# Transin/stromelysin expression in the synovium of rats with experimental erosive arthritis. In situ localization and kinetics of expression of the transformation-associated metalloproteinase in euthymic and athymic Lewis rats.

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**Research Article**

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# Transin/Stromelysin Expression in the Synovium of Rats with Experimental Erosive Arthritis

## In Situ Localization and Kinetics of Expression of the Transformation-associated Metalloproteinase in Euthymic and Athymic Lewis Rats

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## Abstract

**Transin is a neutral metalloproteinase initially isolated from malignantly transformed rat fibroblasts and subsequently shown to be homologous to human stromelysin. We performed Northern blot analysis on synovial tissue specimens from Lewis rats with proliferative and invasive streptococcal cell wall (SCW) arthritis. Transin mRNA was present in abundance, as was the mRNA of the c-*myc* oncogene, which is associated with cellular proliferation. Immunohistochemical staining of synovia from rats with chronic SCW arthritis showed high-level transin expression in the cells of the lining layer and underlying stroma, as well as in chondrocytes and osteoclasts in subchondral bone. Intense nuclear staining for the Myc oncoprotein was also detected with a cross-reactive antibody to v-Myc. Transin stained similarly in the early, rapid-onset, thymus-independent, acute phase of SCW arthritis. In the T cell–dependent adjuvant arthritis, transin expression was noted by day 4, 6 d before the influx of mononuclear cells and the onset of clinical disease. Athymic rats did not express transin. We concluded that transin is a marker of proliferative, invasive arthritis in rats and appears early in the course of disease development, but requires a competent immune system to sustain its expression in these model arthropathies.**

## Introduction

The synovium in rheumatoid arthritis (RA)[1] and two experimental animal models, streptococcal cell wall (SCW) arthritis and adjuvant arthritis (AA) in female LEW/N rats, is characterized by exuberant hypertrophy and hyperplasia of the normally thin, delicate synovium, resulting primarily from proliferation of lining and stromal fibroblast-like mesenchymal cells (synoviocytes) (1–4, and reviewed in reference 5). These cells, as well as new blood vessels, macrophages, and osteoclasts, are the predominant cell populations at the sites of cartilage resorption and bone erosion in diseased joints, where they directly mediate articular destruction (6–12). The aggressive invasiveness of the highly proliferative lesion has suggested to us and others a resemblance to a localized, nonmetastatic neoplasm (13–19). Supporting this notion are the in vitro observations that freshly explanted synoviocytes from rheumatoid and SCW arthritic joints grow rapidly (18–20), do not contact inhibit, tend to form foci, and grow under anchorage-independent conditions (18, 19). Moreover, synovial tissues from both patients with RA (16) and rats with SCW arthritis (18) form short-lived, tumor-like nodules when implanted in nude, athymic mice. These phenomena suggest that synoviocytes may exhibit properties generally associated with malignant tumor cells. In the rheumatoid synovium the process has been termed mesenchymoid transformation (14, 21).

In light of these observations the discovery and characterization of the rat transin gene (22, 23) is significant. Transin was originally isolated on the basis of its high expression, relative to control parental cells, in rat fibroblasts transformed by agents such as Rous sarcoma virus, polyoma virus, and the activated cellular oncogene Ha-*ras* (23). It is likewise highly expressed in malignant versus benign skin tumors induced in a chemical carcinogenesis model, where high transin mRNA levels have been associated with invasiveness and metastasis (24, 25). Thus, high-level expression of the transin gene may be a biochemical correlate of the transformed phenotype. Transin was subsequently demonstrated to be the rat homologue of the human stromelysin (26, 27) gene, which codes for a secreted metalloproteinase isolated from rabbit (28, 29) and human (30, 31) synovium as well as from human skin (26). Functionally, transin/stromelysin is a connective tissue matrix-degrading enzyme of $M_r$ 51,000, active against proteoglycan, type IV (basement membrane) collagen, and denatured, but not native, type I collagen (28). It is identical to the rabbit proactivator protein that participates in the conversion of the enzymatically inactive procollagenase to the fully activated enzyme (32–35). Indeed, sequence analysis demonstrates a striking homology to collagenase itself, suggesting the existence of a family of matrix-degrading metalloproteinases whose normal function is the coordinate modulation of the mesenchymally derived extracellular matrix (32, 36).

Transin/stromelysin may be readily demonstrated in the conditioned medium of cultured synoviocytes from diseased or normal humans and animals (28, 30), and its mRNA may be rapidly induced in cultured cells upon the addition of serum or certain growth factors (23, 32). We have observed its high-level expression in vivo in the synovium of patients with RA, but not in those with typically nonerosive osteoarthritis (37). In the present study we show that the transin protein may be immunohistochemically localized at high levels relative to normal rats in the synovium of rats with SCW or AA. Tissue

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1. *Abbreviations used in this paper:* AA, adjuvant arthritis; RA, rheumatoid arthritis; SCW, streptococcal cell wall.

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staining was most intense at the sites of active or incipient bone and cartilage erosion. It was similarly highly expressed by osteoclasts in the subchondral bone and by chondrocytes in the diseased joints. High-level staining of the c-Myc oncoprotein, another marker of cellular activation, proliferation, and transformation (38, 39), was also demonstrated in the diseased but not in the normal tissues. These histological findings were corroborated by the demonstration of markedly increased tissue levels of the transin and c-*myc* mRNAs in synovia from arthritic joints relative to normal synovia. We also show early transin appearance during the acute phase of SCW arthritis, which is thymus independent (9), and in the synoviocytes and chondrocytes of adjuvant-injected rats well before the histologic appearance of lymphocytes and the development of clinical arthritis. This suggests that high-level transin expression is one of the earliest cellular events in SCW arthritis and AA, implying that bone and cartilage resorption develop early in the course of the disease. Thymus-dependent inflammatory processes, however, appear necessary to sustain the erosive process.

