional growth factors. In these cases the total colony number generally changed less dramatically than did the size of the colonies. Measurement of the colony area allowed for a greater discrimination of changes under various conditions.

Results

Early passage adherent synovial cells from both human and rat arthritic joints form foci and grow to high saturation densities under anchorage-dependent conditions. As previously described for synoviocytes from rats with SCW-induced arthritis (7), early passage human synovial cells from patients with rheumatoid arthritis upon prolonged culture grew beyond a monolayer and formed foci (Fig. 1 *A*). Late passage rheumatoid synoviocytes or rheumatoid synoviocytes treated with all-*trans*-retinoic acid (10^{-6} M), on the other hand, did not grow beyond a monolayer (Fig. 1, *B* and *C*). These morphologic features are consistent with a transformed phenotype in both human and rat early passage cells that was lost upon culture or treatment with retinoic acid. Also compatible with these observations, early passage rheumatoid synoviocytes ($n = 1$) grew to higher saturation densities than later passage cells ($n = 16$) or early passage cells treated with retinoic acid (10^{-6} M) (Table I).

Early passage synovial cells from both human and rat arthritic joints grow under anchorage-independent conditions in serum or PDGF-supplemented medium. Initially, we studied the growth of primary explant and early passage, adherent synovial cells from rats with SCW-induced arthritis for growth under anchorage-independent conditions as previously described (7). These cells grew under anchorage-independent conditions in the presence of 20% FBS (Fig. 2 *A*). Colony growth declined at cell densities greater than 10^5 cells/ml, low serum concentrations and repeated cell passage ($n > 3$) (Fig. 2 *B*).

Similarly, using conditions that support anchorage-independent growth of many human transformed cell lines (i.e., a medium of DME supplemented with 10% FBS and 10% HS), we found that primary explant, adherent rheumatoid synovial cells grew under anchorage-independent conditions (Fig. 2 *C*). Colony growth declined at lower serum concentrations and was negligible in medium supplemented with only 1% FBS and 1% HS (Fig. 2 *D*). Optimal cell concentration was 10^4 cells/ml. It is of interest that colony growth declined at cell densities of 10^5 cells/ml or greater. First and second passage synoviocytes also readily formed colonies, but by the third or fourth cell passage, the synoviocytes began to lose their capacity to grow under anchorage-independent conditions.

The striking serum dependence of colony growth for synoviocytes from both rat and human arthritic joints and the known presence of certain cytokines in serum and rheumatoid synovial fluid led us to examine the effects of various cytokines on the anchorage-independent growth. Previous work has demonstrated PDGF, EGF, and insulinlike growth factors in serum (16, 17), and rheumatoid synovial fluids are known to contain IL-1 (18, 19), TNF-alpha (20), IFN-gamma (21, 22), EGF (23), PDGF, and TGF-beta (24). Therefore, the effects of these cytokines on anchorage-independent synoviocyte growth were analyzed. The initial set of experiments was performed on synoviocytes from arthritic rats. PDGF enhanced colony growth (10–50 ng/ml), and TGF-beta (0.025–25 ng/ml) partially inhibited it (50% maximal inhibitory concentration $\sim 5 \times 10^{-12}$ M; Figs. 3 *A* and 4 *A*). IL-1 (1–1,000 U/ml), TNF-alpha (2–200 U/ml), gamma-IFN (1–1,000 U/ml) and EGF (1–50 ng/ml) did not have any or had only minimal effects over the range of concentrations tested (Fig. 3 *A*). Similar experiments were carried out on synoviocytes from rheumatoid joints in the presence of 10% FBS/10% HS with and without these cytokines (Fig. 3 *B*). PDGF (10–50 ng/ml) in a dose-dependent manner, markedly enhanced colony growth. TGF-beta (0.125–25 ng/ml) had a partial inhibitory effect, with a 50% maximal inhibitory concentration of $\sim 5 \times 10^{-11}$ M (Figs. 3 *A* and 4 *A*). IL-1 (1–1,000 U/ml), TNF-alpha (2–200 U/ml), EGF (1–50 ng/ml), and IFN-gamma (1–1,000 U/ml) had neither statistically significant stimulatory nor inhibitory effects. IL-1 was also tested in the presence of indomethacin (2.5×10^{-6} M), and again there was no apparent effect on either the human or rat cells (Figs. 3 *A* and *B*). Further confirming the importance of PDGF, neutralizing anti-PDGF antibody (10 μg/ml), but not control antibody (10 μg/ml), completely blocked serum-stimulated colony growth of both rat and human synoviocytes (data not shown).

Having established the effects of these various growth factors as additives to medium containing 20% serum, we next studied their effects on primary explant and early passage synoviocytes from rat joints that were grown under low serum conditions (1% FBS) in medium supplemented with insulin, transferrin, selenium, albumin, and linoleic acid (ITS+ 5×). PDGF (10–50 ng/ml) stimulated colony growth in a dose-dependent manner; however, minimal colony growth was observed at 10 ng/ml or less. EGF (1–50 ng/ml) had no or weak effects at all concentrations tested (Fig. 5 *A*). None of the other cytokines, over a wide range of concentrations, promoted any significant colony growth. EGF consistently augmented the growth stimulating effects of PDGF. TGF-beta (2.5 ng/ml) had no significant effect on PDGF-stimulated colony growth. Combinations of TGF-beta and EGF produced colony growth no greater than EGF alone.

Results similar to those of rat synoviocytes grown under low serum were observed with synovial cells from rheumatoid joints (Fig. 5 *B*). In the presence of 1% FBS/1% HS alone, colony formation was negligible; however, the addition of PDGF stimulated significant colony formation at 50 ng/ml, but not at lower concentrations. EGF (1–50 ng/ml) had no effect alone, while EGF enhanced PDGF-stimulated colony growth. TGF-beta (0.25–25 ng/ml) did not stimulate alone or in combination with EGF, but partially inhibited PDGF-stimulated colony growth (maximal effect at 2.5 ng/ml). IL-1 (1–1,000 U/ml), IL-1 with indomethacin (2.5×10^{-6} M), TNF-alpha (2–200 U/ml), and IFN-gamma (1–1,000 U/ml) did not stimulate colony growth.

By contrast, and consistent with previously published data (25, 26), NRK cells exhibited exuberant colony formation in the presence of a combination of EGF (5 ng/ml) and TGF-beta (2.5 ng/ml) or high concentrations of PDGF (50 ng/ml) alone, but not with TGF-beta, EGF, or lower concentrations of PDGF (5 ng/ml) alone (data not shown).

