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Hyperplastic Synoviocytes from Rats with Streptococcal Cell Wall-Induced Arthritis Exhibit a Transformed Phenotype that Is Thymic-Dependent and Retinoid Inhibitable

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It has been suggested that streptococcal cell wall-induced arthritis in LEW/N rats resembles a localized neoplasm consisting of, in part, a proliferative and invasive population of fibroblast-like synoviocytes. To further pursue this concept, the synoviocytes from diseased rats were characterized *in situ* and *in vitro* for various parameters of "transformation." The spindle-shaped synoviocytes were found throughout the synovium and were the predominent cell type at sites of invasion of bone and cartilage by synovium. They stained intensely for vimentin, a microfilament prominently expressed in immature and transformed mesenchymal cells. They stained variably for Ia antigens and did not exhibit T cell surface antigens nor did they stain with histochemical stains characteristic of monocytes or granulocytes. Electron microscopy confirmed their fibroblastlike morphology and suggested high grade metabolic activity. In primary culture, the abnormal synoviocytes were adherent, grew rapidly and did not contact inhibit. Moreover, they grew under anchorage-independent conditions. These abnormal growth characteristics were inhibited by all-trans retinoic acid. Finally, explants of the arthritic synovium formed short-lived tumorlike nodules in athymic nude mice. These observations, considered in the context of other data, support the concept that the pathologic process represents a thymic-dependent, nonmalignant, locally invasive inflammatory neoplasm. (Am J Pathol 1988, 132:38–48)

AN INTRAPERITONEAL INJECTION of an aqueous suspension of peptidoglycan-polysaccharide fragments from the cell walls of selected bacteria, such as group A streptococci (SCW), into a susceptible strain of rats, such as LEW/N females, induces a chronic proliferative and erosive arthritis resembling human rheumatoid arthritis.[1–6] Within 24 hours of administration, cell wall fragments begin to localize in synovial blood vessels, resulting in the development of an acute arthritis that reaches maximum severity by day 3–5 and then substantially subsides by day 10–14. During this period, histologic examination of the affected joints reveals mild synovial lining cell hyperplasia, prominent synovial endothelial cell abnormalities, edema, fibrin deposition, and infiltration of monocytes and polymorphonuclear leukocytes. Between the second and fourth week after SCW injection, a chronic arthritis develops that, although it waxes and wanes, persists for months. The arthritis in this latter phase is associated with persistence of cell-wall antigens in the blood vessels of the synovium. Histologically, this latter phase is characterized by prominent synovial lining cell hyperplasia, villus formation, diffuse and nodular lymphocytic infiltration, marked proliferation of spindle-shaped fibroblast-like synoviocytes, and ultimately bone and cartilage erosion/destruction. In contrast to normal synovium, Ia expression is intense and diffuse.[4,6]

The chronic phase arthritis is thymic-dependent. In

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rats with defective T lymphocyte function such as congenitally athymic nude and cyclosporin A treated LEW/N female rats, SCW induce the transient, rapid-onset, acute arthritis, but the subsequent chronic proliferative and erosive disease is significantly blunted.[4,6] This less severe chronic phase is characterized by markedly less synovial Ia expression and minimal proliferation.[6] Reconstitution of athymic LEW/N rats with T lymphocytes results in the development of a chronic proliferative and erosive arthritis.[7] These data provide strong evidence that the thymus and T lymphocytes play a major role in the development of the chronic erosive and proliferative process, but are not required for development of the rapid-onset, acute phase arthritis.

While lymphocytes, monocytes, plasma cells, mast cells, and eosinophils infiltrate the synovium, the predominant cell observed histologically, particularly at the sites of bone and cartilage destruction, is a spindle-shaped, fibroblastlike cell. We have noted previously that many of these cells have characteristics suggesting a "transformed" phenotype (increased number of mitoses, variation in size and shape, and large pale nuclei with prominent nucleoli).[8] In support of this concept is our report that the neoplasmlike proliferative and erosive disease can be inhibited by *in vivo* treatment with the retinoid, 4-hydroxyphenyl retinamide.[9] Retinoids are known to induce differentiation and to inhibit neoplastic growth of various cell types.[10,11] Moreover, 4-hydroxyphenyl retinamide not only inhibited the proliferative and destructive lesion but also suppressed, in a dose dependent manner, the spontaneous *in vitro* production of collagenase and prostaglandin E2 by the synovial tissues.[9]

In the present investigation, we have pursued more extensively the concept that synovial tissues in this model contain synoviocytes, with characteristics associated with "transformation," that play a major role in mediating bone and cartilage destruction. Using *in situ* histo- and immunochemical stains as well as electron microscopy, we demonstrate that the synovium contains an expanded population of metabolically active and proliferating synoviocytes of mesenchymal origin. We demonstrate that these cells are the dominant cell at sites of invasion of bone and cartilage by synovium. In addition, we show that *in vitro,* these cells exhibit growth characteristics generally associated with transformed cells. Primary explants and early passage synoviocytes grew rapidly, exhibited highly variable morphology, did not contact inhibit and formed "foci." Moreover, these cells grew under anchorage-independent conditions and formed colonies in soft agarose. These abnormal characteristics were reversed by all-trans retinoic acid. Finally, we demonstrate that the synovium from arthritic animals formed short-lived tumorlike nodules when implanted in athymic nude mice. Our data provide further evidence that the SCW-induced arthritis model in LEW/N rats has features in common with mesenchymal neoplasms. It, however, is localized to peripheral joints, is nonmalignant, and is driven by cell-wall-induced, thymic-dependent inflammatory mechanisms.

Materials and Methods

Animals

Specific pathogen-free, inbred LEW/N female rats and athymic (nu/nu) mice were obtained from the Small Animal Section, Veterinary Resources Branch, National Institutes of Health, Bethesda, MD. The animals were housed in cages with filter tops (Lab Products, Maywood, NJ) in an environment free of known microbial pathogens. The rats were approximately 6 weeks old and weighed 90–100 g at the initiation of each experiment. The mice were 4–6 weeks old.