## Methods

*Tissue specimens.* SCW arthritis was induced and scored in female LEW/N and athymic LEW.rnu/rnu rats as previously described (9, 40, 41). AA was induced in female LEW/N rats and clinically scored as described (42). Athymic LEW.rnu/rnu rats, which do not develop AA, were also injected with Freund's adjuvant and tissues collected for histological study. Synovia from rats with acute and chronic SCW arthritis were dissected from 2–4-d and 3–6-wk ankle lesions, respectively, and treated as described below for cell culture, RNA isolation, or tissue fixation. Normal rat synovium was obtained by dissection of prepatellar fat pads (43) and treated similarly. Rats injected with adjuvant (day 0) were killed in pairs at 2-d intervals through the preclinical and clinical stages of disease until the development of maximal clinical arthritis (day 16). Hindlimb specimens were collected for tissue fixation.

*Cell culture.* Synovial tissue specimens weighing ~ 1 g were minced aseptically and digested for 4–5 h with 4 mg/ml collagenase (type III; Worthington Biochemical Corp., Freehold, NJ) in DME at 37°C. After digestion the dissociated cells were centrifuged for 10 min at 500 *g* and suspended in DME supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum and plated onto 6-well cluster dishes (35-mm-diam wells; Costar, Cambridge, MA), at approximately four plates per specimen. The primary culture (explant) synoviocytes were incubated at 37°C in 5% carbon dioxide and the nonadherent population removed. Confluency was typically reached in 7–10 d, at which point the cells were resuspended with trypsin (Gibco Laboratories, Grand Island, NY) and passaged 1:3 to 75-$cm^2$ culture flasks (Costar). These secondary culture synoviocytes were grown to 95% confluence then harvested directly into lysing buffer (44) containing 4 M guanidinium isothiocyanate (Fluka Chemical Corp., Ronkonkoma, NY).

*RNA isolation.* Tissue specimens were either flash-frozen in liquid nitrogen for subsequent storage, or immediately homogenized in lysing buffer with a homogenizer (Brinkmann Instruments, Inc., Westbury, NY) for storage at −70°C or immediate use; specimens stored in liquid nitrogen were later homogenized in lysing buffer as frozen pieces. RNA was isolated by the method of Chomczynski (44). For tissue specimens the second extraction step was replaced by proteinase K digestion followed by multiple extraction with phenol-chloroform in a microfuge tube. RNA was aliquotted and stored as ethanol precipitates at −20°C.

*Northern gel analysis.* 40-μg aliquots of tissue RNA or secondary culture synoviocyte RNA prepared as above were applied to each lane of submerged 1% agarose denaturing gels containing 1.1 M formaldehyde and 1 μg/ml ethidium bromide and electrophoresed at 120 V for ~ 4 h using standard methodology (45). The 28S and 18S ribosomal RNA bands were photographed under ultraviolet illumination before transfer to confirm RNA integrity and that equal amounts had been applied to each lane. Size marking was done with the RNA ladder (Bethesda Research Laboratories, Gaithersburg, MD). The untreated gels were capillary-transferred overnight to Nytran membranes (Schleicher & Schuell, Inc., Keene, NH), which were then baked for 1 h at 80°C. For hybridization with transin, a plasmid containing the complete cDNA of the rat transin gene from pTR1 (the kind gift of Dr. Richard Breathnach) was $^{32}P$-labeled using a nick-translation kit (Bethesda Research Laboratories), and $2 \times 10^6$ cpm/ml applied overnight to the membranes in 50% formamide, 10% dextran sulfate, 5× standard saline citrate (SSC), 2× Denhardt's solution, 0.1% SDS, and 100 μg/ml sheared salmon sperm DNA (Sigma Chemical Co., St. Louis, MO) at 44°C (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0). The membranes were washed with 1× SSC/0.1% SDS four times at 50°C. Probing for c-*myc* used a nick-translated plasmid containing the murine c-*myc* cDNA (46) prepared and hybridized as above except that the washes consisted of 0.1× SSC/0.1% SDS at 65°C. Autoradiographs were exposed for 1–3 d with an enhancing screen at −70°C. Band intensities were quantitated by transmittance densitometry.

*Peptide antisera production and Ig affinity purification.* A 17-mer peptide near the carboxy terminus of the rat transin protein, corresponding to amino acid residues 455–470 of the human stromelysin sequence as given by Whitham et al. (27), with an additional $NH_2$-terminal cysteine residue for conjugation to keyhole limpet hemocyanin via *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (47), was custom synthesized by Biosearch (San Rafael, CA) and an anti-peptide antisera raised in rabbits. IgG was isolated by chromatography on protein A–Sepharose (Sigma Chemical Co.) using 0.1 M Tris (pH 8) as loading buffer followed by elution with 3.5 M magnesium chloride and subsequent dialysis against PBS. The resultant IgG (6/6F) was affinity-purified by adsorption to the immunogenic peptide bound to cyanogen bromide–activated Sepharose 4B (Sigma Chemical Co.) as described (48). The affinity-positive 6/6F IgG was eluted with 3 M potassium thiocyanate containing 0.5 M ammonium hydroxide and the eluate dialyzed against PBS. A control, affinity-negative IgG fraction was isolated as well.

*Immunoprecipitation.* 1-ml samples of [$^{35}S$]methionine-labeled (100 μCi/ml) serum-free medium conditioned by secondary culture SCW synoviocytes for 6 h in the presence of $10^{-8}$ M phorbol myristic acid (Sigma Chemical Co.) were immunoprecipitated with 20 μg anti-transin IgG or control rabbit IgG (ChromPure; Jackson Immunoresearch, West Grove, PA) by standard methodology (49). Blockage with the immunogenic peptide or with an irrelevant peptide was done at a 100× molar equivalent. After reduction with 5% 2-mercaptoethanol specimens were applied to a 10% SDS polyacrylamide gel (49) and electrophoresed at 30 mA constant current. $^{14}C$-Labeled molecular weight markers were obtained from Bethesda Research Laboratories.

