SEROSAL MAST CELLS MAINTAIN THEIR VIABILITY AND PROMOTE THE METABOLISM OF CARTILAGE PROTEOGLYCANS WHEN COCULTURED WITH CHONDROCYTES

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Objective. **To determine the consequences of mast cell (MC)–chondrocyte interactions.**

Methods. **Cocultured cells were analyzed histochemically, morphologically, biochemically, and functionally.**

Results. **Cocultured MC adhered to the chondrocytes and remained viable. Chondrocytes cocultured with nonactivated MC produced more proteoglycans than did chondrocytes cultured alone, and these proteoglycans possessed an intact hyaluronic acid–binding region. In contrast, most of the proteoglycans produced by chondrocytes cocultured with activated MC were degraded.**

Conclusion. **These studies indicate that a complex interaction occurs in which the nonactivated MC stimulates biosynthesis and the activated MC degrades cartilage proteoglycans.**

Mast cells are often present in increased numbers at sites of active cartilage erosion in the joints of patients with rheumatoid arthritis (1–6). The detection of free histamine in the rheumatoid joint (5) and the demonstration of anti-IgE–induced histamine release from synovial mast cells (6) indicate that these mast cells are immunologically competent. Because serine proteases derived from rat serosal mast cells (SMC) degrade fibronectin (7) and type IV collagen (8) and because tryptase derived from human mast cells activates the metalloproteinase stromelysin (9), the mast cell has been implicated in the catabolism of the extracellular matrix of cartilage in rheumatoid arthritis. However, a precise understanding of the role of mast cells in this disease has been hindered by the presence of other types of inflammatory cells in the rheumatoid joint, the lack of an in vitro model system for studying the participation of mast cells in this disorder, and the uncertainty of the phenotype of the mast cells in the diseased tissue.

The discovery that rat SMC can be maintained ex vivo for at least 30 days when cocultured with mouse 3T3 fibroblasts or with rat osteoblast-like cells, but not with mouse keratinocytes, mouse macrophages, or bovine endothelial cells (10,11) suggests that mesenchymal cells may specifically promote the viability of the SMC-like population of mast cells. A complex reciprocal interaction occurs during coculture of mouse bone marrow–derived mast cells and 3T3 fibroblasts, in which the phenotype of the mast cell is dramatically altered histochemically, morpho-

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logically, biochemically, and functionally (12–15). The cocultured fibroblasts also undergo morphologic changes. They increase their plasma membrane expression of globopentaosylceramide, multiply, and produce more type I collagen (13,16,17).

We have cocultured rat SMC with Swarm rat chondrosarcoma chondrocytes to determine whether chondrocytes would supply a microenvironment sufficient to maintain the viability and phenotype of SMC, and whether SMC would affect the metabolism of chondrocyte proteoglycans. The Swarm rat chondrosarcoma chondrocyte has been used extensively for biochemical and molecular biology studies of the proteoglycans (18–21), collagens (22), and other proteins (23,24) that make up the extracellular matrix of cartilage. This cell is carried as a nonmetastatic solid tumor in rats, and $>10^8$ chondrocytes can be routinely obtained from one tumor-bearing animal. Conditions for culturing this cell in the presence (19) and absence (20) of serum have been developed. We demonstrate that Swarm rat chondrosarcoma chondrocytes maintain the viability and phenotype of a significant proportion of rat SMC when the two cell types are cocultured for 7 days; furthermore, the mast cell can stimulate either biosynthesis or degradation of chondrocyte proteoglycans, depending upon whether the mast cell is activated.

MATERIALS AND METHODS

Culture of rat SMC with rat chondrosarcoma chondrocytes. SMC were obtained under sterile conditions from Sprague-Dawley rats (Jackson Laboratory, Bar Harbor, ME) (10). Modified Tyrode's buffer (Tyrode's buffer containing 0.1% [weight/volume] gelatin [Sigma, St. Louis, MO], 100 μg/ml of penicillin, 100 μg/ml of streptomycin, and 20 μg/ml of gentamicin) was sterilized by filtration through a 0.45-μm filter. The abdominal skin was removed from each rat, ~60 ml of modified Tyrode's buffer was injected into the peritoneal cavity, and the peritoneal wash was aspirated. All subsequent steps were carried out under sterile conditions in a laminar flow hood.

The peritoneal fluid was centrifuged at 200*g* for 10 minutes. The supernatant was removed, and the cells were resuspended at a density of $\sim 10^8$ cells/ml in modified Tyrode's buffer. One-milliliter samples were layered on top of 2 ml of 22.5% (w/v) metrizamide (Nyegaard, Oslo, Norway) in modified Tyrode's buffer, and rat SMC were concentrated by sedimentation at 500*g* for 15 minutes (25). Approximately 1×10^6 SMC (95–99% purity) were obtained per animal.

Swarm rat chondrosarcoma chondrocytes were obtained after a trypsin–collagenase digestion of the solid tumor, as previously described (19). Chondrocytes (1–2 million) were suspended in 2 ml of enriched medium (Dulbecco's high-glucose modified Eagle's medium supplemented with 0.25% [w/v] bovine serum albumin, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 15 m*M* HEPES, 10 m*M* *N*,*N*-bis[2-hydroxyethyl]-2-aminoethane sulfonic acid, 10 m*M* *N*-tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid, and 15% fetal calf serum [Gibco, Grand Island, NY]) and cultured in 35-mm plastic culture dishes. For the electron microscopy studies described below, chondrocytes were seeded into 35-mm culture dishes that contained or lacked a Thermanox coverslip (Miles, Naperville, IL).