Induction of Arthritis

The preparation of cell wall peptidoglycan-polysaccharide fragments from group A streptococci and the induction of the polyarthritis were done as described in detail previously.[2] Briefly, a sterile aqueous suspension of sonicated SCW fragments in phosphate buffered saline, (PBS) pH 7.4, was injected intraperitoneally into rats at a dose equivalent to 20 μg of cell wall rhamnose per g body weight. This dose of cell walls has been shown previously to induce severe chronic polyarthritis with nearly 100% incidence in LEW/N female rats.

In Situ Histo- and Immunochemical Evaluation

Whole arthritic joints were snap frozen in a glycerol base embedding medium (O.C.T., Tissue-Tek II, Lab-Tek, Div. of Miles Laboratories, Inc., Naperville, IL) by immersion in a mixture of dry ice and acetone. The frozen blocks were stored at −20 C in sealed containers until sectioned for staining. Sections (8 μm) were cut on a model CTI cryostat (International Equipment Co., Damon Corp. Needham Heights, MA) at −20 C and placed on glass slides. The slides were dried and stored at −20 C until fixed and stained as described below.

Naphthol-AS-D-chloracetate Esterase (ASCDL) Stain for Granulocytes and Mast Cells[13]

The sections were incubated at 25 C for 15 minutes in a solution containing 10 mg naphthol-AS-D-chloracetate (Sigma, St. Louis, MO) in 1 ml N,N-dimethylformamide (Sigma, St. Louis, MO), 0.1 ml hexazotized pararosaniline (Sigma, St. Louis, MO), and 40 ml 0.1M phosphate buffer, pH 6.3, which was filtered before use. The sections were counterstained with 1% methyl green, rinsed with water, air dried, and mounted.

Alpha-Naphthyl Acetate Esterase (ANAE) Stain for Monocytes[13]

The sections were incubated at 37 C for 30 minutes in a solution containing 10 mg alpha-naphthyl acetate (Sigma, St. Louis, MO) in 0.4 ml acetone, 2.4 ml hexazotized pararosaniline, and 40 ml 0.067M phosphate buffer, pH 5.0, which was filtered before use. The sections were counterstained with 0.05% toluidine blue, rinsed with water, air dried, and mounted.

Alpha-Naphthyl Butyrate Esterase (B-EST) Stain for Monocytes[13]

The sections were incubated at 25 C for at least 1 hour in a solution containing 40 mg of alpha-naphthyl butyrate (Sigma, St. Louis, MO) in 2 ml of N,N-dimethylformamide (Sigma, St. Louis, MO), 0.3 ml of hexazotized pararosaniline, and 38 ml PBS, 0.067M, pH 7.0, which was filtered before use. The sections were then washed sequentially with distilled water, 66% ethanol, and 100% ethanol prior to being placed in xylene and mounted.

Acid Toluidine Blue (TB) Stain for Mast Cells[14]

The sections were fixed in Mota's solution for 10 minutes prior to incubation at 25 C for 30 minutes in a solution containing 0.5% toluidine blue (Sigma, St. Louis, MO) and 0.7M hydrochloric acid in distilled water (pH < 1.0), which was filtered before use. The sections were counterstained with 1% methyl green, rinsed with water, air dried, and mounted.

Monoclonal Antibody Stains

The sections were fixed in acetone at room temperature for 5 minutes. The cellular antigens were demonstrated by the use of saturating amounts of monoclonal antibodies against vimentin (Chemicon, El Segundo, CA), Ia (nonpolymorphic, OX-6), pan-T lymphocyte (W3/13 and OX-19), T helper/inducer lymphocyte and activated macrophage (W3/25), and T syppressor/cytotoxic lymphocyte (OX8) (Serotec, Indianapolis, Ind.) used in combination with an immunoperoxidase staining kit as described previously (ABC Vecta Stain Kit; Vector Laboratories, Inc., Burlingame, CA).[15,16] In brief, color was developed by immersing the sections in a solution of 0.05% wt/vol 3,3-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO), 0.04% wt/vol nickel chloride, and 0.003% hydrogen peroxide in 0.05 M Tris/0.15 M NaCl buffer, pH 7.4. The sections were counterstained with 2% methyl green in methanol, dehydrated in 3 changes of absolute ethanol, cleared in xylene, and mounted in Permount (Fisher Scientific Co., Fair Lawn, NJ) under a glass coverslip. Control sections were stained with no primary antibody, or with mouse ascites fluid or normal mouse IgG.

Electron Microscopic Evaluation

Portions of synovium were dissected from the ankles of LEW/N rats 5 weeks after cell wall injection. Tissue was immediately fixed in one half strength Karnovsky's fixative, minced into 0.5 mm pieces, and processed as described previously for transmission electron microscopy.[17]

Synoviocyte Isolation and Propagation *In Vitro*

Synovium from arthritic animals was removed under sterile conditions, minced into 1 mm pieces, and placed in Dulbecco's minimal essential medium (DMEM) (Gibco Laboratories, Grand Island, NY) containing penicillin (100 units/ml), streptomycin (100 mcg/ml), glutamine (2mM), 20% fetal bovine serum (FBS), and collagenase (200 units/ml) (Cooper Biomedical, Malvern, PA). The tissue was incubated at 37 C for 4 hours with frequent mixing, then filtered through sterile gauze to remove undigested pieces and washed twice in DMEM containing antibiotics, glutamine, and 10% FBS (complete medium). The cells were cultured overnight in tissue culture flasks (Costar, Cambridge, MA) at 37 C in 5% CO_2 and the nonadherent cells removed. The adherent cells were gently washed 3 times and cultured in complete medium containing 5% FBS. The cultures were observed daily and passaged every 7–10 days.