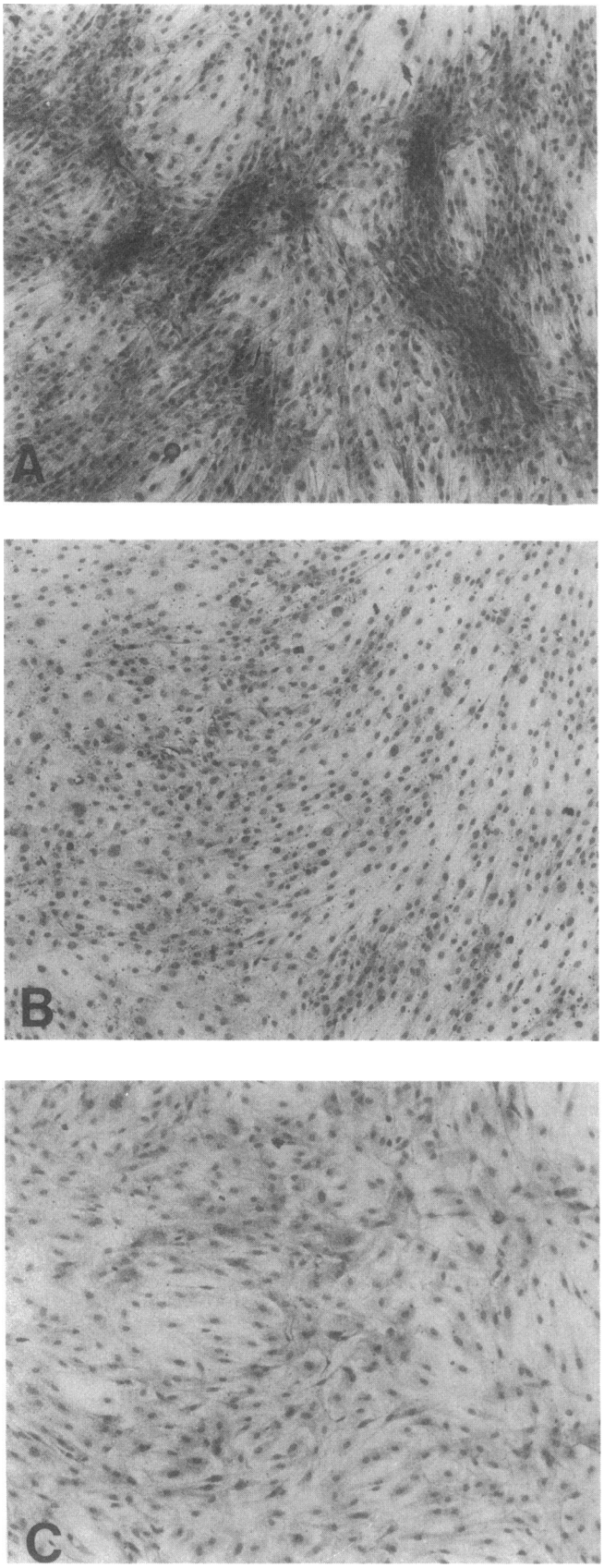


Figure 1. Representative experiment (n = 2) demonstrating anchorage-dependent growth of 1st passage human rheumatoid synoviocytes (*A*), 16th passage synoviocytes (*B*), and 1st passage synoviocytes grown in medium containing all-*trans*-retinoic acid (10^{-6} M) (*C*).

Table I. Saturation Density of Adherent Rheumatoid Synovial Cells

Cell passage and treatment	Cell density at saturation
	cells/well ($\times 10^3$)
1st Passage	13.28±1.24
16th Passage	7.37±0.78
1st Passage plus retinoic acid*	8.36±0.31

Values represent synovial cell number (±SD) in wells of a 96 well plate after their growth had plateaued (10 d), as assessed by duplicate cell counts using a hemacytometer. Results shown are representative of replicate experiments (n = 2).
* All-*trans*-retinoic acid 10^{-6} M.

Inhibition of anchorage-independent synovial cell growth by retinoids. Since retinoids are known to (*a*) induce differentiation of many cell types (27), (*b*) inhibit anchorage-independent growth of many neoplastic cell lines (28), (*c*) induce slow, contact-inhibited growth of SCW-arthritic derived synoviocytes (7) and (*d*) markedly inhibit SCW-induced arthritis in LEW/N rats in vivo (29), we examined their effects on the anchorage-independent growth of both human and rat synovial cells (Fig. 6, *A* and *B*). As shown, all-*trans*-retinoic acid almost completely inhibited colony growth of both rat and human arthritic synovial cells at concentrations of 10^{-6} M or greater (50% maximal inhibition was observed between 10^{-8} and 10^{-9} M retinoic acid). These observations are additional evidence supporting our view that synoviocyte anchorage-independent growth reflects the acquisition of molecular and cellular characteristics associated with the transformed phenotype. Retinoids inhibit the expression of this phenotype.

Normal synoviocytes also form colonies when grown under anchorage-independent conditions. To judge the significance of anchorage-independent growth of synovial cells from human and rat arthritic joints, we also analyzed other cell types and cell lines for anchorage-independent growth in medium supplemented with 20% FBS. As expected, the transformed cell lines, HeLa and SSV-transformed NRK cells, grew well under anchorage-independent conditions. Unexpectedly, and of potential importance, early passage rat synovial cells from normal joints consistently grew with the same efficiency as synovial cells from arthritic joints (data not shown, n = 3). Similarly, we obtained colony formation with early-passage synovial cells from a patient with a traumatic knee injury and a patient with osteoarthritis. These cells grew under anchorage-independent conditions with efficiency equal to that of the early passage synoviocytes from arthritic joints. By contrast, dermal and lung fibroblasts, as well as late-gestation rat embryonic limb bud mesenchymal cells, did not form colonies when grown under conditions that produced high-grade colony formation with early passage synovial cells (20% FBS) or under low serum conditions (1% FBS) (data not shown, n = 2). Thus, synoviocytes from inflamed, noninflamed, and normal joints

Cells were grown as described in Methods until saturation density had been reached and were then fixed and stained with Giemsa (original photograph, ×60).

have the potential to grow under anchorage-independent conditions; the other types of normal fibroblast-like mesenchymal cells, that we studied, appear much more resistant to growth under similar conditions.