Chondrocyte cultures were maintained for 24–48 hours at 37°C in a humidified atmosphere of 6% CO_2 to ensure that no contaminating cells were present and that the chondrocytes had recovered from the proteolytic isolation procedure. The culture medium was replaced daily. For most experiments, the purified rat SMC were suspended in enriched medium, and 2-ml portions of the cell suspensions were seeded into separate 35-mm culture dishes containing the confluent layer of rat chondrocytes that had been plated 24–48 hours earlier (final SMC:chondrocyte ratio 1:2).

Mast cell/chondrocyte cocultures were maintained at 37°C in a humidified atmosphere of 6% CO_2 for up to 7 days. The culture medium was replaced every 24 hours with 2 ml of fresh enriched medium. At the end of the coculture in some experiments, the chondrocytes and adherent SMC were detached from the culture dish and separated from one another by treating the cocultures with 0.05% trypsin in calcium-free and magnesium-free Hanks' balanced salt solution for 10 minutes at 37°C. To distinguish the two cell types at the light microscopic level, the cell suspension was incubated with alcian blue followed by Safranin (26,27), two cationic dyes that preferentially stain SMC. The mast cells and chondrocytes were enumerated with a hemacytometer. The viability of the nonadherent mast cells, the adherent mast cells, and the chondrocytes was determined by trypan blue exclusion.

Electron microscopy. SMC/chondrocyte cocultures were prepared for electron microscopy by two different techniques. In the first method, the cocultured cells in 35-mm culture dishes were fixed by the addition of an equal volume of mixed aldehydes (1.5% formaldehyde and 2% glutaraldehyde in 0.1*M* cacodylate buffer, pH 7.4) for 2 hours at room temperature. Cells were kept overnight at 4°C in 0.1*M* cacodylate containing 7% sucrose, postfixed in acetate–veronal buffered OsO_4 for 2 hours at 4°C, stained en bloc with uranyl acetate, dehydrated in graded alcohols and glycol methacrylate, and embedded in Epon 812 (28,29). After polymerization, the hardened plastic samples were removed and cut into pieces, which were then were reembedded and sectioned.

The second method examined cocultures on Thermanox coverslips. Cocultured cells were fixed as above, but were dehydrated in ethanol and propylene oxide before being embedded in Epon 812. The coverslips were embedded face down on a resin-filled well. After polymerization, the coverslips were removed by plunging the blocks into liquid N_2. The plastic was cut and reembedded before sectioning. Silver thin sections were cut on diamond knives, picked up on naked copper grids, stained with uranyl acetate and lead citrate, coated with carbon, and examined with a JEOL 100C/ASID electron microscope operating at 80 kV.

Proteoglycan analysis. Rat chondrosarcoma chondrocytes cultured for 7 days in the presence or absence of rat

SMC were incubated for 2 hours at 37°C in enriched media containing 50 μCi/ml (1.85×10^6 Bq/ml) of ^{35}S-sulfate (New England Nuclear, Boston, MA). The supernatants were removed, and the cell layers were incubated with 150 μl of 1% Zwittergent 3-12 (Calbiochem-Behring, La Jolla, CA) containing 0.1*M* 6-aminohexanoic acid, 0.1*M* EDTA, 5 m*M* benzamidine HCl, 1 m*M* sodium iodoacetamide, 1 m*M* phenylmethylsulfonyl fluoride, 0.1*M* sodium acetate, pH 6.0, for ~1 minute, followed by 1.35 ml of 4*M* guanidine HCl, 0.1*M* sodium sulfate, and 0.1*M* Tris HCl, pH 7.0 (GST buffer) (18–20). The amount of ^{35}S-sulfate incorporated into proteoglycans was determined by chromatographing samples of each supernatant and cell layer extract on replicate 10-ml Sephadex G-25 columns equilibrated in GST buffer.

Because rat SMC synthesize protease-resistant, heparin proteoglycans of $\sim 7.5 \times 10^5$ M_r (25,30) and chondrocytes synthesize protease-sensitive, chondroitin sulfate proteoglycans of $\sim 2 \times 10^6$ M_r (18,19), the hydrodynamic sizes of the released and cell layer–associated ^{35}S-labeled proteoglycans were determined, as well as their sensitivity to Pronase, chondroitinase ABC, and heparinase. For glycosaminoglycan analysis, replicate samples of each preparation were incubated with chondroitinase ABC (Miles) (31) or heparinase (Miles) (32), and the digests were chromatographed on Sephadex G-25 columns, as described above. The protease sensitivities of the ^{35}S-labeled proteoglycans were determined after incubation of 25-μl samples for 1 hour at 37°C in 25 μl of enriched medium containing or lacking 25 μg of Pronase (Calbiochem-Behring) (33). Each Pronase digest was chromatographed under dissociative conditions on a 1 × 100-cm column of Sepharose CL-2B (Pharmacia, Piscataway, NJ) equilibrated in GST buffer; 0.5-ml fractions were collected and analyzed for radioactivity.

When Swarm rat chondrosarcoma chondrocytes are cultured by themselves, they continuously exocytose into the culture medium their ^{35}S-labeled proteoglycans (19). These exocytosed proteoglycans are bound to hyaluronic acid as macromolecular aggregates, possessing a hydrodynamic size of $\sim 5 \times 10^8$ M_r (19). Samples of the ^{35}S-labeled proteoglycans released into the supernatants by rat chondrocytes cultured in the absence and presence of rat SMC were chromatographed on a 1 × 100-cm column of Sepharose CL-2B equilibrated under the associative conditions of 0.5*M* sodium acetate, pH 7.0, to determine if the released ^{35}S-labeled proteoglycans were bound to hyaluronic acid.