Quantification of Synoviocyte Growth Rates in Monolayer Culture

Ninety-six well microtiter tissue culture plates (Costar, Cambridge, MA) were seeded with 2×10^3 viable cells in complete medium supplemented with 10% FBS. The cells were allowed to adhere during incubation at 37 C overnight in 5% CO_2. The medium was removed by aspiration each day and replaced

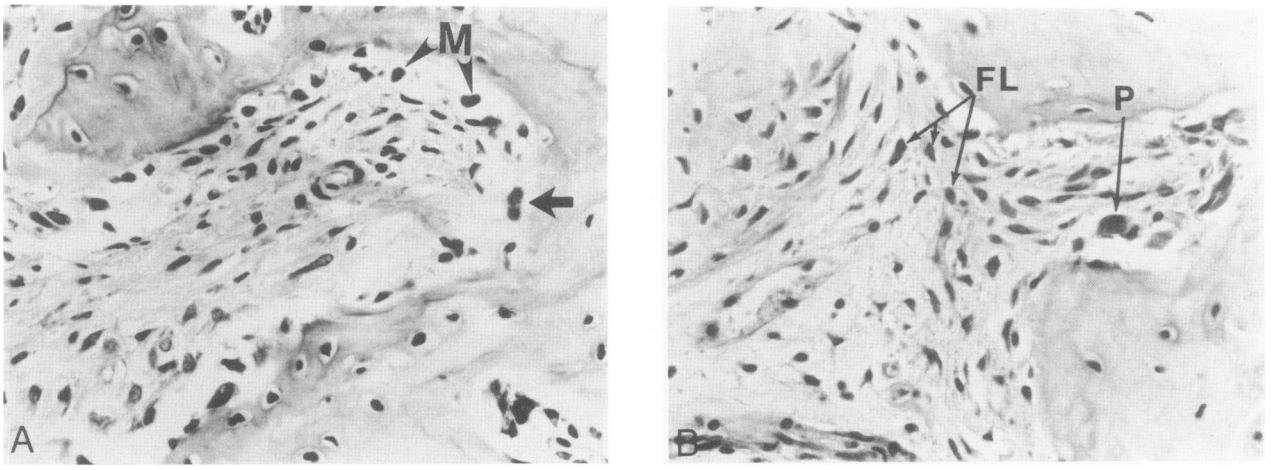


Figure 1—Representative stained histologic sections of synovium from LEW/N female rat injected 5 weeks previously with cell walls. **A**—High-power magnification (×500 on original photograph). Two macrophages are marked M (ANAE positive). A cell in mitosis is marked with an arrow. **B**—Synovium at a site of erosion into cartilage and bone. A polymorphonuclear leukocyte (ASCDL positive) is marked P and 2 fibroblastlike cells are marked FL. (×220 on original photograph)

with fresh complete medium. Each day 6 wells were harvested by trypsinization. The cells from 3 wells were combined and the numbers of viable cells per well were determined in duplicate using a hemacytometer. Additional wells were prepared for assessment of morphology after staining with Giemsa or naphthol blue black.

Synoviocyte Growth Under Anchorage-Independent Conditions

Primary adherent synoviocytes from arthritic animals were cultured under anchorage-independent conditions using soft agarose.[18] Culture dishes (35 mm, 6 well cluster dishes, Costar, Cambridge, MA) were precoated with 1 ml/well of complete DMEM containing 20% FBS and 1.0% agarose at 40 C (Sea Plaque, FMC Corporation, Rockland, ME), Primary cultures of synoviocytes were trypsinized, collected by centrifugation, resuspended in complete DMEM containing 20% FBS at 10^6 cells/ml, and passed through a 25-gauge needle. A small aliquot of the resulting cell suspension was added to 3 ml of 1.67X DMEM containing 33% FBS warmed to 37 C. Two milliliters of 1–6% agarose in water was added and the mixture allowed to gel for 10 minutes in the refrigerator before being placed in a humidified incubator at 37 C and 5% CO_2. Growth of colonies was generally apparent in 7 days. They reached maximum size in 10–14 days. The optimal cell concentration for synoviocyte colony growth was determined to be 1×10^4 cells/well.

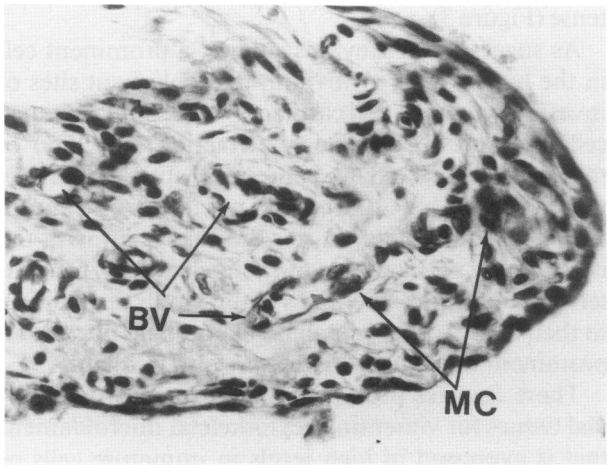


Figure 2—Representative stained histologic section of synovium from a LEW/N female rat 4 weeks after cell wall injection (×500 on original photograph). It demonstrates increased vascularity with perivascular inflammatory infiltrates. The labels denote blood vessels (BV) and mast cells (MC) (acid toluidine blue positive).

Growth of Arthritic Synovium in Athymic Nude Mice

Synovial tissue from arthritic animals was removed under sterile conditions from rats 3–5 weeks after SCW administration. The tissue was minced into 2 mm pieces and washed twice in DMEM. Athymic nude mice were anesthetized using ether and 5–6 mm incisions were made in the mid-upper back area. A small pocket was made by tunneling with forceps and 4 of the 2 mm pieces of synovial tissue were placed in each pocket. The incision was then closed with sutures. Animals were observed daily for growth at the implantation site. Incisions that did not have synovial tissue implants or were implanted with normal synovium served as control sites.