Synovial cells from rheumatoid and SCW-arthritic joints do not form tumors in nude mice. Second passage synovial cells cultured from both rheumatoid and SCW-arthritic joints were injected subcutaneously into nude mice (5×10^6 cells/injection). After 1 mo of observation, none of the four mice injected developed tumors. Control nude mice injected with HeLa or SSV-transformed NRK cells uniformly developed tumors within 2 wk.

Discussion

We, as well as others, have become increasingly impressed with the similarities between invasive tumors and the proliferative and invasive lesion characteristic of rheumatoid arthritis (8, 9, 30–34). Mitoses are frequent in both. The major proliferating cell in situ is spindle shaped and is characterized by large pale nuclei with prominent nucleoli, reminiscent of tumor cells. Moreover, these "fibroblastoid" cells are the predominant cell type at sites of bone erosions (35). They are often in close proximity to macrophages and proliferating blood vessels. We have pursued the hypothesis that a possible mechanism driving this tumorlike expansion and invasive behavior of synovial cells is that locally secreted paracrine growth factors, derived from infiltrating macrophages and lymphocytes as well as from endothelial and other cells, induce synovial cells to exhibit phenotypic characteristics in common with transformed cells, resulting in a locally proliferative and invasive inflammatory lesion. Thus, the rheumatoid lesion resembles a locally invasive tumor.

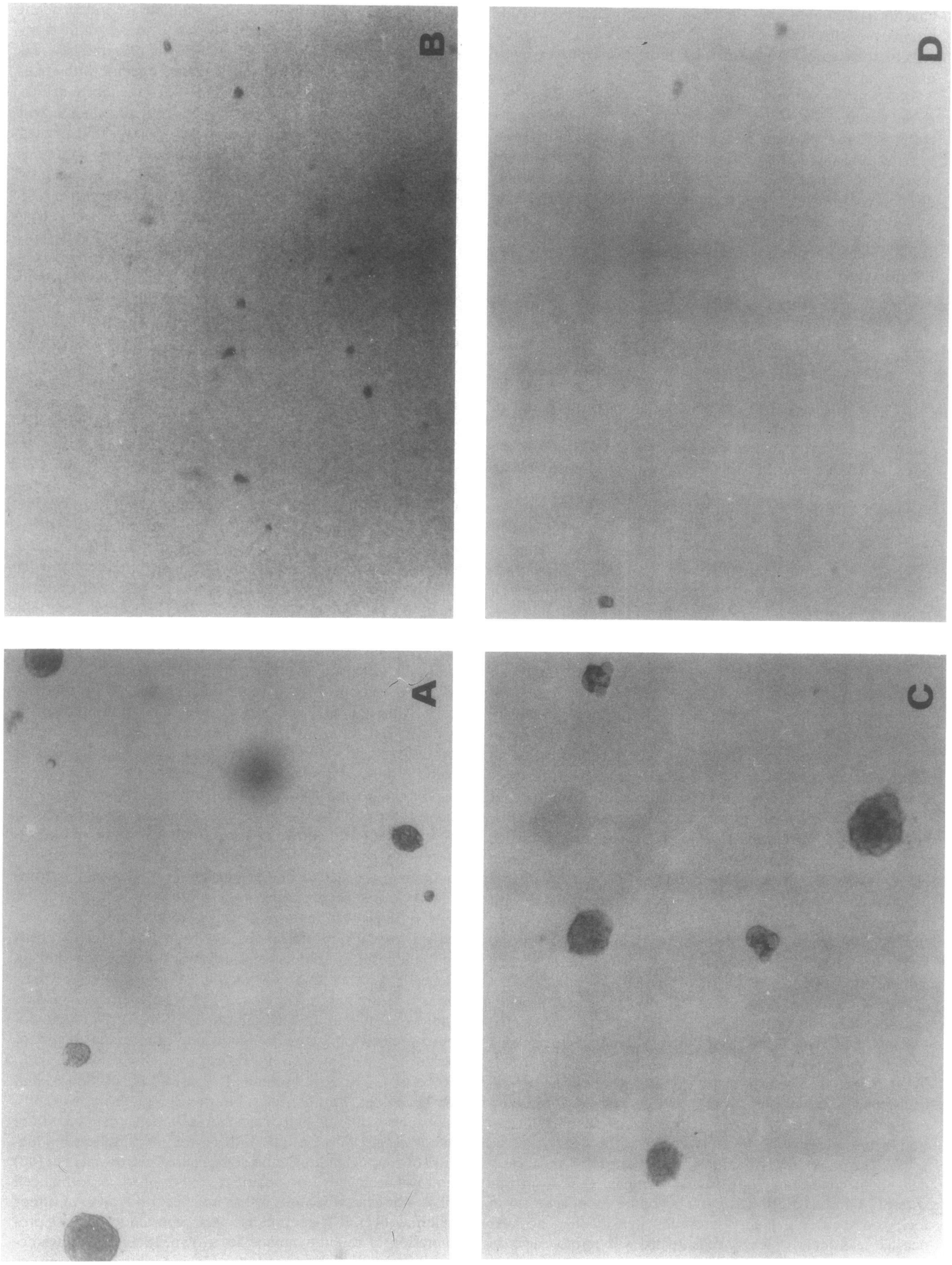
We have also been impressed that tumorlike synovial growth and local invasion of bone and cartilage, closely resembling rheumatoid arthritis, can be induced in genetically predisposed rats by systemic injection of peptidoglycan-polysaccharide fragments from the cell walls of certain bacteria, e.g., group A streptococci. Like rheumatoid arthritis, the proliferative and invasive lesion is predominantly composed of spindle-shaped cells with numerous mitoses, large pale nuclei, and prominent nucleoli. They are, like rheumatoid arthritis, usually in close proximity to macrophages and proliferating blood vessels (7). These observations have led us to further examine the synovial cells for characteristics associated with neoplastic transformation.

High saturation density, growth beyond a monolayer, and anchorage-independent growth have been correlated with a transformed phenotype in vitro. Anchorage-independent growth of cells in vitro is one of the most specific criteria for assessing the cell's potential for expression of a transformed phenotype in vivo. It has been shown to be highly correlated with a cell's ability to form tumors in athymic nude mice. This in vitro assay has been utilized extensively to define several transforming growth factors (36) as well as mechanisms underlying neoplastic cell growth including cellular transformation by oncogenes.