Treatment of cocultured rat SMC with compound 48/80. Adherent SMC that had been cocultured with chondrosarcoma chondrocytes for 4–7 days were washed twice with 2 ml of Tyrode's buffer containing 1.8 m*M* $CaCl_2$, 0.2 m*M* $MgSO_4$, and 0.1% w/v gelatin (Sigma), and were then incubated for 10 minutes with buffer containing 1.5 μg/ml of the mast cell degranulating reagent, compound 48/80 (Sigma) (34). Both the mast cells and chondrocytes remained viable following treatment with compound 48/80, as assessed by their continued exclusion of trypan blue dye. Adherent cocultured SMC were activated with compound 48/80 rather than with anti-IgE because of the magnitude of the activation–secretion response of rat SMC to this pharmacologic reagent. The amount of histamine released into the culture medium and the amount that remained associated with the cell layer after SMC activation were determined with a radioenzymatic assay (35). The net percent release of histamine was calculated by subtracting the amount of histamine released spontaneously from the amount of histamine released by cocultured SMC activated with compound 48/80, dividing this value by the total amount of histamine in the coculture, and multiplying the result by 100.

Replicate cocultures were radiolabeled with ^{35}S-sulfate, as described above, before being challenged with compound 48/80. The ^{35}S-labeled chondrocyte-derived proteoglycans that were released into the supernatants and the ^{35}S-labeled chondrocyte-derived proteoglycans that remained associated with the cell layers after treatment of the cells with compound 48/80 were quantified by Sephadex G-25 chromatography. The overall hydrodynamic sizes of the chondrocyte proteoglycans released into the supernatant during these SMC activations and their ability to interact with hyaluronic acid were assessed by chromatographing samples of the supernatants on separate Sepharose CL-2B columns that had been equilibrated under dissociative and associative conditions, respectively.

Culture of bovine cartilage with rat SMC lysates. Bovine cartilage was cultured in the absence and presence of rat SMC to determine if SMC-derived proteases would induce the degradation and release of proteoglycans when they were immobilized in the extracellular matrix of cartilage. Purified rat SMC were suspended in enriched medium, and then were frozen and thawed 7 times to disrupt the mast cells. Alternatively, SMC were incubated with compound 48/80 as described below, the activated SMC were centrifuged at 150*g* for 5 minutes, and the granule core–enriched supernatants were separated from the cell pellets. Disks (~1 mm in thickness and 8 mm in diameter) of living or of dead (frozen and thawed) bovine nasal septum cartilage were cultured (36–38) for up to 8 days in 1.5 ml of enriched medium containing lysates or granule core–enriched supernatants from 10^6 SMC. The medium was harvested at days 4 and 8 of the culture, and the proteoglycans that were released from the extracellular matrix of the cartilage were quantified with a spectrophotometric microassay (39).

In some experiments, SMC lysates either were boiled for 10 minutes or were incubated for 30 minutes at 37°C with 1 m*M* diisopropyl fluorophosphate (Sigma) before being added to the dead cartilage. *Salmonella typhosa* lipopolysaccharide, by itself, induces living bovine chondrocytes, but not dead chondrocytes, to increase their breakdown of cartilage (38). As a control in each experiment, replicate disks of living bovine cartilage were cultured in 1.5 ml of enriched medium alone or containing 25 μg of this endotoxin (Sigma). The cartilage proteoglycans released into the culture medium were quantified at days 4 and 8.

Statistical analysis. Results are expressed as the mean ± SD. The significance of the difference between the means was evaluated by Student's 2-tailed *t*-test.

RESULTS

Coculture of rat SMC and rat chondrosarcoma chondrocytes. When purified rat SMC were cocultured for 7 days with rat chondrosarcoma chondrocytes, 28

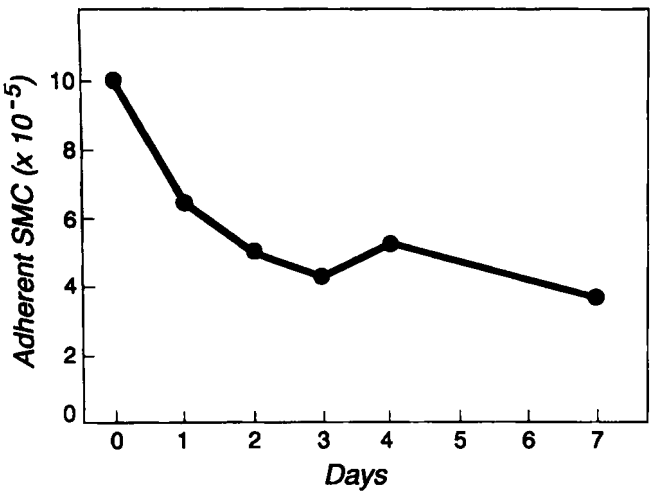

Figure 1. Results of a representative experiment showing quantitation of viable, adherent serosal mast cells (SMC) during 7 days of coculture with a confluent layer of Swarm rat chondrosarcoma chondrocytes after dispersion of the cells with trypsin. Values are the mean from duplicate cultures. The greatest number of viable, adherent SMC at day 7 of coculture was obtained in this experiment, as opposed to the 3 other experiments.

± 9% (mean ± SD, n = 4) of the mast cells adhered to the layer of chondrocytes (Figure 1). The other ~70% of the seeded SMC were nonadherent and thus were removed during the culture medium changes that were done every 24 hours. Both the adherent and the nonadherent SMC were viable, as assessed by their exclusion of trypan blue dye. As can be seen in Figure 1, there was no dramatic change in the number of adherent SMC during days 2–7 of the coculture.