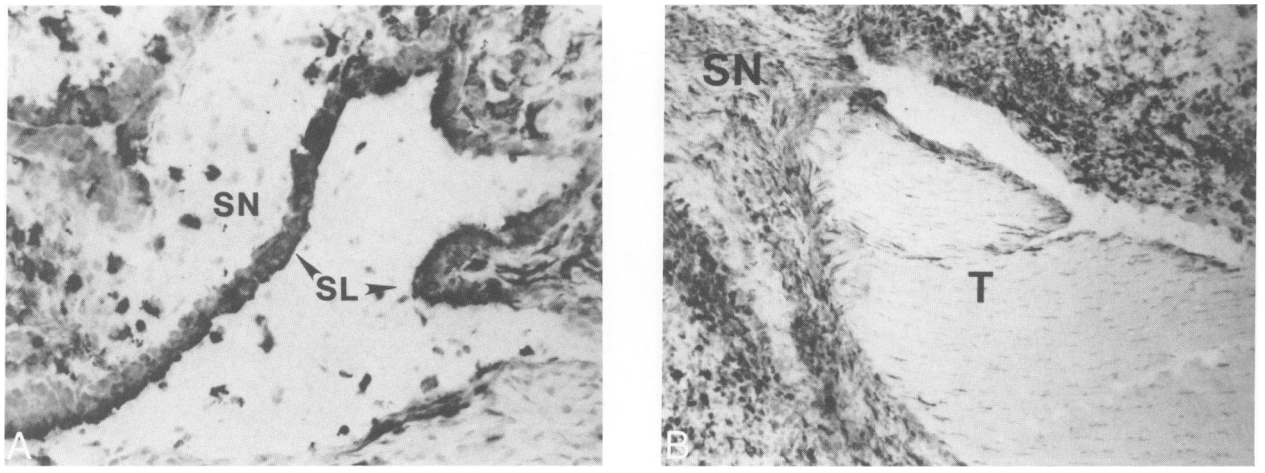


Figure 3—Representative immunoperoxidase stains *in situ* of Ia antigen synovial tissue from a LEW/N female rat 3 weeks after cell wall administration. **A**—High grade staining on the lining layer (SL) and more variable staining in the sublining synovium (SN). **B**—Diffuse and intense staining throughout the synovium (SN) surrounding a tendon (T). (×32 on original photograph)

Results

Evaluation of the Joints by *In Situ* Histochemical and Immunochemical Staining

Sections of whole ankle joints were obtained from cell-wall-injected LEW/N female rats during the most severe proliferative and erosive arthritis (3–6 weeks). They were examined microscopically after staining with the various histochemical reagents (hematoxylin and eosin, B-EST, ANAE, ASCDL, and acid TB) and monoclonal antibodies (anti-vimentin, OX-6, W3/13, OX-19, W3/25, and OX-8). PBS-injected LEW/N female rats served as controls.

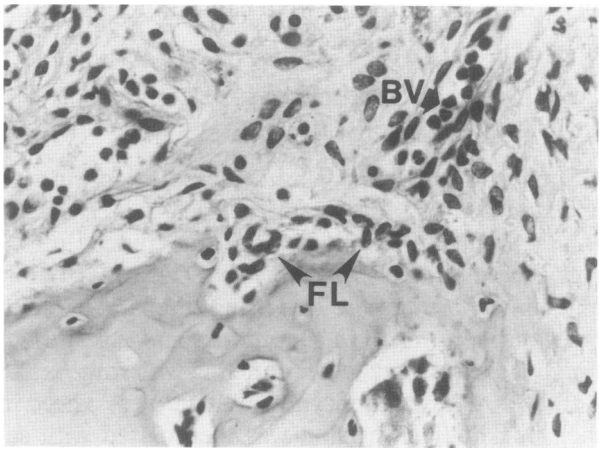


Figure 4—Representative hematoxylin and eosin stained section of synovium 4 weeks after cell wall injection demonstrating typical "fibroblastlike" synoviocytes as the major cell population directly adjacent to an area of erosion into cartilage. Several synoviocytes (FL) and a blood vessel (BV) are marked. Note the lack of inflammatory cells. (×400 on original photograph)

Although staining varied from microscopic field to field, monocytes, and macrophages (ANAE and B-EST positive cells) were prominent throughout the synovial tissue. Granulocytes (ASCDL positive cells) were strikingly less frequent (Figures 1A and 1B). Occasional mast cells (acid TB positive cells) were noted primarily around nerves and blood vessels and in mononuclear cell clusters (Figure 2). As in previous studies,[4,8] diffuse and nodular infiltrates of cells expressing W3/13 and W3/25, putative pan-T, and T helper/inducer markers were also prominent. OX-8 positive cells (T suppressor/cytoxic cells) were significantly less frequent than W3/13, OX-19, or W3/25 positive cells. Although variable from field to field, Ia expression (OX-6) was, in general, diffuse and intense (Figure 3).

As suggested in previous studies, a prominent cell in the hyperplastic synovium, particularly at sites of invasion into bone and cartilage, was a spindle-shaped cell[5,8] (Figure 4). Although a fraction (15–25%) of these cells appeared to express Ia antigen (OX-6), most of these cells did not stain with this or any of the histochemical stains, nor did they stain with the monoclonal antibodies to mononuclear cells. Characteristic morphologic features of these cells, in addition to their spindle shape, included large, pale nuclei and prominent nucleoli. Mitoses were also frequent.

These features prompted us to examine the synovial tissues for vimentin, a cytoskeletal microfilament that is expressed in high levels in immature cells of mesenchymal origin.[19,20] As shown in Figure 5, vimentin expression was diffuse and intense throughout the synovium, particularly on synoviocytes at sites of invasion into bone.