We have previously demonstrated that under anchorage-dependent conditions early passage synovial cells from rats with SCW-induced arthritis grow beyond a monolayer, form foci, and grow to a higher saturation density than do later passage cells or early passage cells treated with retinoic acid. In addition, we have shown early passage synoviocytes from rats with SCW-arthritis grow under anchorage-independent conditions (7). In the present study, we further examined anchorage-independent growth of rat synovial cells and have extended our studies to synoviocytes from patients with rheumatoid arthritis. We have shown that under anchorage-dependent conditions early passage synoviocytes from patients with rheumatoid arthritis, grew beyond a monolayer and formed foci, grew to a higher saturation density, and grew under anchorage-independent conditions. In contrast to malignantly transformed cells, the synoviocytes from both species lost, upon repeated passage in culture, these phenotypic markers of transformation. Anchorage-independent growth of early passage cells required serum or medium supplemented with appropriate growth factors, suggesting that tumor-like growth in vivo is driven not by autocrine, but by paracrine growth factors produced as a result of the inflammatory process. The failure of the synoviocytes to form tumors in nude mice is consistent with this hypothesis.

Serum is known to contain at least three growth factors: insulin-like growth factors, PDGF, and EGF (16, 17). We have shown previously that PDGF and EGF, but not IL-1, TNF-alpha, IFN-gamma, or TGF-beta are both important in supporting anchorage-dependent growth of synoviocytes (37, Remmers, E. F., R. Lafyatis, D. E. Yocum, A. B. Roberts, G. K. Kumkumian, J. P. Case, M. B. Sporn, and R. L. Wilder. Manuscript submitted for publication). Further, we have demonstrated the presence of PDGF in rheumatoid synovial fluid in concentrations well above the range required for synovial cell stimulation (30–100 ng/ml) as well as local synovial production of a PDGF-like molecule (24). We report here that PDGF is also able to induce anchorage-independent growth of synovial cells even under suboptimal serum conditions. These data give support to the notion that PDGF contributes in vivo to the development of a tumor-like phenotype by synovial cells. Although EGF supported low-grade colony growth of synovial cells from rat arthritic joints, it did not show activity on human cells. EGF did, however, appear to act synergistically with PDGF on both rat and human cells.

Similar to our results with anchorage-dependent growth, none of the other cytokines tested that are believed by many to be important in rheumatoid arthritis stimulated anchorage-independent cell growth. IL-1, which increases synovial cell production of collagenase and prostaglandin E_2 (38), had no stimulatory effect on anchorage-independent growth of arthritic synovial cells even in the presence of indomethacin, which was added to block prostaglandin E_2 production. This contrasts with the results of others demonstrating enhanced anchorage-dependent proliferation of synovial cells by IL-1 in the presence of serum plus indomethacin (39). However, our own data indicate that IL-1 is only minimally mitogenic for synoviocytes under anchorage-dependent conditions in the absence of serum (37, Remmers, E. F., R. Lafyatis, D. E. Yocum, A. B. Roberts, G. K. Kumkumian, J. P. Case, M. B. Sporn, and R. L. Wilder. Manuscript submitted for publication). TNF-alpha, also, did not support anchorage-independent growth of synovial cells. Similar to IL-1, this cytokine stimulates production of prostaglandin E_2 and collagenase by synovial cells (40) and has been shown to augment growth of some normal cell lines; however, it inhibits or has no effect on growth of tumor cell lines (41). IFN-gamma has been reported to increase proliferation of rabbit synoviocytes grown under anchorage-dependent conditions and to induce Ia and inhibit collagen syn-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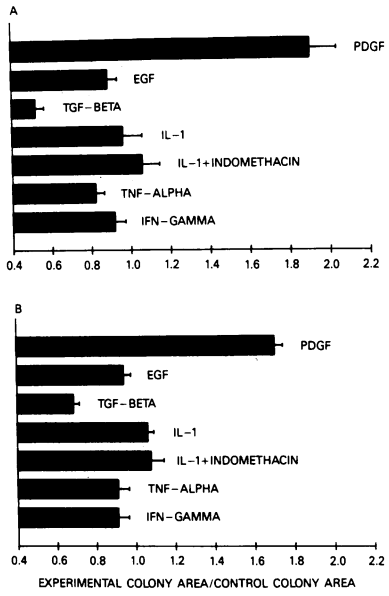

Figure 3. Representative experiment (n = 3) demonstrating anchorage-independent growth of synoviocytes from an arthritic rat joint grown in medium supplemented with 20% FBS and various cytokines (*A*). *B* shows anchorage-independent growth of synoviocytes from a rheumatoid joint grown in medium supplemented with 10% FBS/10% HS and various cytokines. The data presented represent the greatest relative stimulatory or inhibitory activities observed with the various supplements: PDGF (50 ng/ml), EGF (10 ng/ml), TGF-beta (2.5 ng/ml), IL-1 (10 U/ml), IL-1 + indomethocin (2.5×10^{-6} M), TNF-alpha (20 U/ml), and IFN-gamma (100 U/ml). Each bar represents the average of four readings (±SEM) across the test well in a single experiment utilizing an image analyzer to measure the area of all colonies/field (20 mm^2) divided by the area of all colonies/field in the control well (serum alone, no supplemental cytokine added).

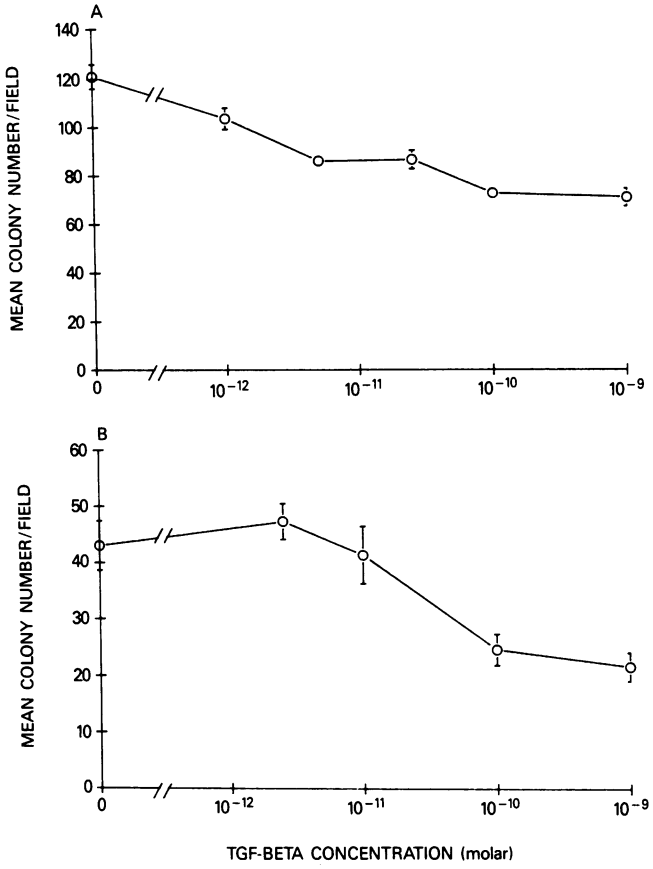

Figure 4. Representative experiment (n = 3) demonstrating anchorage-independent colony formation of synoviocytes from an arthritic rat joint grown in medium supplemented with 20% FBS and various concentrations of TGF-beta (*A*), and from a rheumatoid joint grown in medium supplemented with 10% FBS/10% HS and various concentrations of TGF-beta (*B*). Each point represents the average of four readings across the test well in a single experiment (±SEM) utilizing an image analyzer to count the number of colonies > 60 μm in diameter/field (20 mm^2).