The secretory granules of the entire population of SMC were Safranin positive throughout the coculture period (Figure 2). Although the numbers of chondrocytes increased in cultures in the presence and absence of SMC, at day 7, there was no significant difference between the numbers of chondrocytes in both cultures. SMC and chondrocytes were the only cell types detected in the day-1 to day-7 cultures.

Ultrastructure of cocultures. Because the SMC were extremely scarce relative to the chondrocytes in the cocultures from day 4 and day 7, only a few cells were examined despite extensive sampling of the cocultures. SMC were seen on the outer surface of the layer of chondrocytes or were (apparently) unattached in the culture medium (Figure 3A). They were not seen between the chondrocytes and the plastic culture dish, and thus did not appear to migrate into the culture. SMC that contacted the chondrocytes appeared to do so via microvilli or microplicae (Figure 3B). The ultrastructure of the cocultured mast cells resembled that of freshly isolated SMC, and although a few granules had decreased electron density, the SMC appeared fully granulated.

Biochemical characterization of the ^{35}S-labeled proteoglycans produced by cocultured chondrocytes. Rat chondrosarcoma chondrocytes exposed to rat SMC for 2 hours incorporated as much ^{35}S-sulfate into proteoglycans as did the control cultures. However, chondrocytes cocultured for 24 hours with SMC incorporated 58 ± 11% more (mean ± SD, n = 3; $P < 0.05$) ^{35}S-sulfate into proteoglycans than did the corresponding chondrocytes cultured without SMC. Chondrocytes cocultured for 7 days with SMC incorporated 45 ± 18% more (mean ± SD, n = 6; $P < 0.005$) ^{35}S-sulfate into proteoglycans than the chondrocytes cultured without SMC. The chondrocytes cultured alone for 7 days spontaneously released 23 ± 12% (mean ± SD, n = 6; $P < 0.01$) of their total ^{35}S-labeled proteoglycans into the culture medium during the 2-hour pulse with ^{35}S-sulfate, whereas chondrocytes cocultured for 7 days with SMC spontaneously released only 8 ± 2% of their ^{35}S-labeled proteoglycans. The released and cell layer–associated ^{35}S-labeled proteoglycans from the

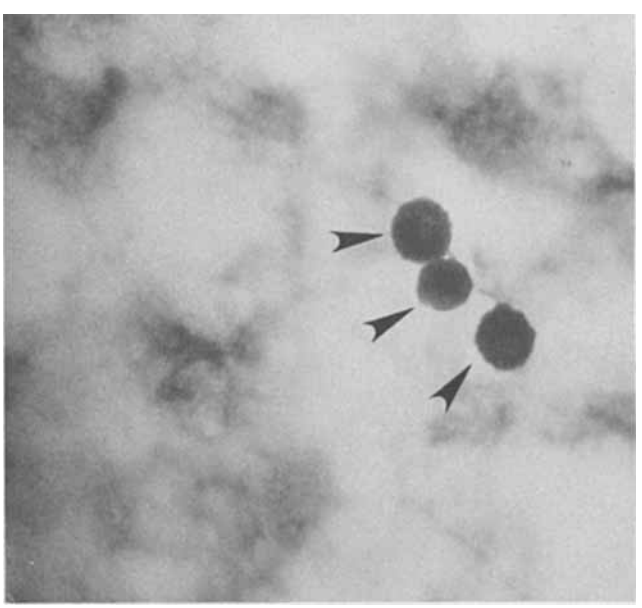

Figure 2. Histochemistry of cocultured rat serosal mast cells (SMC). Rat SMC that had been cocultured with rat chondrosarcoma chondrocytes for 7 days were stained with alcian blue and then with Safranin. **Arrows** show 3 Safranin-positive SMC (original magnification × 900).