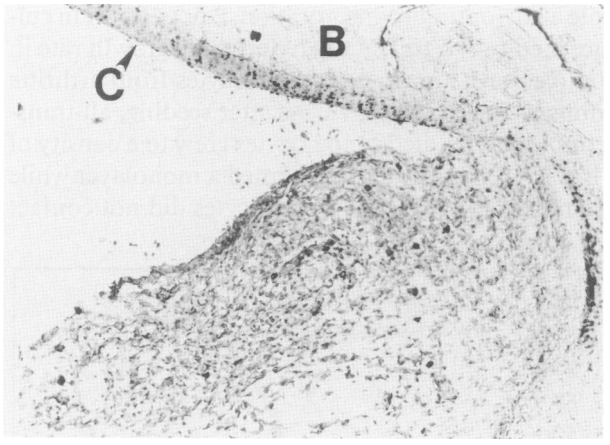


Figure 5—Demonstration of vimentin expression in the synovium during the early chronic phase disease (4–5 weeks) in LEW/N rat. Note the intense expression throughout the synovium and adjacent to the bone and cartilage. The labels denote cartilage (C) and bone (B). (×50 on original photograph)

In contrast to synovium from the diseased joints, synovium from control rats expressed vimentin weakly. Weak Ia staining was observed on the synovial lining layer only and on an occasional fibroblastlike cell in the sublining stroma. Cells staining positively with W3/13, OX-19, W3/25, or OX-8, or the histochemical stains ANAE, B-EST, or ASCDL were rare. Mast cells (acid TB-positive cells), however, were noted around nerves and some blood vessels.

Evaluation of the Joints *In Situ* by Electron Microscopy

Electron microscopic analysis of synovium from arthritic rats confirmed that the fibroblastlike cells were the most abundant cell present, particularly at sites of synovial erosion of bone. These cells were characterized by a spindle shape, large nuclei with little condensed chromatin, and by extensive endoplasmic reticulum. They were often juxtaposed to macrophages containing vacuoles filled with large amounts of unidentified amorphous material (Figures 6 and 7). In addition, vessels showed prominent endothelial cells and multilaminated basement membranes (Figure 8).

Characteristics of the Synoviocytes from Arthritic Rats *In Vitro*

Following dissociation into single cells, preliminary experiments indicated that the spindle-shaped synoviocytes were plastic adherent. These cells, which represented 60–80% of the total synovial cells, were characterized using the same histo- and immunochemical stains used for *in situ* staining of the joints. As *in situ*, primary explant cells expressed vimentin and variable

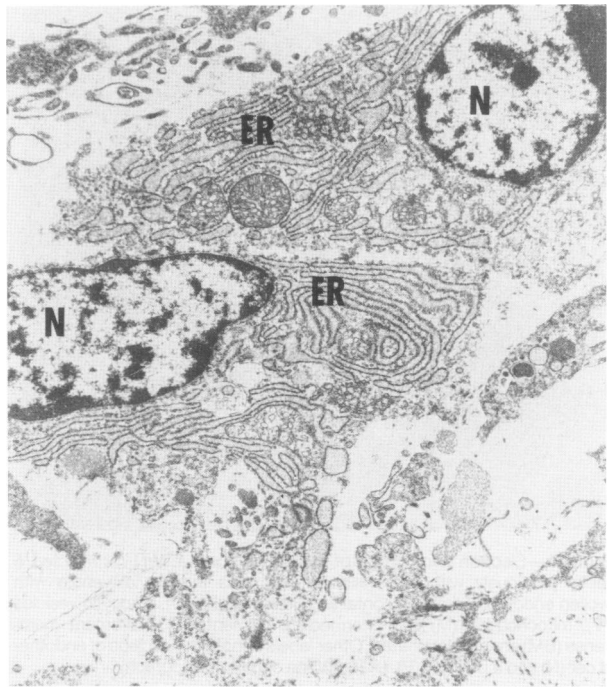


Figure 6—Representative electron micrograph of synovium from LEW/N rat injected 5 weeks previously with cell walls. There are 2 large synoviocytes with typical large pale nuclei and extensive endoplasmic reticulum (labelled N and ER, respectively). (×18,000 on original photograph)

intensities and frequencies of Ia. The Ia positive staining declined rapidly in culture and was infrequent by 7 days. Variable frequencies of macrophages (ANAE, B-EST positive, or cells that phagocytosed latex beads) were also present in the initial explants (20–30%), but they did not proliferate in culture. By the

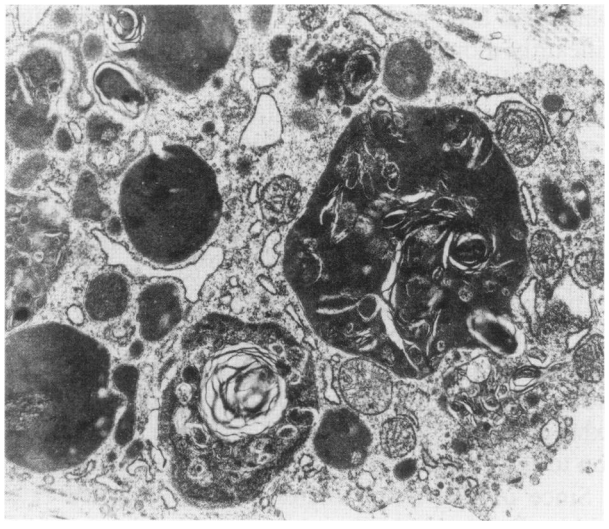


Figure 7—Electron micrograph of synovium from LEW/N rat injected 5 weeks previously with cell walls. A macrophagelike cell has phagocytic vacuoles packed with dense, particulate amorphous and membranous debris. (×30,000 on original photograph)