thesis of human synoviocytes grown under anchorage-dependent conditions (42, 43). This cytokine had no effects on the growth of either human or rat arthritic synovial cells in our studies.

TGF-beta, initially isolated on the basis of its capacity to induce anchorage-independent, but not anchorage-dependent growth of NRK cells, has since been shown to have pleiotropic effects depending on the cell type examined (44). On some cell types, it appears to stimulate differentiation. On dermal fibroblasts it increases secretion of collagen and when injected intradermally it causes fibrosis (45). We have recently demonstrated physiologically significant levels of TGF-beta in rheumatoid synovial fluid (25–75 ng/ml) and have also immunolocalized TGF-beta diffusely throughout specimens of rheumatoid synovium, as well as synovium from rats with SCW arthritis (24). Our data presented here demonstrate that TGF-beta partially inhibited anchorage-independent growth of both human and rat synoviocytes. In view of the local production of this cytokine by proliferative synovium, our data suggest that TGF-beta may be involved in suppressing in vivo synovial cell proliferation and inducing a more differentiated phenotype, (i.e., slower growth, increased production of collagen, and decreased production of matrix-degrading enzymes), consistent with the more mature stages of wound repair.

The contrasting effects of PDGF, IL-1, TNF-alpha, EGF, TGF-beta, and IFN-gamma on synovial cells in vitro point out the complexity of the regulatory pathways involved in the tissue destruction characteristic of rheumatoid and SCW-induced arthritis. PDGF, IL-1, TNF-alpha and TGF-beta are produced by activated macrophages (46, 47), which are found in abundance in the synovium of rheumatoid and SCW-induced arthritic joints. IL-1 and TNF-alpha induce prostaglandin E_2 and collagenase production in synoviocytes and represent a pathway by which bone destruction might be mediated by soluble factors. In contrast, PDGF may play a critical role in driving the phenotypically transformed cell growth that is also a characteristic feature of rheumatoid and SCW-arthritis. Transformation has been associated with the production of proteases in other cell types. Membrane-bound, fibronectin-degrading proteases are induced by Rous sarcoma virus transformation of chick embryo fibroblasts (48) and secretion of transin/stromelysin is induced by transformation of rat fibroblasts (49, 50). These proteases, which are thought to be involved in tissue invasion by cancer cells, might, similarly, be involved in bone invasion by synovial cells that have been

Figure 2. Representative experiment (n = 6) demonstrating anchorage-independent growth in soft agarose of primary explant synoviocytes from an arthritic rat joint grown in medium containing 20% FBS (*A*) or 1% FBS (*B*) and from a rheumatoid joint grown in the presence of medium containing 10% FBS/10% HS (*C*) or 1% FBS/1% HS (*D*). Cells were cultured for 14 d. Colony growth is clearly apparent in *A* and *C*, but not *B* and *D* (original photograph, ×100).

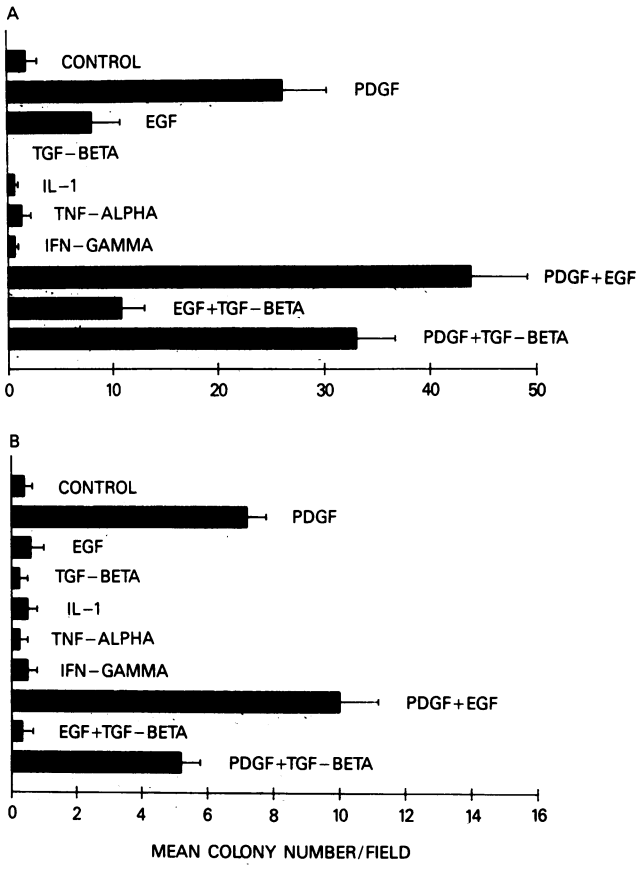

Figure 5. Representative experiment (n = 2) demonstrating anchorage-independent growth of synoviocytes from an arthritic rat joint grown in medium supplemented with 1% FBS, 5× ITS+ and various growth factors (*A*). *B* shows data for synoviocytes from a rheumatoid joint grown in the presence of 1% FBS, 1% HS, 5× ITS+ and various cytokines. The data presented represent the greatest relative stimulatory activity observed with PDGF (50 ng/ml), EGF (10 ng/ml), TGF-beta (2.5 ng/ml), IL-1 (10 U/ml), TNF-alpha (20 U/ml), and IFN-gamma (100 U/ml). Each bar represents the average of four readings across the test well in a single experiment (±SEM) utilizing an image analyzer to count the number of colonies > 60 μm/field (20 mm²).