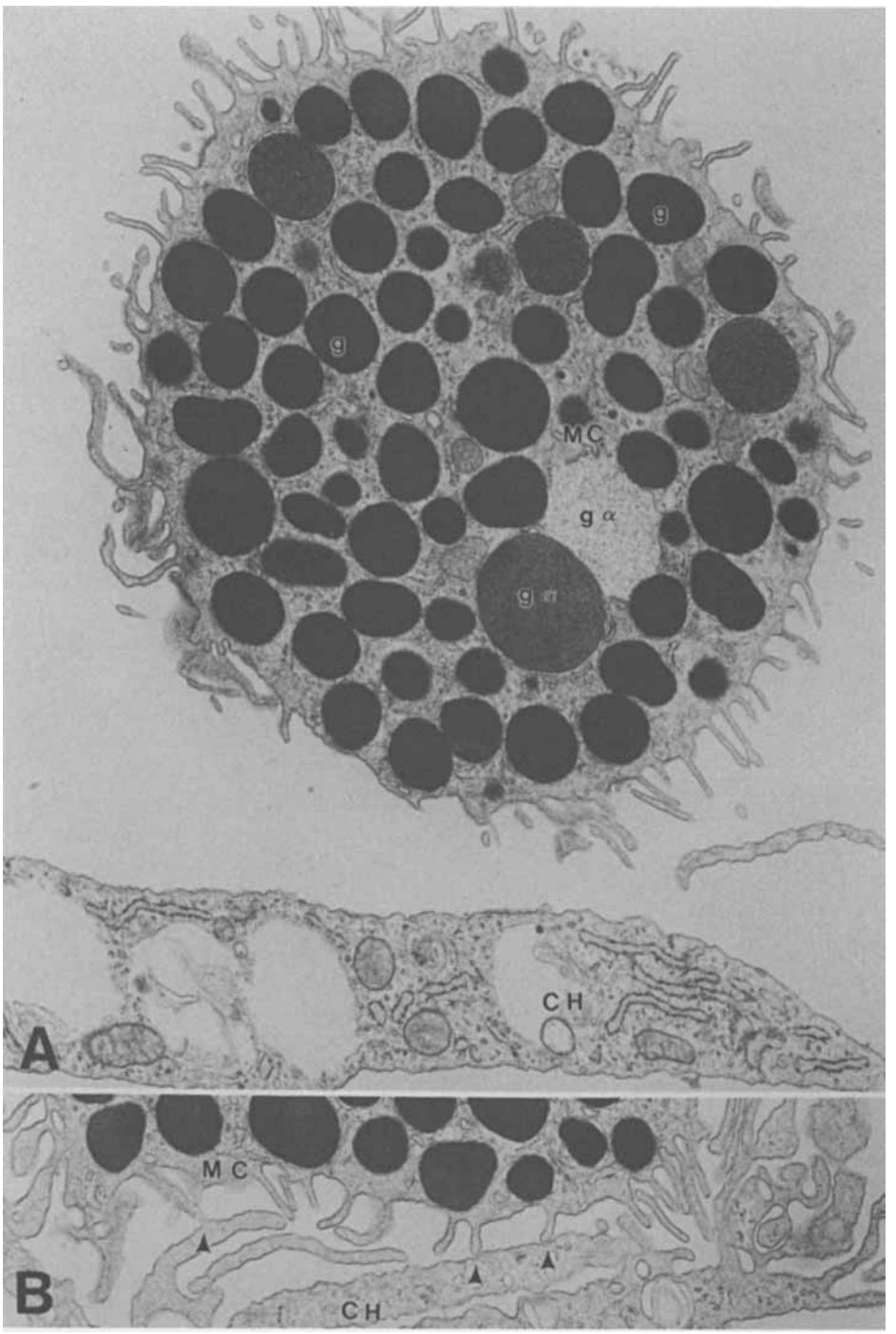

Figure 3. Electron micrographs of a 4-day coculture of rat serosal mast cells (SMC) and chondrosarcoma chondrocytes. **A,** The mast cell (**MC**) does not contact the chondrocyte (**CH**). It has large numbers of electron-dense granules (**g**); some of the granules are of lower electron density (**gα**) than is usually seen in freshly isolated rat SMC (original magnification × 12,000). **B,** An SMC contacts a chondrocyte with the tips of its microvilli or microplicae (**arrows**) (original magnification × 18,000).

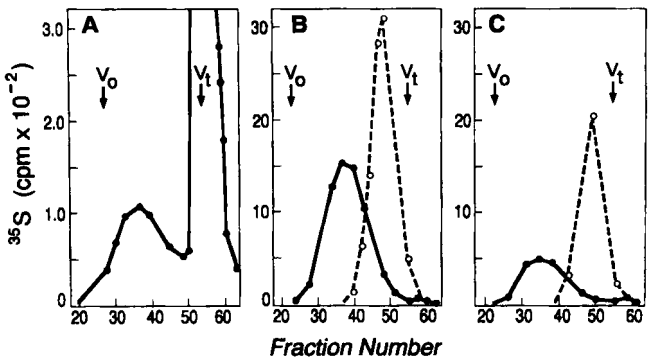

Figure 4. Dissociative Sepharose CL-2B chromatography of ^{35}S-labeled proteoglycans. The ^{35}S-labeled proteoglycans that had been spontaneously released into the culture medium (**A**) or remained associated with the cell layers (**B** and **C**) from chondrocytes cultured in the presence (**A** and **B**) and absence (**C**) of serosal mast cells were chromatographed as described in Materials and Methods. Cell-layer proteoglycans (**B** and **C**) were chromatographed before (solid line) and after (broken line) treatment with Pronase. The large total volume (V_t) peak in **A** is unincorporated ^{35}S-sulfate. V_o = void volume.

cocultured cells (Figures 4A and 4B) and the chondrocytes cultured in the absence of SMC (Figure 4C) eluted from the Sepharose CL-2B column with a K_{av} of 0.41, equivalent to an M_r of $\sim 2 \times 10^6$.

Samples of the ^{35}S-labeled proteoglycans produced by chondrocytes cultured in the absence (Figure 4C) and presence (Figure 4B) of SMC were incubated with Pronase and analyzed for a change in their hydrodynamic sizes. Essentially all of the ^{35}S-labeled proteoglycans produced by both types of cultures were susceptible to degradation by this protease, filtering on the Sepharose CL-2B column with K_{av} values of 0.41 and 0.81 before and after Pronase treatment, respectively (Figure 4). The ^{35}S-labeled proteoglycans released into the culture medium during the 2-hour radiolabeling of chondrocytes cultured in the presence (Figure 5A) and absence (Figure 5B) of SMC, filtered in the void volume of the Sepharose CL-2B column that had been equilibrated under associative conditions.

In 3 experiments, the ^{35}S-labeled proteoglycans present in the cell layer and supernatant of the cocultured cells were not susceptible to digestion by heparinase. However, 91 ± 2% and 92 ± 3% (mean ± SD, n = 3) of the total ^{35}S-labeled proteoglycans produced by chondrocytes cultured in the absence and presence of SMC, respectively, were converted to ^{35}S-labeled disaccharides by chondroitinase ABC. Because almost all of the ^{35}S-labeled macromolecules produced by the