*Immunohistochemistry.* Tissue specimens were preserved in 10% formalin (Formalde-Fresh; Fisher Scientific Co., Pittsburgh, PA). After decalcifying where necessary in citrate/formate, specimens were embedded in paraffin and sectioned onto gelatin-coated microscope slides at a thickness of 6 μm. Immunoperoxidase staining was done with the Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA) using the manufacturer's suggested protocol and reagents. Nickel chloride (0.04%) was included in the experiments using decalcified hindlimb specimens to intensify specific staining relative to the counterstain. Comparative studies of tissues from normal versus arthritic animals were done treating the respective tissue sections identically and simultaneously. Transin immunostaining was done optimally with the affinity-positive 6/6F IgG at 20–30 μg/ml. Control antibodies consisted of the affinity-negative IgG fraction prepared as described above, an antibody to an irrelevant antigen, or a purified rabbit IgG (Jackson Immunoresearch), used at the same concentrations as the specific antibody and under identical incubation conditions. All yielded negative

results. Counterstaining was done with aqueous hematoxylin (Gill #1; Fisher Scientific Co., Orangeburg, NY) or light green SF (Roboz Surgical Instruments Co., Inc., Washington, DC). Anti-Myc immunostaining used a commercially available affinity-purified Ig made in sheep by immunization with a synthetic peptide corresponding to amino acid residues 56–67 of v-Myc (50), whose homology to c-Myc is highly conserved among species (Cambridge Research, Valley Stream, NY). Optimal staining was achieved at a concentration of 25 μg/ml. Control slides were incubated with an equal concentration of sheep IgG (ChromPure; Jackson Immunoresearch), or with the specific antibody preincubated with a 100× molar excess of the immunogenic peptide. These were negative in all cases. Counterstaining was with light green SF.

## Results

*Transin mRNA expression in the synovium of rats with chronic SCW arthritis.* Fig. 1 (*top*) demonstrates that the 1.9-kb transin mRNA was detected readily in the synovial tissue of LEW/N rats with the chronic phase of SCW arthritis (lane *2*). In contrast, the synovial tissue from control animals expressed the message to a much lower degree (lane *1*), sevenfold less by densitometry. The bottom of the figure shows the ethidium bromide–stained 18S rRNA bands, confirming that equivalent amounts of RNA were applied to each lane. The finding of high-level transcription of the tumor-associated matrix-degrading metalloproteinase in the abnormal tissue compares with its similar skewed distribution in malignant tumors relative to benign tumors (24, 25).

*Synovial tissue levels of myc mRNA.* Presence of the 2.3-kb mRNA of the nuclear protooncogene, c-*myc,* is shown in Fig. 1 (*middle*). It was sixfold more abundant in SCW arthritic rat synovium versus normal synovium. This again suggests that the diseased synovium in invasive arthritis contains a population of rapidly proliferating and activated cells.

*Presence of the transin and myc transcripts in cultured synoviocytes.* Hyperplasia of the fibroblast-like mesenchymal cells of the synovial lining layer and underlying stroma is an invariable feature of clinical SCW arthritis (9, 10) and AA (12), as it is of RA (51). This has been demonstrated to be due to in situ proliferation of the synoviocytes (1–4, reviewed in reference 5), which, along with blood vessels and lesser numbers of macrophages, are the predominant cell populations at the interface of synovium with cartilage and bone (6, 18). To verify that synoviocytes synthesize transin and c-*myc* mRNAs, fibroblast-like cells from rats with SCW arthritis grown in secondary culture were analyzed for transin and c-*myc* mRNA expression. Fig. 2 demonstrates that cultured synoviocytes from the diseased tissue expressed the transin (*top*) and c-*myc* (*bottom*) mRNAs in the presence of serum. Since normal synoviocytes, when activated by agents such as phorbol esters or serum, may also be induced in vitro to express the transin/stromelysin and c-*myc* mRNAs (32, 35, 52, and results not shown), this implies that the synoviocyte is intrinsically able to express transin and c-*myc* when exposed to an appropriate growth factor milieu provided in vitro by serum. In vivo, synoviocytes in diseased joints are apparently exposed to the appropriate inducing factors. Synoviocytes in normal joints are not.

*Characterization of an antiserum to a COOH-terminal transin peptide.* To determine the specificity of the anti-peptide antiserum before its use in in situ localization of transin, immunoprecipitation of transin from the conditioned medium of phorbol ester–stimulated cultured SCW synoviocytes was carried out (Fig. 3). Affinity-purified 6/6F IgG specifically

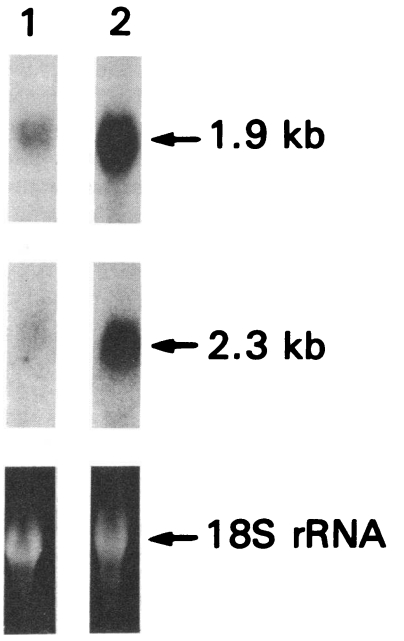



*Figure 1.* Detection of transin and c-*myc* mRNA in synovia from normal rats and rats with chronic SCW arthritis. Tissue RNA was size-separated on a formaldehyde Northern gel and transferred to a Nytran membrane, and nick-translated probes to transin and c-*myc* prepared and hybridized to the membrane sequentially as described in Methods. The resulting autoradiographs are shown. The top panels show the 1.9-kb transin message, sevenfold more abundant by densitometry in the arthritic rat synovium (lane *2*) than in the normal rat synovium (lane *1*). The middle panels show a sixfold greater abundance of the 2.3-kb c-*myc* message in arthritic (lane *2*) versus normal rat (lane *1*) synovia. The bottom panels show the ethidium bromide–stained 18S rRNA bands before transfer, verifying the application of equal quantities of RNA to each lane.