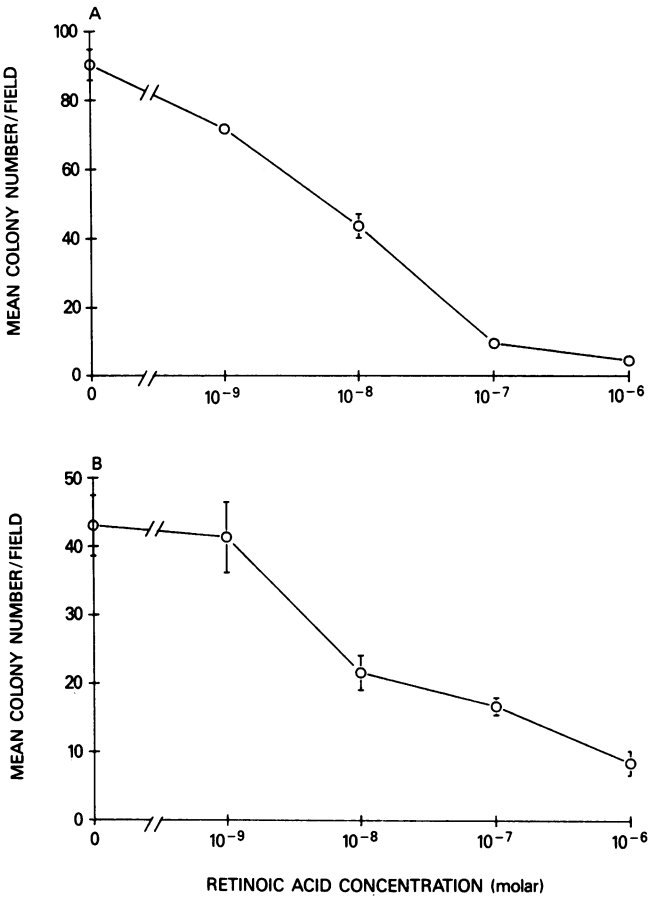

Figure 6. Representative experiment (n = 3) demonstrating anchorage-independent growth of synoviocytes from an arthritic rat joint grown in medium supplemented with 20% FBS and various concentrations of all-*trans*-retinoic acid (*A*). *B* shows similar data for synoviocytes from a rheumatoid joint grown in medium supplemented with 10% FBS/10% HS and various concentrations of all-*trans*-retinoic acid. Each point represents the average of four readings across the test well in a single experiment (±SEM) utilizing an image analyzer to count the number of colonies > 60 μm in diameter/field (20 mm²).

phenotypically transformed by PDGF. In addition, PDGF has been reported to increase collagenase production by dermal fibroblasts (51).

Our data are also of significance in light of the results on normal rat and human synovial cells. To our initial surprise, we found that these cells also formed colonies in soft agarose, indicating that normal synovial cells can be stimulated to express a characteristic generally associated with transformation. These results contrast sharply with the failure of dermal, lung, and late-gestation rat embryo limb bud fibroblasts to form colonies under the same conditions. These data suggest that early passage rat and human synoviocytes may be relatively unique in that they readily acquire properties of transformed cells when stimulated with the appropriate paracrine growth factors. Our data show that these factors are present in serum, and can be replaced, in large part, by basal media supplemented with PDGF, EGF, insulin, transferrin, and selenium. These results are consistent with our view that the cytokines present in the inflammatory milieu of the rheumatoid or SCW-induced arthritic joint induce quiescent synoviocytes to behave like a locally invasive tumor. Synovial cells cultured from noninflammatory joints are stimulated by these same paracrine factors.

An important distinction should be emphasized here between autocrine and paracrine growth factor-driven transformation of cells. The homology of some viral oncogenes to growth factors suggests that expression of the malignant phenotype in some virally transformed tumors is induced through dysregulated autocrine production of a growth factor (30, 52). A similar mechanism of transformation is suggested for the U-2 osteosarcoma cell line in that this cell line produces PDGF and expresses PDGF receptors (53). In contrast to these cells, which are able to secrete their own growth factors, synoviocytes from rheumatoid and SCW-induced arthritic joints as well as normal synoviocytes apparently do not constitutively secrete adequate amounts of PDGF to support their own (autocrine) growth and require a paracrine source of growth factors for acquisition and maintenance of the transformed phe-

notype. Thus, primary paracrine, versus autocrine, mechanisms distinguish the growth of synovial cells in arthritic joints from the growth of an autonomously growing, malignant tumor.

Lastly, our observations on the effects of retinoids need comment. These agents, known to inhibit the anchorage-independent growth of several cell lines derived from malignant tumors, are shown here to inhibit anchorage-independent growth of synoviocytes and induce monolayer growth. Previous work in our laboratories has shown that treatment of rats with retinoids before or after induction of SCW-induced arthritis significantly inhibits the disease (29). Histologically, synovial cell hyperplasia and synovial invasion of periarticular bone are inhibited and synovial fibrosis is induced. Production of prostaglandin E_2 and collagenase, both potential mediators of tissue destruction, are markedly suppressed. In the experiments presented here, which are consistent with the known effects of retinoids in inducing differentiation, all-*trans*-retinoic acid completely inhibited anchorage-independent growth of both human and rat synoviocytes at concentrations achievable in vivo. Taken together these data suggest that inhibition of synovial cell transformation may be the primary mechanism by which retinoids suppress arthritis in vivo. If this is the case, suppression of transformed synovial cell growth might be an effective therapeutic strategy in rheumatoid arthritis, and inhibition of soft agarose synovial cell growth might provide an in vitro assay system in the development of new therapeutic agents. Unfortunately, the actions of retinoids on many other cell types makes it impossible to definitively assign inhibition of synovial cell transformation as the primary target of this drug in vivo at this time.

In summary, our data, considered in the context of published data, provide further evidence supporting the view that synovial cells in rheumatoid and SCW-induced arthritis have acquired a cellular phenotype generally associated with invasive tumors. The acquisition of the abnormal phenotype in vivo is probably regulated as it is in vitro by paracrine growth factors such as PDGF and TGF-beta. It is likely that localized phenotypic transformation of synovial cells is a critical aspect of the tissue destruction characteristic in rheumatoid arthritis and the essentially identical destructive disease process observed in SCW-induced arthritis in LEW/N female rats. Our data indicate that synovial cells, in sharp contrast to several other fibroblast-like cells, are relatively unique in their susceptibility to exhibit properties generally associated with autonomously transformed cells. These data support the concept that even among fibroblastoid mesenchymal cells, the type and differentiation state of the target cell determines the biological effects of a given growth factor (or factors), i.e., the biologic response, even of "fibroblasts," is not necessarily an intrinsic property of the cytokine. We expect that further investigation of mechanisms underlying these observations will lead to new insights into the pathogenesis and therapy of rheumatoid arthritis, related arthritides, and probably other inflammatory diseases.