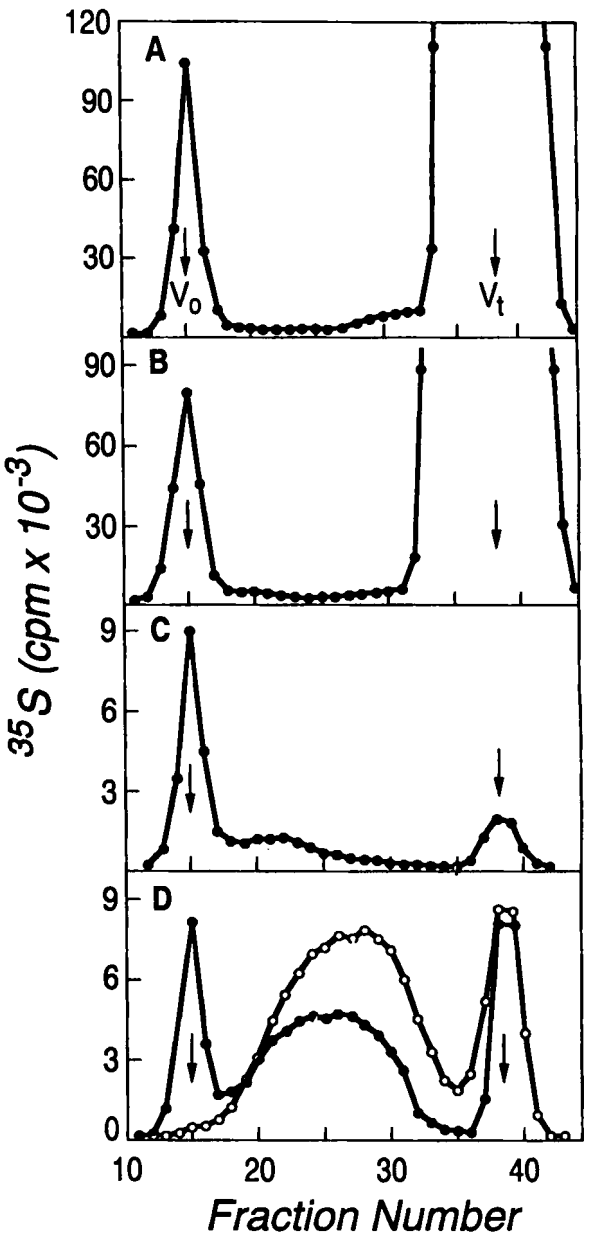

Figure 5. Associative Sepharose CL-2B chromatography of ^{35}S-labeled proteoglycans. The ^{35}S-labeled proteoglycans that had been released into the culture medium from chondrocytes cultured in the presence (**A**) and absence (**B**) of serosal mast cells (SMC) were chromatographed under associative conditions on a Sepharose CL-2B column. ^{35}S-sulfate–labeled chondrocytes alone (**C**) or SMC/chondrocytes (**D**) were washed and challenged for 10 minutes with buffer containing compound 48/80, and then the gel filtration profiles of the released ^{35}S-labeled macromolecules were determined. **D**, A sample of the challenge supernatant from one experiment was chromatographed on the Sepharose CL-2B column immediately after the cocultured cells were exposed to compound 48/80 (●) or after the supernatant was stored for 24 hours at −20°C (○). The total volume (V_t) peaks represent residual unincorporated ^{35}S-sulfate. V_o = void volume.

cocultured cells were 2×10^6 M_r Pronase-sensitive chondroitin sulfate proteoglycans, it was concluded that they originated from the chondrocytes.

Compound 48/80 activation of cocultured rat SMC. After 1 week of coculture, the mast cell degranulating agent compound 48/80 was added to see if the metabolism of the chondrosarcoma chondrocyte proteoglycans was acutely altered by products released from the degranulated SMC. Under conditions in which the net percent release of histamine from the cocultured mast cells was 41 ± 13% (mean ± SD, n = 3) after 10 minutes of activation, the amounts of ^{35}S-labeled proteoglycans released into the supernatant from compound 48/80–challenged SMC/chondrocyte cocultures and from compound 48/80–challenged chondrocytes were essentially the same. Almost all of the ^{35}S-labeled proteoglycans released from the buffer-treated cocultures (data not shown) or from compound 48/80–treated chondrocytes alone (Figure 5C) filtered in the excluded volume of the Sepharose CL-2B column calibrated under associative conditions. In contrast, only 10–30% of the ^{35}S-labeled proteoglycans released from the compound 48/80–treated cocultures filtered in the excluded volume of the column (Figure 5D). In addition, if the supernatants from activated SMC/chondrocyte cocultures were stored at −20°C for 24 hours before being analyzed, no aggregated proteoglycans could be detected (Figure 5D). The monomer proteoglycans possessed a K_{av} of 0.56 ± 0.4 (mean ± half the range, n = 2), and thus their hydrodynamic size was slightly smaller than the proteoglycans released by chondrocytes cultured in the absence of SMC.

Mast cell–induced release of cartilage proteoglycans. As shown in Figure 6 for 1 of 6 experiments (each of which was done in quadruplicate), significantly more ($P < 0.01$, n = 6) cartilage proteoglycan was released into the medium when dead bovine nasal cartilage was cultured for 8 days in the presence of rat SMC lysates than when cultured in the absence of SMC lysates or in the presence of boiled SMC lysates. The ability of nonboiled lysates of rat SMC to stimulate the release of extracellular-matrix cartilage proteoglycans into the culture medium was not significantly different whether living or dead bovine cartilage was used (data not shown). In a 4-day assay, the granule core–enriched supernatants from compound 48/80–activated SMC induced almost as much release of cartilage proteoglycan as *Salmonella typhosa* lipopolysaccharide or whole lysates of SMC (Figure 7). However, the ability of SMC lysates to induce the release of bovine cartilage proteoglycans was substantially diminished when the lysates were pre-exposed to 1 m*M* diisopropyl fluorophosphate (Figure 7).