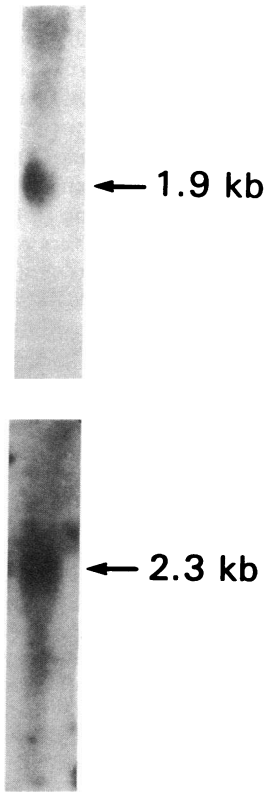



*Figure 2.* Detection of the transin and c-*myc* mRNAs in synoviocytes in vitro. RNA isolated from secondary culture SCW arthritis synovial fibroblast-like cells (see Methods) were analyzed by Northern gel. The autoradiograph reveals that transin (*top*) and c-*myc* (*bottom*) are readily detected during in vitro culture in serum-containing medium.

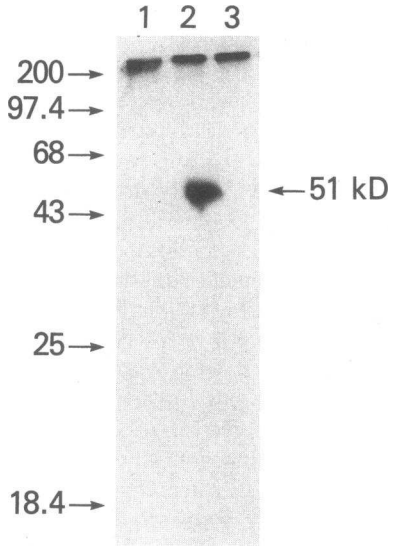



*Figure 3.* Affinity-purified 6/6F specifically immunoprecipitates transin from cultured synoviocytes. The [$^{35}$S]-methionine-labeled conditioned medium of primary culture synovial fibroblast-like cells grown in the presence of phorbol myristic acid ($10^{-8}$ M) was immunoprecipitated with control rabbit IgG (lane *1*), affinity-purified 6/6F IgG (lane *2*), or the specific IgG in the presence of a 100× molar excess of the immunogenic peptide (lane *3*) as detailed in Methods. The 51-kD band (*arrow*) corresponding to secreted protransin is precipitated only by the unblocked, specific antibody (lane *2*). Molecular weight markers are shown at left.

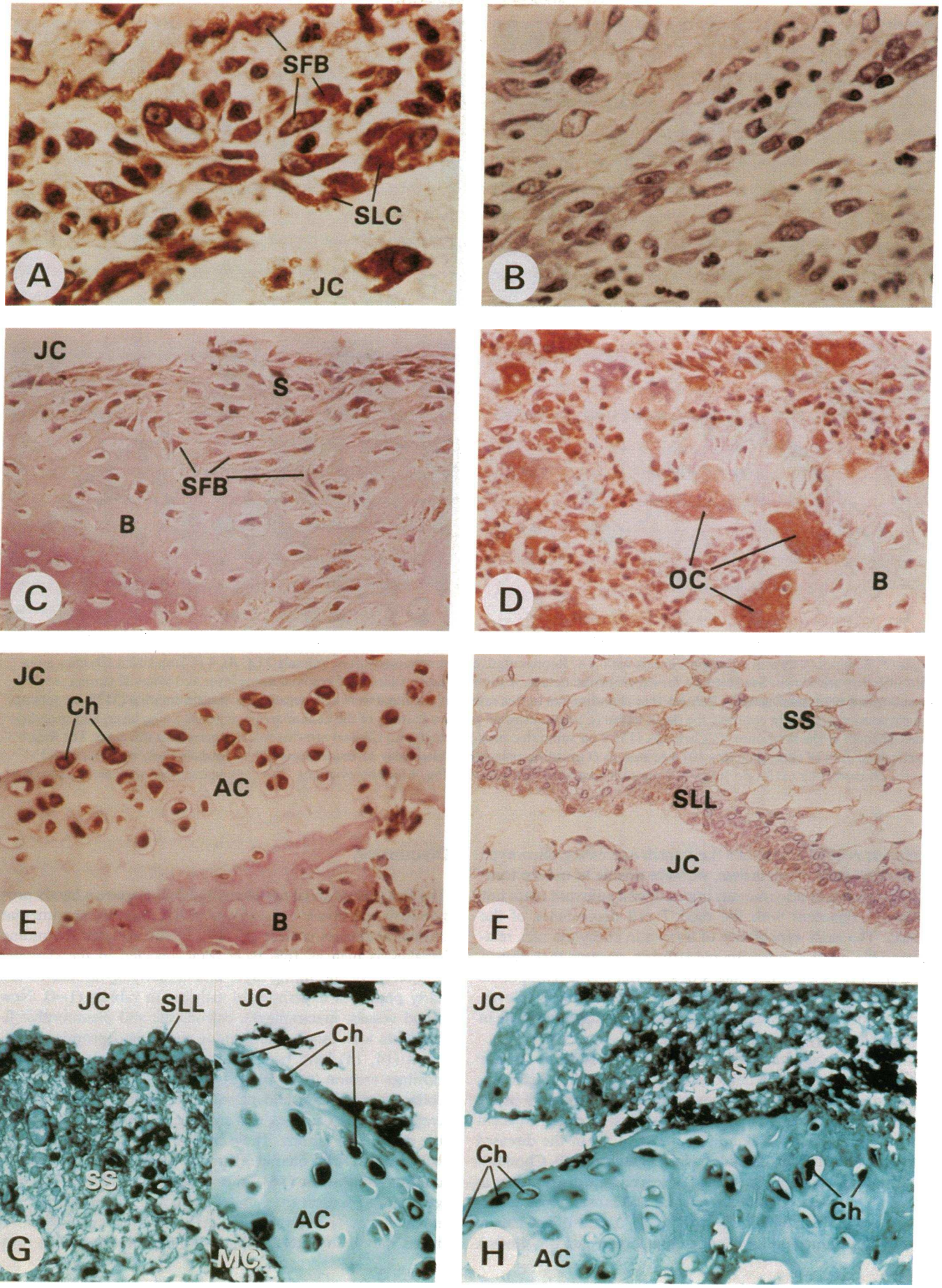
immunoprecipitated the 51-kD transin proenzyme. A single band was detected in the presence of the specific antibody (lane *2*), but was not observed in the control IgG immunoprecipitate (lane *1*), or when the specific antibody was blocked by prior incubation with the immunogenic peptide (lane *3*). In a separate experiment an irrelevant peptide failed to block the specific immunoprecipitation (data not shown). Identical results were obtained in a similar immunoprecipitation of *ras*-transformed normal rat kidney cells, consistent with the known induction of transin by transformation with that oncogene (23; data not shown).