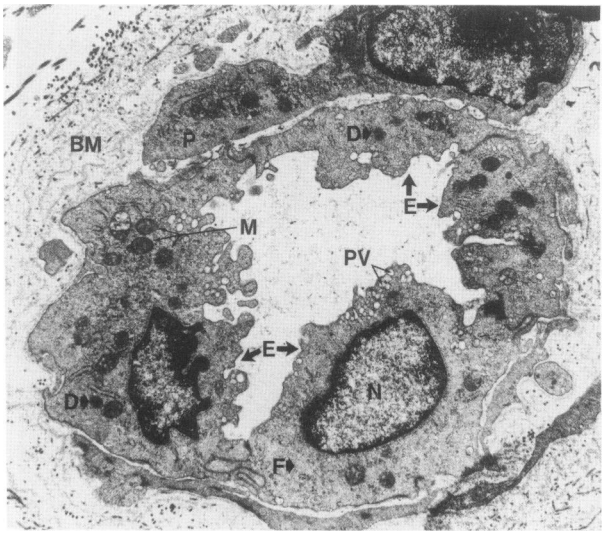
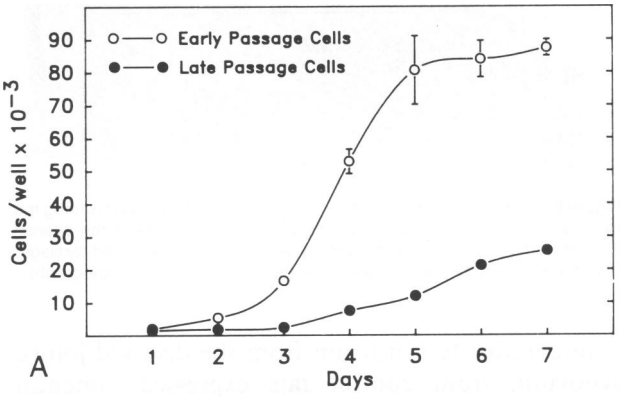


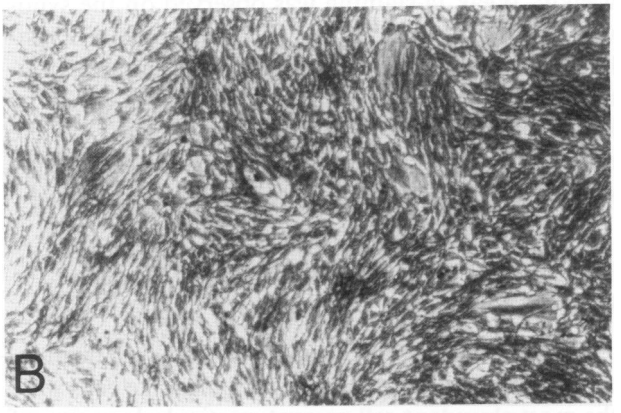
Figure 8—Electron micrograph of synovium from LEW/N rat injected 5 weeks previously with cell walls demonstrates a synovial venule with swollen endothelial cells (E) containing dense bodies (D), pinocytic vesicles (PV), mitochondria (M) and intermediate filaments (F). Basement membranes (BM) are multilaminated. Other labels denote the nucleus of endothelial cell (N) and a pericyte (P). (×30,000 on original photograph)

time the initial tissue explants reached confluence in culture, cells with macrophage characteristics represented less than 0.1% of total cells. Cells staining with other markers, including the various mononuclear cell markers identified by monoclonal antibodies, were absent in the primary explant adherent cell population.

The majority of the adherent synoviocytes with the spindle-shaped morphology exhibited large, pale nuclei and prominent nucleoli. Primary explants and early passage synoviocytes from arthritic animals, which had been passaged less than 4 times, grew significantly more rapidly and attained higher cell density than late passage synoviocytes (cells which have been passaged in culture greater than 10 times) (see Figure 9A). For example, 7 days after seeding 2×10^3 cells per well, the early passaged cells reached a density of 88×10^3 cells per well and did not form a monolayer, whereas late passage cells formed a typical monolayer at a density of 26×10^3 cells per well. In addition, the morphology of the adherent synoviocytes from arthritic rats changed dramatically after continued passage in culture. Late passage cells covered a much higher surface area per synoviocyte and exhibited more cytoskeletal organization in comparison to early passage synoviocytes (Figures 9B and C).

Since *in vivo* treatment of cell wall-injected rats with retinoids markedly inhibits the development of chronic arthritis and suppresses established arthritis,[9] we also examined their effects on the growth and morphology of early passage synoviocytes. All-trans-retinoic acid, (optimal concentration, 2.5×10^{-6}M in culture medium) significantly reduced the growth rate in comparison to untreated synoviocytes from arthritic animals. For example, 7 days after seeding, all-trans-retinoic acid-treated synoviocytes grew to a density of 41×10^3 cells per well and formed a monolayer while the nonretinoid-treated synoviocytes did not contact





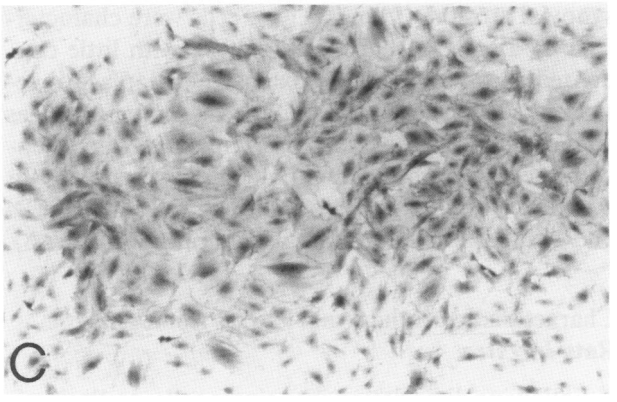
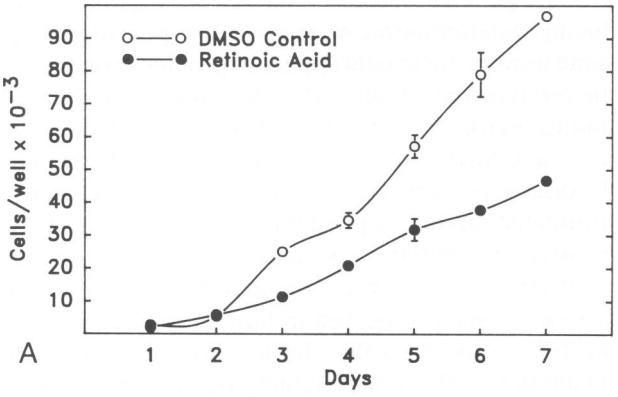
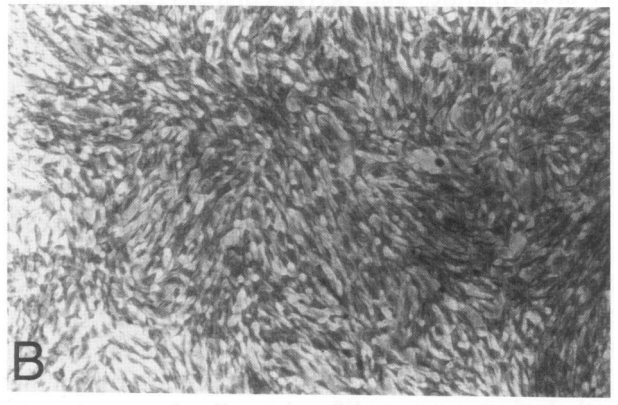