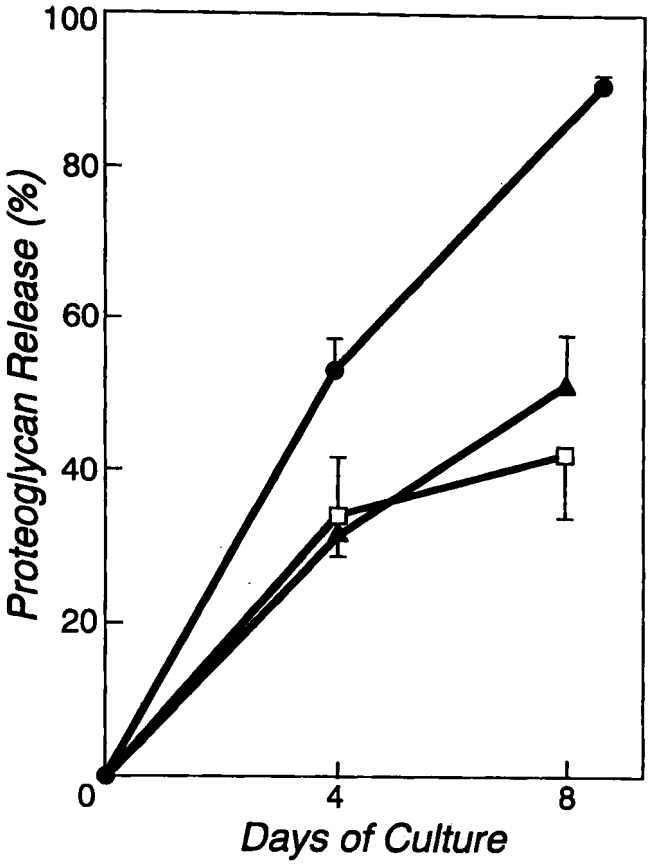

Figure 6. Time-dependent release of cartilage proteoglycans. Dead (frozen and thawed) bovine nasal cartilage was cultured for up to 8 days in the absence (□) or the presence of nonboiled (●) or boiled (▲) lysates of 10^6 rat serosal mast cells (SMC). The results are the mean and SD of an experiment performed in quadruplicate. In this experiment, significantly more ($P < 0.01$) proteoglycan was released from cartilage cultured 8 days in the presence of SMC lysates, as compared with the control samples.

DISCUSSION

The discovery that the viability and phenotype of rat SMC could be maintained when these mast cells were cocultured with either mouse 3T3 fibroblasts (10) or rat osteoblast-like cells (11) was the impetus to determine if a similar in vitro approach could be used to study the bilateral interaction of rat chondrocytes with rat SMC. No viable mast cells could be detected when rat SMC were cultured for 7 days in enriched medium in noncoated culture dishes or in culture dishes coated with gelatin, fibronectin, type III colla-

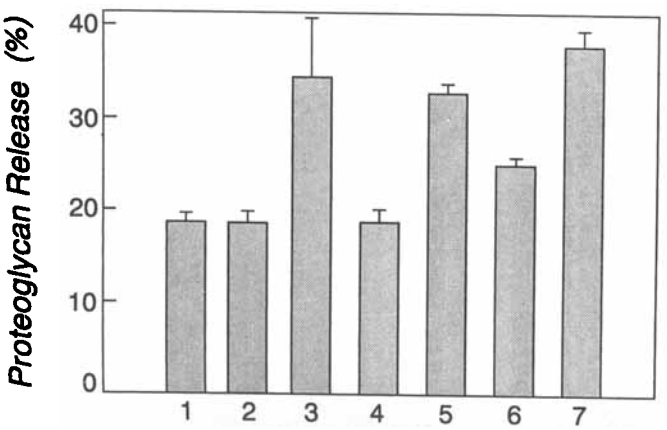

Figure 7. Serosal mast cell (SMC)–derived serine proteases induce the release of proteoglycans from cartilage. The release of cartilage proteoglycans was determined when living (lane 1) or dead (lane 2) bovine nasal cartilage was cultured for 4 days in enriched medium alone; when living (lane 3) or dead (lane 4) cartilage was cultured in the presence of enriched medium containing 25 μg/ml of *Salmonella typhosa* lipopolysaccharide; and when dead cartilage was cultured in the presence of lysates of 10^6 SMC (lane 5), 1 m*M* diisopropyl fluorophosphate–treated lysates of SMC (lane 6), or supernatants derived from compound 48/80–activated SMC (lane 7). Values are the mean and SD of an experiment performed with 5 separate samples of cartilage. In this experiment, significantly more ($P < 0.001$) proteoglycan was released from cartilage cultured in the presence of SMC lysates (lane 5) or supernatants derived from compound 48/80–activated SMC (lane 7), as compared with the control sample (lane 2).

gen, or an extracellular matrix prepared from mouse 3T3 fibroblasts (10). In this study, 28% of the initially seeded rat SMC were viable and adhered to the layer of Swarm rat chondrosarcoma chondrocytes after 7 days of coculture (Figure 1). In contrast to fibroblast coculture in which rat SMC (10), mouse bone marrow–derived mast cells (13), and human lung mast cells (40) migrate through the layers of fibroblasts, SMC were unattached or preferentially remained bound to the surface of the outer layer of chondrocytes (Figure 3). Because rat SMC were less adherent in the chondrocyte cocultures than in the fibroblast cocultures, a higher percentage of the SMC were removed from the cultures when the medium was changed during the first 2 days of the culture. Nevertheless, the adherent, cocultured SMC excluded trypan blue and were morphologically (Figure 3) similar to freshly isolated SMC. The cocultured SMC were functionally competent cells that could be induced to exocytose ~40% of their histamine upon exposure to compound 48/80. After 1 week of coculture, all of the SMC resembled the starting cells, in that they contained large numbers of electron-dense granules (Figure 3) that stained with Safranin (Figure 2). It remains to be determined if chondrocytes derived from normal cartilage can also maintain the viability and phenotype of SMC ex vivo.