*Immunolocalization of the transin protein in situ in SCW arthritis.* Formalin-fixed tissue sections from rats with chronic SCW arthritis demonstrated intense specific transin synovial staining (Fig. 4, *A–E*). Cells in the lining cell layer and synovial fibroblasts of the underlying stroma showed dark brown cytosplasmic staining (*A*) that was absent on the control slide (*B*). Spindle-shaped synovial fibroblasts at the bone-resorbing interface of synovium with bone showed similar staining (*C*). Transin was therefore present in synoviocytes at the sites of active tissue destruction in chronic arthritis. Osteoclasts similarly expressed the protein (*D*), as did chondrocytes in a section of articular cartilage not under active invasion in this chronic lesion (*E*). By contrast, the normal rat synovium exhibited absent to negligible staining in the synovial lining layer and underlying synovial stroma as shown in Fig. 4 *F*.

*Induction of the transin-expressing phenotype in SCW arthritis is thymus independent.* The arthritis induced by injection of an aqueous suspension of SCW fragments consists of two phases, a rapid-onset, acute, thymus-independent phase, and a later-developing chronic phase that requires thymus-derived lymphocytes (9, 10). In parallel with the development of acute-phase arthritis, transin expression was widespread and intense (Fig. 4 *G*). Black intracellular staining was detected in the synovium and cartilage, as demonstrated by this day 4 specimen. In addition, cellular elements within the bone marrow demonstrated specific, high-level staining (not shown). Similarly, athymic Lewis rats, which develop a severe acute, but markedly blunted chronic-phase arthritis, showed high-level transin staining of synoviocytes, cartilage, and bone marrow by day 2 (Fig. 4 *H*), which subsequently declined with time (not shown). These results demonstrated that transin expression in the SCW-induced arthritis model closely paralleled clinical arthritis during the thymic independent and dependent phases.

*Kinetics of transin expression in AA.* The injection of a suspension of heat-killed mycobacteria in mineral oil induces chronic, destructive peripheral arthritis in LEW/N rats that appears clinically ~ 10 d after adjuvant injection (12, 53, 54). The lesion is considered to be fully T cell–mediated and may be passively transferred by lymphocytes (53, 55, 56). To determine when high-level transin expression was first detectable in AA, rats were killed in pairs at 2-d intervals after injection of adjuvant and examined histologically (Fig. 5 *A–D*). Intense transin staining in chondrocytes and synoviocytes was first observed 4 d after adjuvant injection (*C*), where chondrocytes adjacent to positively-staining synovial lining cells, but not distant chondrocytes, were positive. Synovial hyperplasia was not observed at this time, nor was encroachment of cartilage or bone by synoviocytes. As in the SCW model, the bone marrow showed intense, specific staining. By contrast, in day 2 animals (*A, B*) or earlier (data not shown), transin staining was not demonstrated. Day 12 animals with synovial hyperplasia and early macroscopic arthritis exhibited high-level transin staining in synovial cells and in the chondrocytes of cartilage adjacent to encroaching synovium (*D*). Since the influx of mononuclear cells occurs with the onset of clinical arthritis (12; data not shown), the detection of transin as early as 4 d after adjuvant injection strongly implied that local, intraarticular T cells