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References

1. Wilder, R. L., J. B. Allen, and C. Hansen. 1987. Thymus-dependent and -independent regulation of Ia antigen expression in situ by cells in the synovium of rats with SCW-arthritis. *J. Clin. Invest.* 79:1160–1171.

2. Schwab, J. H., J. B. Allen, S. K. Anderle, F. Daldorf, R. Eisenberg, and W. J. Cromartie. 1982. Relationship of complement to experimental arthritis induced in rats with streptococcal cell walls. *Immunology.* 46:83–88.

3. Clark, R. L., J. T. Cuttino, S. K. Anderle, W. J. Cromartie, and J. H. Schwab. 1979. Radiologic analysis of arthritis in rats after systemic injection of streptococcal cell walls. *Arthritis Rheum.* 25:1064–1072.

4. Allen, J. B., D. G. Malone, S. M. Wahl, G. B. Calandra, and R. L. Wilder. 1985. Role of the thymus in streptococcal cell wall-induced arthritis and hepatic granuloma formation. *J. Clin. Invest.* 76:1042–1056.

5. Ridge, S. R., J. B. Zabriske, A. L. Oronsky, and S. S. Kerwar. 1985. Streptococcal cell wall arthritis: studies with nude (athymic) inbred Lewis rats. *Cell. Immunol.* 96:231–234.

6. Yocum, D. E., J. B. Allen, S. M. Wahl, G. B. Calandra, and R. L. Wilder. 1986. Inhibition by cyclosporin A of streptococcal cell wall induced arthritis and hepatic granulomas in rats. *Arthritis Rheum.* 29:262–273.

7. Yocum, D. E., R. Lafyatis, E. F. Remmers, H. R. Schumacher, and R. L. Wilder. 1988. Hyperplastic synoviocytes from rats with streptococcal cell wall-induced arthritis exhibit a transformed phenotype that is thymic-dependent and retinoid inhibitable. *Am. J. Pathol.* 132:38–48.

8. Harris, E. D., Jr. 1985. Pathogenesis of Rheumatoid Arthritis. *In* Textbook of Rheumatology. 2nd ed. W. N. Kelly, E. D. Harris, Jr., S. Ruddy, and C. B. Sledge, editors. W. B. Saunders Co., Philadelphia, PA. 886–915.

9. Fassbender, H. G., M. Simmling-Annefeld, and E. Stofft. 1980. Transformation der Synovialzellen bie rheumatoiden Arthritis. *Verh. Deutsch Ges. Path.* 64:193–212.

10. Freedman, V. H., and S. Shin. 1974. Cellular tumorigenicity in nude mice: correlation with cell growth in semi-solid medium. *Cell.* 3:355–359.

11. Neugut, A. I., and I. B. Weinstein. 1979. The use of agarose in the determination of anchorage independent growth. *In Vitro.* 15:351–355.

12. Colburn, N. H., W. F. Vorder Bruegge, J. R. Bates, R. H. Gray, J. D. Rossen, W. H. Kelsey, and T. Shimada. 1978. Correlation of anchorage-independent growth with tumorigenicity of chemically transformed mouse epidermal cells. *Cancer Res.* 38:624–634.

13. Cifone, M. A., and I. J. Fidler. 1980. Correlation of patterns of anchorage-independent growth with *in vivo* behavior of cells from a murine fibrosarcoma. *Proc. Natl. Acad. Sci. USA.* 77:1039–1043.

14. Assoian, R. K., A. Komoriya, C. A. Meyers, D. M. Miller, and M. B. Sporn. 1983. Transforming growth factor-beta in human platelets. *J. Biol. Chem.* 258:7155–7160.

15. Wilder, R. L., G. B. Calandra, A. J. Garvin, K. D. Wright, and C. T. Hansen. 1982. Strain and sex variation in the susceptibility to streptococcal cell wall-induced arthritis in the rat. *Arthritis Rheum.* 25:1064–1072.

16. Froesch, E. R., C. Schmid, J. Schwander, and J. Zapf. 1985. Actions of insulin-like growth factors. *Annu. Rev. Physiol.* 47:443–467.

17. Oka, Y., and D. N. Orth. 1983. Human plasma epidermal growth factor/beta-urogastrone is associated with blood platelets. *J. Clin. Invest.* 72:249–259.

18. Miossec, P., C. A. Dinarello, and M. Ziff. 1986. Interleukin-1 lymphocytic chemotactic activity in rheumatoid arthritis synovial fluid. *Arthritis Rheum.* 29:461–470.

19. Wood, D. D., E. J. Ihrie, C. A. Dinarello, and P. L. Cohen. 1983. Isolation of an interleukin-1 like factor from human joint effusions. *Arthritis Rheum.* 26:975–983.

20. Firestein, G. S., V. Tsai, and N. J. Zvaifler. 1987. Cellular immunity in the joints of patients with rheumatoid arthritis and other forms of chronic synovitis. *Rheum. Dis. Clin. N. Am.* 13:191–213.

21. Firestein, G. S., and N. J. Zvaifler. 1987. Peripheral blood and synovial fluid monocyte activation in inflammatory arthritis. II. Low levels of synovial fluid and synovial tissue interferon suggest that gamma interferon is not the primary macrophage activating factor. *Arthritis Rheum.* 30:864–871.

22. Sanders, K. M., J. Carmack, and B. H. Littman. 1986. Gold sodium thiomalate: effects on macrophage responses to interferon-gamma and production of C2. *Arthritis Rheum.* 29:D19. (Abstr.)

23. Yocum, D. E., L. M. Tobin, and M. Korc. 1988. Epidermal growth factor production in rheumatoid arthritis and osteoarthritis. *Arthritis Rheum.* 31:S105. (Abstr.)

24. Lafyatis, R., N. Thompson, E. Remmers, K. Flanders, A. Roberts, M. Sporn, and R. Wilder. 1988. Demonstration of local production of PDGF and TGF-beta by synovial tissue from patients with rheumatoid arthritis. *Arthritis Rheum.* 31:S62. (Abstr.)

25. Assoian, R. K., G. R. Grotendorst, D. M. Miller, and M. B. Sporn. 1984. Cellular transformation by coordinated action of three peptide growth factors from human platelets. *Nature (Lond.).* 309:804–806.