Figure 9—Growth of early passage and late passage adherent synoviocytes from arthritic rats in culture. **A**—Numbers of early and late passage cells per well were determined each day as described in Materials and Methods. Each point represents the average of duplicate counts and error bars depict the standard error of the mean. Points without error bars have standard errors less than or equal to 1900 cells per well. **B and C**—Photographs showing early passage and late passage cell morphology respectively at day four. Cells were stained with naphthol blue black. (×20 on original photograph)

inhibit, formed foci, and grew to a density of 95×10^3 cells per well (see Figure 10A). Moreover, a striking change in morphology was noted in the retinoid-treated cells. The retinoid-treated cells were large, flattened and covered an increased surface area compared to the nonretinoid-treated cells (Figures 10B and 10C).





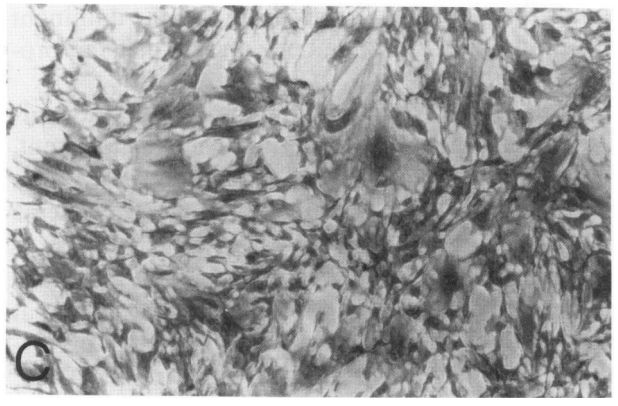


Figure 10—Effect of all-trans-retinoic acid on the growth of early passage adherent synovial cells from arthritic rats. **A**—Early passage cells were cultured in medium containing 2.5 μM all-trans-retinoic acid or control volumes of dimethyl sufoxide (solvent vehicle). The number of cells per well was determined each day and depicted as described in Materials and Methods and the legend to Figure 9. **B and C**—Photographs comparing control and treated cells respectively, stained with naphthol blue black on the fourth day after drug addition. (×20 on original photograph)

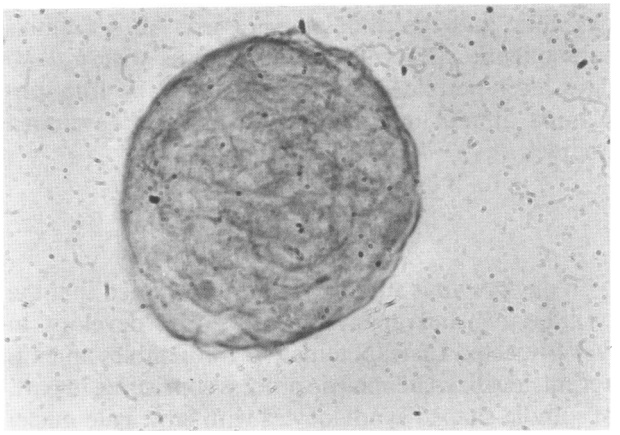


Figure 11—Representative colony from soft agar culture of synoviocytes. Synoviocytes were originally taken from synovium of LEW/N rats injected 5 weeks previously with cell walls. The colony is approximately 150 microns in diameter. (×540 on original photograph)

Synoviocyte Growth in Soft Agarose

Transformed cells characteristically grow under anchorage-independent conditions and form colonies. It was, therefore, of interest to determine if the synoviocytes from arthritic animals in primary culture grew under these conditions and formed colonies. As shown in Figure 11, the synoviocytes did, indeed, grow under anchorage-independent conditions in soft agarose. Colony growth was dependent on the number of cells plated with an optimum of 1×10^4 per well. The number and size of the colonies decreased at higher cell densities with no apparent colony growth at cell densities of 1×10^6 or higher per well.

Of additional interest was the observation that late passage cells ($N > 10$) did not form colonies in soft agarose. Moreover, all-trans-retinoic acid at 5×10^{-6}M completely suppressed the formation of colonies by primary explant synoviocytes.

Growth of Synovium from Arthritic Rats in Athymic Nude Mice

To further examine the tumorlike properties of the synovium from arthritic animals, we implanted the diseased tissue into nude, athymic mice. Synovial tissue was removed under sterile conditions from rats 3–5 weeks after cell wall administration and 2 mm pieces implanted into the subcutaneous tissue of 5 nude mice. Additional mice that were treated the same surgically without the implantation of synovium or were implanted with normal synovium served as controls. Development of a nodule, at the site of implantation, was observed in 4 of the 5 mice 10 days after implantation. The nodules continued to enlarge for 3–4 weeks after implantation, at which time the area became ec-

chymotic and the nodules regressed. All 4 animals subsequently died of unknown causes within 7–10 days after nodule regression. Neither the fifth implanted animal nor the control animals developed nodules.

Discussion

In our previous studies, we have suggested that the proliferative and erosive arthritis that develops in SCW-injected LEW/N female rats resembles an exuberant, localized neoplastic process consisting, in part, of spindle-shaped synoviocytes with large pale nuclei and prominent nucleoli.[5,6,8] To pursue this concept, we have further characterized the spindle-shaped synoviocytes present in the synovium of LEW/N rats with SCW-induced arthritis using *in situ* immunochemical and histochemical stains, electron microscopy, *in vitro* culture and staining techniques, and cell transfer studies into nude, athymic mice. Our results confirm that the most abundant cell in synovium of arthritic rats is an immature, spindle-shaped mesenchymal cell that exhibits characteristics generally associated with transformed cells. In culture, these cells grew rapidly, exhibited variable morphology, did not contact inhibit and tended to form "foci." Moreover, they grew under anchorage-independent conditions. These characteristics are typically exhibited by transformed cells and are not exhibited by normal cells.