*Figure 4.* In situ localization of transin in synovia from normal rat synovium and rats with acute and chronic SCW arthritis. Formalin-fixed specimens were stained immunohistochemically with affinity-purified anti-transin as described in Methods. *A–E,* Transin staining in chronic SCW arthritis. Brown cytoplasmic staining was evident in synovial lining cells (*SLC*) and spindle-shaped synovial fibroblasts (*SFB*) in *A. JC,* joint cavity. Control slide *B* was incubated with control antibody and is negative (magnification 782× on submitted photomicrographs). *C* demonstrates that spindle-shaped fibroblast-like cells interfacing with bone stained positively (313× on submitted photomicrograph). *S,* Synovium; *B,* bone. *D* shows osteoclasts (*OC*) adjacent to bone, also stained strongly for transin (313× on submitted photomicrograph). *E* shows articular cartilage, where chondrocytes expressed intense cytoplasmic transin staining (313× on submitted photomicrograph). *AC,* Articular cartilage; *Ch,* chondrocytes. Normal rat synovium (*F*) showed negligible transin staining of the synovial lining layer (*SLL*) and underlying synovial stroma (*SS;* 313× on submitted photomicrograph). *G,* which was developed in the presence of nickel chloride as described in Methods (*left*) demonstrates black cytoplasmic staining of the lining layer and stroma in a specimen from a rat with acute SCW arthritis (day 4). *G* (*right*) demonstrates cytoplasmic staining of chondrocytes and marrow cavity in a section of articular cartilage from the same animal. *MC,* Marrow cavity. (313× on submitted photomicrograph.) Similar results in an athymic rat (*H,* stained in the presence of nickel chloride) with acute SCW arthritis (day 2 lesion depicted) demonstrated that transin expression in this model is T cell independent (313× on submitted photomicrograph). The counterstain in *A–F* was aqueous hematoxylin; in *G* and *H,* light green SF. See Methods for details.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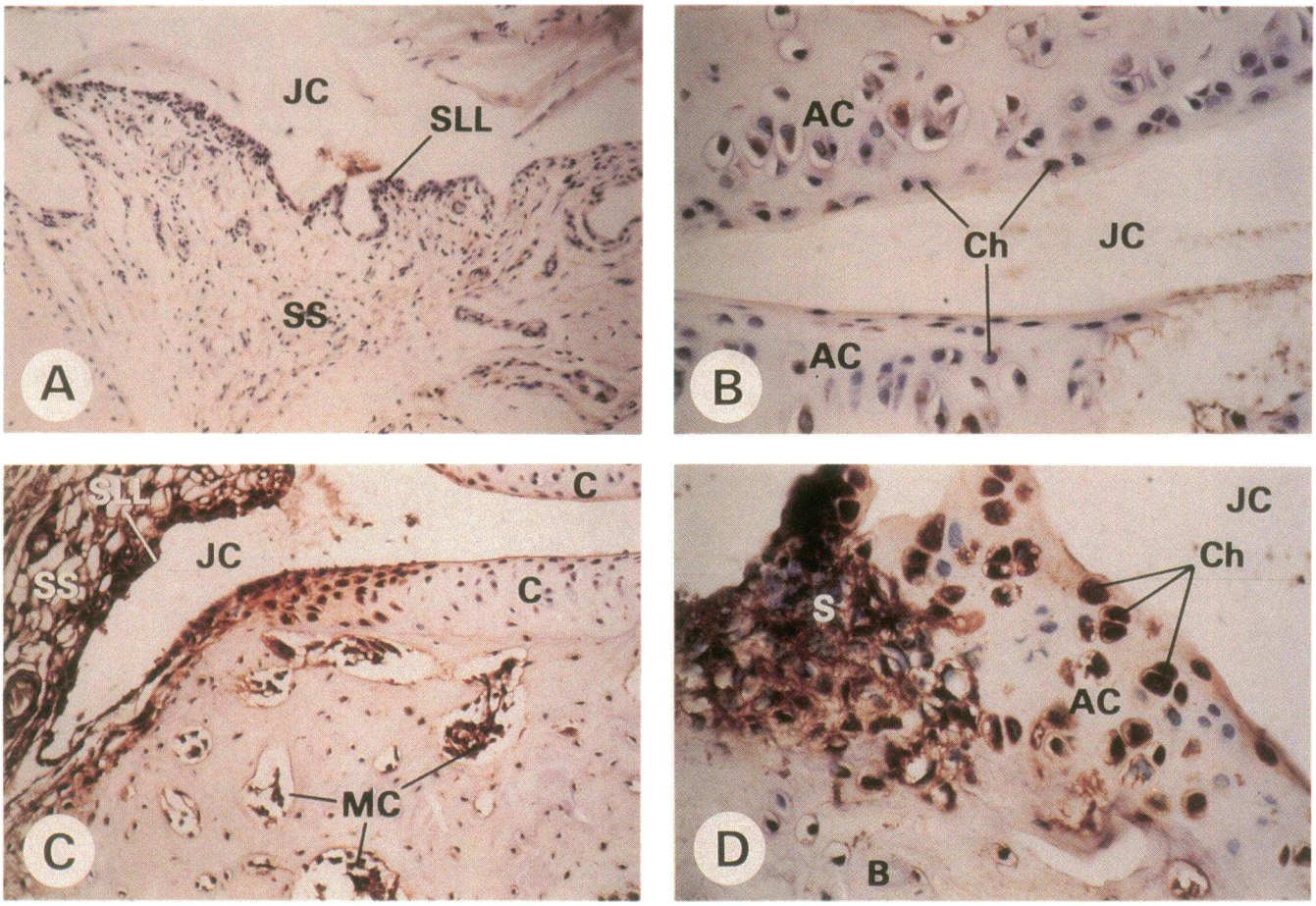


*Figure 5.* Kinetics of transin expression in rats with AA. Representative joint specimens from animals 2 (*A, B*), 4 (*C*), and 12 d (*D*) after adjuvant injection are shown. See Methods for experimental details. *A* shows negative staining of synovial lining layer and synovial stroma (155× on submitted photomicrograph). *B* shows that chondrocytes in this same day 2 animal were also negative for transin staining (313× on submitted photomicrograph). In contrast, the day 4 lesion (*C*) demonstrated positive staining of the bone marrow, synovial lining layer, and chondrocytes adjacent to the synovium. *C,* Cartilage. (155× on submitted photomicrograph.) Clinical arthritis was not present at this time. The hyperplastic synovium of clinical (≥ day 10) AA, seen in *D* invading articular cartilage (day 12 lesion depicted), stained similarly positive, as did nearby chondrocytes (313× on submitted photomicrograph). See legend to Fig. 4 for abbreviations. The counterstain was aqueous hematoxylin.

were not necessary to evoke the transin-expressing phenotype within the joint. Nonetheless, we were unable to induce transin expression in athymic rats injected with adjuvant (data not shown), consistent with the rigid T cell dependence of the lesion. The early appearance of transin in euthymic rats, relative to the onset of macroscopic arthritis, suggests that transin is a marker of the early stages of synovial cell activation. Its appearance preceded proliferation and hyperplasia, the development of clinical disease, and the loss of cartilage and bone in these genetically susceptible animals.