Because almost all of the ^{35}S-labeled macromolecules produced by the cocultured cells were ~2 × 10^6 M_r chondroitin sulfate proteoglycans that were susceptible to Pronase (Figure 4), they originated from the chondrocytes rather than from the SMC. When chondrocytes were cultured with living SMC, the SMC/chondrocyte cocultures produced significantly more ^{35}S-labeled proteoglycans, and a higher percentage of these proteoglycans remained associated with the cell layer. Because the numbers of chondrocytes in the cocultures were similar to those in the control cultures and because the percent increases in ^{35}S-sulfate incorporation were similar after 1 and 7 days of coculture, it was concluded that SMC preferentially induced the chondrocytes to increase their per-cell rate of proteoglycan synthesis. Whether the formation of other matrix components, such as types II and IX collagen, is stimulated or inhibited by the cocultured chondrocytes remains to be determined. The accelerated biosynthesis of proteoglycans by the cocultured chondrocytes may well contribute to a more extensive extracellular matrix, which could physically entrap the proteoglycans more efficiently.

Although not studied in detail, direct cell-to-cell contact probably is not needed for the SMC-induced effect on the proteoglycan biosynthesis. As shown in Figure 3A, the cocultured SMC resided on the outer surface of the layer of chondrocytes or were unattached in the culture medium. To obtain an ~50% overall increased rate of biosynthesis under conditions in which proteoglycan synthesis is already very high (19,20), most of the chondrocytes in the culture would have to be affected. Since the chondrocytes substantially outnumber the SMC, it is difficult to envision a mechanism by which SMC can induce so many of the chondrocytes in the culture to increase their proteoglycan biosynthetic rates if direct plasma membrane contact between the two cell types is needed during the 2-hour radiolabeling. Most likely, the cocultured SMC are constitutively releasing a soluble cytokine that affects the majority of the chondrocytes in the culture.

Gel filtration analyses (Figures 4 and 5) were performed to determine if the accelerated biosynthesis was a compensatory mechanism for a possible mast cell–induced degradation of extracellular matrix proteoglycans. The hyaluronic acid–binding region of

chondrocyte proteoglycans is most sensitive to proteolytic degradation (18,41). Therefore, it was postulated that the ^{35}S-labeled proteoglycans that were spontaneously released into the culture medium from the nonactivated cocultured cells should possess a diminished ability to bind to hyaluronic acid if proteolysis had taken place. Swarm rat chondrocyte proteoglycans have a hydrodynamic size exceeding 10^8 M_r when aggregated with hyaluronic acid (18–20), and they will therefore filter in the excluded volume of a Sepharose CL-2B column that has been equilibrated under associative conditions of 0.5*M* sodium acetate. The proteoglycans present in the culture medium of the cocultured cells filtered in the excluded volume of an associative Sepharose CL-2B column (Figure 5A), and therefore, no significant proteolysis of the chondrocyte proteoglycans occurred during coculture when the SMC were not induced to exocytose their proteases.

The observation that mast cells are often increased in number at sites of cartilage erosion in the arthritic joint has led to the proposal that mast cells actively participate in the loss of extracellular matrix proteoglycans (3). The in vitro studies described here indicate that a complex interaction probably occurs between mast cells and chondrocytes, in which the mast cell can stimulate either biosynthesis or catabolism of cartilage proteoglycans. The SMC/chondrocyte coculture studies reveal that if the mast cells are induced to degranulate, the chondrocyte proteoglycans lose their ability to bind to hyaluronic acid and become slightly smaller in hydrodynamic size (Figure 5D). Although it is possible that the chondrocyte proteoglycan's inability to bind hyaluronic acid is the result of destruction of the peptide core by oxygen radicals (42), it probably is a consequence of proteolytic cleavage of the hyaluronic acid–binding region by an exocytosed, mast cell–derived proteoglycanase.

Because Swarm rat chondrocytes cultured in the absence of SMC continuously and rapidly release their newly synthesized proteoglycans into the culture medium (19,20), SMC/bovine cartilage experiments were performed to determine if mast cell–derived factors would induce the release of proteoglycans even when these proteoglycans are immobilized in the extracellular matrix of normal cartilage. As shown in Figures 6 and 7, rat SMC possess factors which, when exocytosed from an activated mast cell, can stimulate an increased release of proteoglycans from the extracellular matrix of cartilage. The ability to inhibit this induced release of cartilage proteoglycans by >50% with pretreatment of the SMC lysates with 1 m*M* diisopropyl fluorophosphate indicates that at least one of these mast cell–derived factors is a serine protease.

Most of the proteoglycans in the synovial fluid of patients with rheumatoid arthritis no longer have a hyaluronic acid–binding region (43), and therefore, a mast cell–derived serine protease may contribute to the pathogenesis of the rheumatoid joint. The SMC/chondrocyte coculture experiments indicate that SMC can induce the chondrocytes to increase their rate of biosynthesis of cartilage proteoglycans. This in vitro observation may be a consequence of the transformed nature of the chondrocytes used in this study. However, various populations of activated and nonactivated, in vitro–derived mouse mast cells have been found to express multiple cytokines, including transforming growth factor β (44–50). Because recombinant transforming growth factor β induces rabbit chondrocytes to increase their rate of biosynthesis of glycosaminoglycans (51), this cytokine might be the mast cell–derived factor that stimulates chondrosarcoma chondrocytes to increase their rate of biosynthesis of aggrecan proteoglycans.

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