26. Rizzino, A., E. Ruff, and H. Rizzino. 1986. Induction and modulation of anchorage-independent growth by platelet-derived growth factor, fibroblast growth factor, and transforming growth factor-beta. *Cancer Res.* 46:2816–2820.

27. Sporn, M. B., A. B. Roberts, N. S. Roche, H. Kagechika, and K. Shudo. 1986. Mechanism of action of retinoids. *J. Am. Acad. Dermatol.* 15:756–764.

28. Sporn, M. B., and A. B. Roberts. 1983. Role of retinoids in differentiation and carcinogenesis. *Cancer Res.* 43:3034–3040.

29. Haraoui, B., R. L. Wilder, J. B. Allen, M. B. Sporn, R. K. Helfgott, and C. E. Brinckerhoff. 1985. Dose-dependent suppression by the synthetic retinoid, 4-hydroxyphenyl retinamide, of streptococcal cell wall-induced arthritis in rats. *Int. J. Immunopharmacol.* 7:903–916.

30. Sporn, M. B., and A. B. Roberts. 1986. Peptide growth factors and inflammation, tissue repair and cancer. *J. Clin. Invest.* 78:329–332.

31. Harris, E. D., Jr. 1976. Recent insights into the pathogenesis of the proliferative lesion in rheumatoid arthritis. *Arthritis Rheum.* 19:68–72.

32. Brinckerhoff, C. E., and E. D. Harris, Jr. 1981. Survival of human rheumatoid synovium implanted into nude mice. *Am. J. Pathol.* 103:411–418.

33. Hamilton, J. A. 1983. Hypothesis: *In Vitro* evidence for the invasive and tumor-like properties of the rheumatoid pannus. *J. Rheumatol.* 10:845–851.

34. Brinckerhoff, C. E., L. A. Sheldon, M. C. Benott, D. R. Burgess, and R. L. Wilder. 1985. Effect of retinoids on rheumatoid arthritis, a proliferative and invasive non-malignant disease. *Ciba Found. Symp.* 113:191–211.

35. Bromley, M., and D. E. Wooley. 1984. Histopathology of the rheumatoid lesion identification of cell types at sites of cartilage erosion. *Arthritis Rheum.* 27:857–863.

36. Anzano, M. A., A. B. Roberts, C. A. Meyers, A. Komoriya, L. C. Lamb, J. M. Smith, and M. B. Sporn. 1982. Synergistic interaction of two classes of transforming growth factors from murine sarcoma cells. *Cancer Res.* 42:4776–4778.

37. Lafyatis, R., E. F. Remmers, A. Roberts, M. Sporn, and R. Wilder. 1988. Regulation of rheumatoid synovial cell proliferation by cytokines. *Arthritis Rheum.* 31:S48. (Abstr.)

38. Dayer, J.-M., B. de Rochemonteix, B. Burrus, S. Demczuk, and C. A. Dinarello. 1986. Human recombinant interleukin-1 stimulates collagenase and prostaglandin E_2 production by human synovial cells. *J. Clin. Invest.* 77:645–648.

39. Baker, D. G., and B. Freundlich. 1987. Comparison of tumor necrosis factor and interleukin-1 stimulated adherent synovial cell proliferation. *Arthritis Rheum.* 30:S82. (Abstr.)

40. Dayer, J.-M., B. Beutler, and A. Cerami. 1985. Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E_2 production by human synovial cells and dermal fibroblasts. *J. Exp. Med.* 162:2163–2168.

41. Sugarman, B. J., B. B. Aggarwal, P. E. Hass, I. S. Figari, M. A. Palladino, Jr., and H. M. Shepard. 1985. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells *in vitro. Science (Wash.).* 230:943–945.

42. Brinckerhoff, C. E., and P. M. Guyre. 1985. Increased proliferation of human synovial fibroblasts treated with recombinant immune interferon. *J. Immunol.* 134:3142–3146.

43. Amento, E. P., A. K. Bhan, K. G. McCullagh, and S. M. Krane. 1985. Influences of gamma interferon on synovial fibroblast cells: Ia induction and inhibition of collagen synthesis. *J. Clin. Invest.* 76:837–848.

44. Sporn, M. B., A. B. Roberts, L. M. Wakefield, and B. de Crombrugghe. 1985. Some recent advances in the chemistry and biology of transforming growth factor-beta. *J. Cell Biol.* 105:1039–1045.

45. Roberts, A. B., M. B. Sporn, R. K. Assoian, J. M. Smith, N. S. Roche, L. M. Wakefield, U. I. Heine, L. A. Liotta, V. Falanga, J. H. Kehrl, and A. Fauci. 1986. Transforming growth factor type beta: Rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro. Proc. Natl. Acad. Sci. USA.* 83:4167–4171.

46. Nathan, C. F. 1987. Secretory products of macrophages. *J. Clin. Invest.* 79:319–326.

47. Assoian, R. K., B. E. Fleurdelys, H. C. Stevenson, P. J. Miller, D. K. Madtes, E. W. Raines, R. Ross, and M. B. Sporn. 1987. Expression and secretion of type beta transforming growth factor by activated human macrophages. *Proc. Natl. Acad. Sci. USA.* 84:6020–6024.

48. Chen, J., and W. Chen. 1987. Fibronectin-degrading proteases from the membranes of transformed cells. *Cell.* 48:193–203.

49. Matrisian, L. M., N. Glaichenhaus, M. Gesnel, and R. Breathnach. 1985. Epidermal growth factor and oncogenes induce transcription of the same cellular mRNA in rat fibroblasts. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:1435–1440.

50. Whitham, S. E., G. Murphy, P. Angel, H.-J. Rahmsdorf, B. J. Smith, A. Lyons, T. J. R. Harris, J. J. Reynolds, P. Herrlich, and A. J. P. Docherty. 1986. Comparison of human stromelysin and collagenase by cloning and sequence analysis. *Biochem. J.* 240:913–916.

51. Bauer, E. A., T. W. Cooper, J. S. Huang, J. Altman, and T. F. Deuel. 1985. Stimulation of *in vitro* human skin collagenase expression by platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA.* 82:4132–4136.

52. Huang, J. S., S. S. Huang, and T. F. Deuel. 1984. Transforming protein of simian sarcoma virus stimulates autocrine growth of SSV-transformed cells through PDGF cell-surface receptors. *Cell.* 39:79–87.

53. Betsholtz, C., B. Westermark, B. Ek, and C. H. Heldin. 1984. Coexpression of a PDGF-like growth factor and PDGF receptors in a human osteosarcoma cell line: implications for autocrine receptor activation. *Cell.* 39:447–457.