*Relative abundance of the nuclear Myc antigen in chronic SCW synovium.* Fig. 6 demonstrates widespread, specific nuclear staining for Myc in the synovial lining layer and in the underlying stroma (*A*), and in the fibroblast-like cells adjacent to an eroding edge of bone (*C;* cf. Fig. 4 *C*) in the diseased tissue. Control antibody slides were negative (*B, D*). Chondrocytes and osteoclasts also stained positively; the distribution, in general, paralleled that of transin and was most intense at sites of active or incipient joint erosion. In contrast, normal rat synovium stained diffusely and at low levels (data not shown).

## Discussion

The fact that synovial mesenchymal cells acquire a highly proliferative and invasive phenotype in RA and the experimental animal analogues is clear (13–19). In situ tritiated thymidine uptakes as high as 16% have been detected in the synovial fibroblasts of the lining layer and underlying stroma in the early phases of experimental arthritis in rabbits (1–4). New blood vessels, macrophages, osteoclasts, and pleomorphic fibroblasts with large, pale-staining nuclei and prominent nucleoli (14, 18) have been detected at the sites of bone and cartilage erosion (6, 7, 14, 18). Degradative enzymes such as collagenase (57–59) and plasminogen activator (60) are secreted in high quantities, and are believed pivotal in producing the destructive lesions. These in situ observations, combined with such in vitro characteristics as rapid growth, absence of contact inhibition (18), and anchorage independence of growth (19) suggest that, under the conditions of disease, the normally quiescent mesenchymally derived synovial fibroblasts may exhibit dysregulated proliferation and local inva-

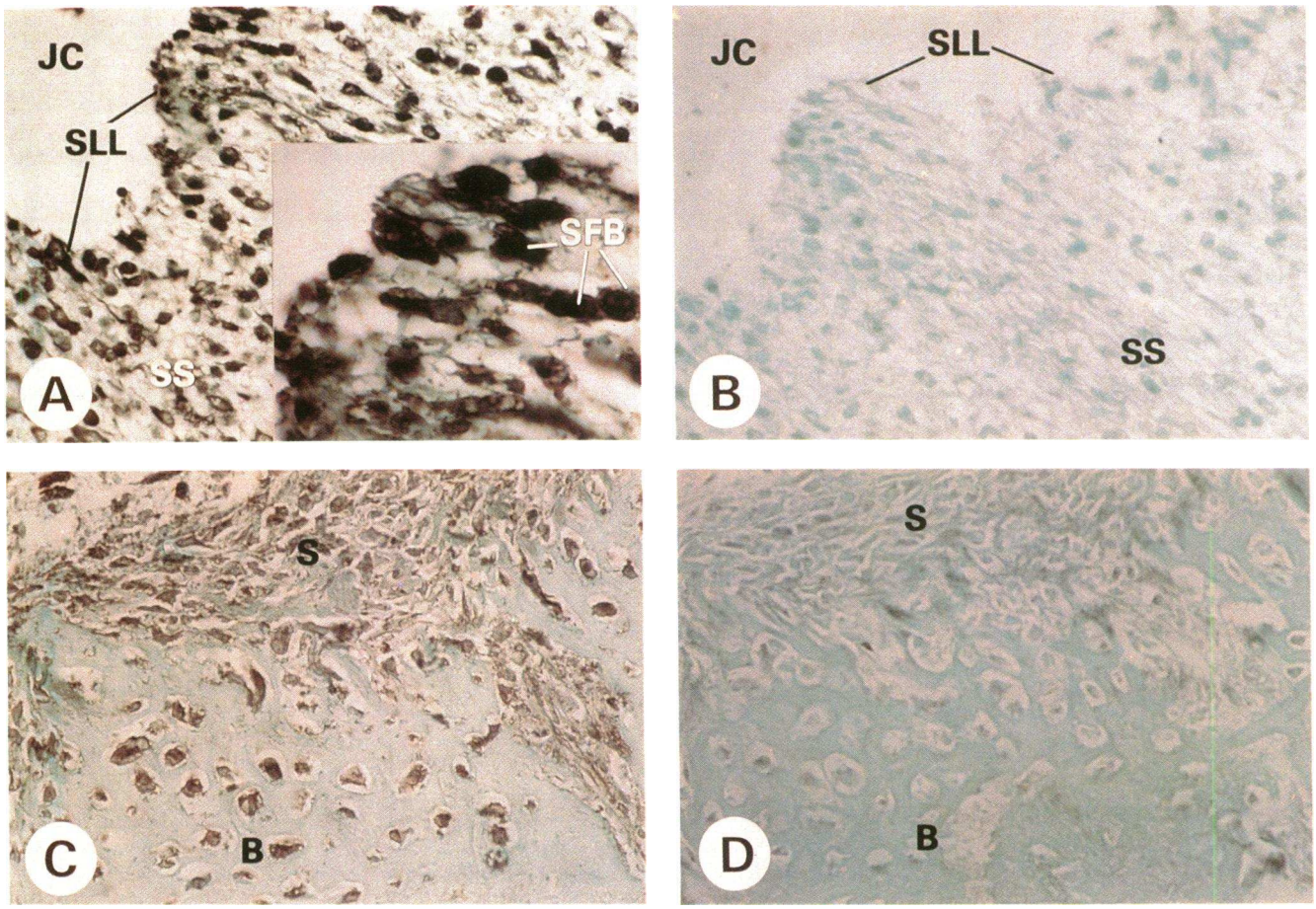


*Figure 6.* Myc immunostaining in rats with chronic SCW arthritis. *A* shows intense brown nuclear staining of cells in the lining layer and underlying stroma (313×; *inset,* 782× on submitted photomicrograph). Control slide *B* is negative. *C* is a section similar to that depicted in Fig. 4 *C,* showing that fibroblast-like cells at an erosive margin with bone expressed Myc (313× on submitted photomicrograph). The presence of some cytoplasmic staining in this section is attributed to fixation artifact (39). *D,* the antibody control, is negative. The counterstain was light green SF. See legend to Fig. 4 for abbreviations and Methods for experimental details.