To further examine the tumorlike properties of the synovium, we implanted the synovium into the subcutaneous tissue of nude, athymic mice. Similar to transformed tumor cells, nodules developed that continued to increase in size for up to 3 weeks after implantation. Normal cells and tissues did not form tumors.

An important difference, however, was observed that distinguished the abnormal synoviocytes from malignantly transformed cells. By the sixth to ninth passage in culture, the synoviocytes exhibited slower, contact inhibited monolayer growth, and failed to grow under anchorage-independent conditions. The argument can be raised that early passaged synoviocytes did not convert to a more normal phenotype but were overgrown by normal cells. Excessive cell death, however, was not exhibited by primary explant or early passaged cells, and the rapid initial growth rate should not have allowed overgrowth by more slowly growing normal cells. Since the cells lost the transformed phenotype, it is unlikely that the transformed phenotype was virally induced. It is highly probable that the transformed phenotype was progressively lost during *in vitro* culture of the synoviocytes. Further study will be needed to identify and examine the effects of inflammatory mediators, like interleukin-1, tumor necrosis factor, platelet-derived growth factor, etc. with respect to synoviocyte transformation.

An additional important observation was that the transformed phenotype exhibited in culture was reversed by the retinoid, all-trans-retinoic acid. This agent induced the cells to grow more slowly, to form confluent monolayers, and inhibited anchorage-independent growth. Because retinoids are capable of inducing differentiation of many cell types, including some tumors, these data appear to provide insight into the mechanisms of our earlier demonstration that retinoids markedly inhibit SCW-induced arthritis *in vivo*[20] and further support our general hypothesis that the disease process can be characterized as a cell-wall-stimulated, thymic-dependent, nonmalignant, locally invasive, inflammatory neoplasm.

Inflammatory arthritis, similar to the SCW-induced disease in rats, is observed in human rheumatoid arthritis.[21,22] Histologically, the acute or early phases of rheumatoid arthritis are characterized by microvascular abnormalities and perivascular accumulation of monocytes and granulocytes. Established disease is characterized by perivascular nodules of T lymphocytes, primarily T helper/inducer cells.[23] Bromley and Woolley have shown that the predominant cells at the erosive junction between synovium and the cartilage/bone are spindle-shaped, fibroblastlike cells and lesser numbers of macrophages.[24] In addition to these cell types, they have also demonstrated the presence of chondroclasts and osteoclasts in some areas of subchondral bone/cartilage erosions.[25] These studies strongly suggest, as do our studies in the rat model, that while immune cells play an important role in the pathologic process, proliferating cells of mesenchymal connective tissue origin appear to play the dominant role in directly mediating bone and cartilage resorption.

Similar to the synovium from the SCW-induced arthritic rats, rheumatoid synovium contains adherent, spindle-shaped, fibroblastlike cells that grow *in vitro* without contact inhibition and spontaneously release large amounts of neutral proteases.[26–28] These abnormal properties are lost on continued passage of the cells in *in vitro* culture. Moreover, Brinckerhoff et al have reported that implantation of rheumatoid synovium into nude athymic mice results in short-lived, tumorlike growth. These various observations have led several authors, including us, to hypothesize that rheumatoid synoviocytes exhibit properties generally associated with transformed tumor cells,[29–33] but these observations are insufficient to adequately establish that rheumatoid synoviocytes are, in fact, phenotypically transformed. Significantly, our laboratory has re-

cently grown early, but not late, passage, rheumatoid synoviocytes *in vitro* under anchorage-independent conditions. These data establish further analogies between human rheumatoid and SCW-induced arthritis in rats and provide strong support for the concept that the abnormal synoviocytes in both conditions are phenotypically transformed (Lafyatis R, Remmers EF, Yocum DD, Roberts AN, Sporn M, Wilder RL: Anchorage-independent growth of adherent rheumatoid synovial cells, in preparation).

Since the human rheumatoid adherent synoviocytes, like the synoviocytes from SCW-induced arthritic rats, lose the characteristics of the transformed phenotype on extended culture, the critical question that is still unresolved, is what paracrine growth factors drive the synoviocytes to acquire phenotypic characteristics generally associated with tumors. In an attempt to address this question, Brinckerhoff, using normal rabbit synoviocytes, demonstrated an increased rate of cell growth and morphologic changes suggesting transformation when transforming growth factor-beta was added to the cultures in combination with either transforming growth factor-alpha or epidermal growth factor.[34] The acquisition of these abnormal characteristics were prevented by all-trans-retinoic acid, a situation reminiscent of our observations with the SCW-induced rat synoviocytes. Further studies are clearly indicated to determine if these cytokines, as well as many others, play a role in driving the exuberant tumorlike invasive disease process.

Evidence of synoviocyte transformation in other animal models of arthritis is limited. O'Sullivan et al reported that the arthritic synovium of the MLR/lpr/lpr mouse was composed of transformed mesenchymal cells.[35] The sole criteria for transformation in that study, however, was histologic assessment of hematoxylin and eosin stained sections. Histo- and immunochemical staining, electron microscopy, and *in vitro* cell culture and transfer studies were not done. Nevertheless, these limited data provide further support for the concept that synoviocyte transformation is a feature of proliferative and invasive forms of arthritis.

Our data provide further evidence that the SCW model closely resembles the proliferative and erosive disease process observed in rheumatoid arthritis. Moreover, the data support the concept that the pathologic process represents an inflammatory, locally invasive but nonmalignant neoplasm. The model presents an opportunity to study the mechanisms by which immune and inflammatory processes regulate synovial mesenchymal cell growth and bone and cartilage resorption under controlled circumstances.

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