siveness, i.e., properties reminiscent of transformed cells. Clearly not malignancies, the invasive arthropathies are not autonomous, but are driven by paracrine factors generated in the inflammatory milieu of the arthritic joint (18, 19, 61). In contrast to malignant tumor cells, the abnormal phenotype is reversible. For example, upon extended passage in culture synovial fibroblasts lose the capacity to grow under anchorage-independent conditions (19), the best in vitro correlate of the transformed phenotype.

Perhaps not unexpectedly the expression of transin/stromelysin, a metalloproteinase highly associated with invasive tumors, was found in high levels in proliferative and invasive synovia. The nuclear oncoprotein c-Myc was similarly highly expressed. Transcription of the c-*myc* gene is associated with cellular activation and proliferation in normal and malignant cells (38). Transin expression was not restricted to the fibroblast-like cells. It was also present in osteoclasts as well as in chondrocytes, particularly those juxtaposed to sites of active joint resorption (Fig. 4 *G* and 5 *D*). Indeed, transin expression was detected in chondrocytes before synoviocyte proliferation and invasion, but adjacent to synovial lining cells that similarly expressed transin intensely (Fig. 5 *C*). As well as implying a possible paracrine role for the synovium in activating chondrocytes, the time course and distribution of transin expression suggested that it is possibly one of the earliest biochemical markers of synoviocyte activation/transformation in these invasive arthropathies. Transin expression by chondrocytes probably indicates active/incipient cartilage resorption. Chondrocytes secrete transin/stromelysin in vitro (62). The biochemical characterization of transin/stromelysin as an important mediator of matrix degradation (29, 30, 32, 33), its close association with malignant transformation (23–25), and its marked expression in invasive arthritis, as demonstrated here, suggest that it is a valuable biochemical marker for cellular transformation and activation in invasive arthropathies as in malignant tumors.

Transin was detected at low levels in normal synovium and cartilage. Since it is present in normal skin (26) and synovium, it probably plays a role in the physiologic regulation of connective tissue matrix degradation and remodeling. Indeed, since cultured synoviocytes from normal as well as arthritic animals expressed transin and c-*myc* mRNA, it appears that in vitro culture in serum exposes the cells to growth factors that can induce transin/stromelysin and c-*myc* expression (22, 23, 32, 63). The factors responsible for their aberrant, pathologic expression in situ in diseased animals are as yet incompletely

defined, but presumably are a consequence of the inflammatory process (61). Similarly, synoviocytes from normal as well as diseased rats will grow under anchorage-independent conditions under the appropriate growth factor influence, e.g., platelet-derived growth factor (19). These observations are consistent with our contention that in situ synoviocyte transformation is contingent on exogenous factors to which, in the case of disease, the native, quiescent synoviocyte is exposed. Serum, a complex mixture of cytokines and growth factors, provides transin- and c-*myc*-inducing factors not available to the native synoviocyte in the normal animal, which lacks the inflammatory milieu.

Since transin expression in situ appeared early during the thymus-independent phase of SCW arthritis in both euthymic and athymic Lewis rats, our data clearly demonstrate that, under certain circumstances, synoviocyte and chondrocyte transin expression can proceed both spatially and temporally independent of T cell immunocompetence. Since the initial histopathological lesion in SCW arthritis is cell wall–induced microvascular injury (9), the data provide more support for the concept that serum-, platelet-, or blood vessel–derived growth factors induce initial transin expression in synovial mesenchymal cells. Our data also demonstrate that the thymus and T cell immunocompetence are required to fully stimulate and perpetuate transin expression. Additional insights were generated with studies of AA, which is widely believed to be a completely thymus-dependent lesion (53, 55, 56). It has been clearly demonstrated that adjuvant injected intradermally is distributed widely and rapidly (within hours) throughout the body, including deposition into the joints (64, 65). Nonetheless, antigen distribution to the draining lymph nodes has been shown necessary for establishment of systemic disease, since removal of these nodes before day 5 ablates disease development (66, 67). This is about the time when delayed-type hypersensitivity to mycobacterial antigens develops (68). In light of these observations, our detection of synovial transin expression as soon as 4 d after adjuvant injection in euthymic but not athymic rats suggests that systemic factors, possibly elaborated by proliferating lymphocytes in regional lymph nodes (66), initiate transin expression in the joints. As in the SCW model, the biochemical synovial abnormality required thymus-dependent lymphocytes to fully induce and sustain transin expression in parallel with the development of clinical AA on or around day 10.

The relevance of animal models such as SCW arthritis and AA in understanding the mechanisms and possible avenues for therapy in human arthropathies, such as RA, is, of course, problematic. Many agents of great benefit in treating RA are also effective in these animal models. It is of interest, therefore, that retinoids have been effective in treating both SCW and AA (69, 70) and are major downregulators of transin/stromelysin expression (32). Moreover, they reverse other parameters of the transformed phenotype, such as anchorage independence of growth (19). Since transin/stromelysin expression is one of the earliest events in the animal models, this further supports the view that the biochemical mechanisms involved in connective tissue destruction are operative very early in the course of inflammatory joint diseases such as RA. These observations support the need for early definitive treatment with agents that suppress the expression of the proliferative and invasive phenotype. Retinoids or other agents that promote cell and tissue differentiation may represent a rational therapeutic approach toward this goal.

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