Identification of Link Proteins in Canine Synovial Cell Cultures and Canine Articular Cartilage

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ABSTRACT Link proteins are glycoproteins in cartilage that are involved in the stabilization of aggregates of proteoglycans and hyaluronic acid. We have identified link proteins in synovial cell cultures from normal canine synovium using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, immunofluorescence, and immunolocation with specific antibodies by electrophoretic transfer. We have also found evidence for the synthesis of link proteins in these cultures by fluorography of radiolabeled synovial cell extracts. We have identified a 70,000-mol-wt protein in canine synovial cell culture extracts that has antigenic cross-reactivity with the 48,000-mol-wt link protein. Three link proteins were identified in normal canine articular cartilage. These results indicate that link proteins are more widely distributed in connective tissues than previously recognized and may have biological functions other than aggregate stabilization.

Link proteins are glycoproteins found in all hyaline cartilage examined to date (1–4). They stabilize the binding of proteoglycan monomers to hyaluronic acid to form aggregates, and appear to interact with both proteoglycan and hyaluronic acid (1, 5–7). On polyacrylamide gel electrophoresis in a discontinuous Laemmli system (8) under nonreducing conditions, link proteins isolated from bovine nasal cartilage and human articular cartilage migrate as two major bands with apparent molecular weights of ~48,000 (LP 1)[1] and 44,000 (LP 2), respectively (5, 7, 9–11). A small amount of a third link protein with greater electrophoretic mobility (LP 3) is also found in some preparations (10–12). The larger of the two principal link proteins (LP 1) contains more carbohydrate than the smaller (LP 2), but amino acid analysis and peptide mapping indicate that these proteins are otherwise very similar (9, 10, 13, 14). It has been noted that the apparent electrophoretic mobility of the link proteins decreases after treatment with reducing agents such as mercaptoethanol (7, 10).

Link proteins from bovine articular cartilage (2), bovine epiphyseal plate cartilage (15), avian xiphoid cartilage (12), bovine aorta (16), and the Swarm rat chondrosarcoma (17) are all closely related to the prototype link proteins from bovine nasal cartilage immunologically and/or according to amino acid analysis. In most hyaline cartilage, the larger of the two major link proteins (LP 1) predominates, but in bovine epiphyseal plate (15) and human hip cartilage (3), there are nearly equal amounts of both proteins, and in avian xiphoid cartilage (12), the smaller link protein (LP 2) is more abundant. In the Swarm rat chondrosarcoma, only one link protein is present, and its electrophoretic mobility corresponds to that of LP 2 (17).

Link proteins are also present in some noncartilagenous tissues. The bovine aorta contains material that has an electrophoretic mobility similar to that of the larger link protein, LP 1, and that cross-reacts with antiserum to link proteins from bovine nasal cartilage (16). Immunofluorescent studies reveal material that reacts with antiserum to link proteins in bovine sclera, anterior uveal tract, optic nerve endoneurium, and some of the ophthalmic arteries (18). Older reports, using a relatively nonspecific antiserum, describe a synovial fluid component that appears to be antigenically related to link proteins (19, 20).

Because of the ubiquity of link proteins in various connective tissues rich in glycosaminoglycans, and because proteoglycan synthesis is known to occur in human synovial cell cultures (21), we investigated the presence of link proteins in canine synovial cell cultures. We report herein the identification and synthesis of proteins with the antigenic and electrophoretic characteristics of link proteins in these cultures.

[1] *Abbreviations used in this paper*: GuCl, guanidinium chloride; LP, link protein; 2-ME, 2-mercaptoethanol.

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MATERIALS AND METHODS

Tissues: Canine synovial villi were dissected from normal canine knee joints immediately after the animals were killed with an overdose of sodium pentothal. Canine articular cartilage was obtained from the distal femoral condyles of adult mongrel dogs immediately after they were killed. Bovine articular cartilage was obtained from the ankle joints of 2–3-yr-old steers immediately after slaughter. All cartilage was frozen at −70°C until used.

Tissue Culture: Canine synovial cells were obtained by digestion of minced synovium with 0.1% collagenase (Type IV, Sigma Chemical Co., St. Louis, MO) and 0.25% trypsin (Gibco Laboratories, Grand Island, NY), and were cultured in CMRL 1066 medium (Gibco Laboratories) containing 20% newborn calf serum (22). All culture media were supplemented with 1% penicillin-streptomycin (Gibco Laboratories), 2 mM L-glutamine, and 20 mM HEPES buffer (Sigma Chemical Co.) (22). Confluent cultures were serially passaged (5–6 times) in this medium containing 20% newborn calf serum, or were labeled after one passage with 6 μCi/ml [^{3}H]leucine (5 Ci/mmol) (New England Nuclear, Boston, MA) in L-15 medium (Gibco Laboratories) containing 1% newborn calf serum for 24 h. For immunofluorescence studies, cells were subcultured once on multiwell slides (Lab-Tex, Miles Scientific Div., Naperville, IL).

Extraction Conditions and Density Gradients: Synovial cell cultures were freeze-thawed three times after removal of the culture medium. The synovial cells were extracted with 4 M guanidinium chloride (GuCl) (Schwarz/Mann Div., Orangeburg, NY) containing 0.01 M EDTA (Aldrich Chemical Co., Milwaukee, WI), 0.005 M benzamidine HCl (Sigma Chemical Co.), and 0.1 M 6-aminocaproic acid (Aldrich Chemical Co.), as protease inhibitors (23), in 0.05 M sodium acetate, pH 5.8, for 48 h at 4°C (10). The protease inhibitors were replenished after 24 h. The extract was then filtered and dialyzed against distilled water. The culture medium was also dialyzed exhaustively against distilled water. Synovial cell cultures labeled with [^{3}H]-leucine were handled in a similar fashion.

Bovine and canine articular cartilage were finely ground in liquid nitrogen, extracted with 4 M GuCl as described above, and dialyzed against 0.4 M GuCl. Cesium chloride (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, NY) was added to yield a density of 1.65 g/ml, and associative (0.4 M GuCl) gradients were formed in a VTi 50 or VTi 65 rotor (Beckman Instruments Inc., Palo Alto, CA) in an ultracentrifuge (model L5-65; Beckman Instruments Inc.) at 120,000 *g* for 24 h at 4°C (10). The resulting gradient was divided into fifths. An aliquot of the bottom fraction (A1) was removed and dialyzed against distilled water for subsequent studies. GuCl was added to the bottom two fractions (A1A2) to yield a molarity of 4 M, and cesium chloride was added to yield a density of 1.50 g/ml. A dissociative (4 M GuCl) density gradient was formed under the same centrifugation conditions as above. The resulting gradient was divided into fourths. The top of this gradient (A1D4) was dialyzed against distilled water and lyophilized (10).

Protein was quantitated by a colorimetric assay (Bio-Rad Laboratories, Richmond, CA) (24).

Gel Electrophoresis: Samples containing 20–40 μg of protein were dissolved in the SDS-polyacrylamide gel electrophoresis sample buffer, containing 62.5 mM Tris-Cl, pH 6.8, with 1% SDS and 12% glycerol. An aliquot of each sample was treated with 1% (vol/vol) 2-mercaptoethanol (2-ME) (Bio-Rad Laboratories). Samples were then applied to 9% polyacrylamide slab gels with 3% stacking gels and were electrophoresed in a Laemmli system (8). The gels were stained with Coomassie Blue R-25 (Bio-Rad Laboratories). Molecular weights were calculated by comparing relative migrations with standard reference proteins (Bio-Rad Laboratories).

For peptide mapping studies, samples of [^{3}H]leucine-labeled extract were electrophoresed on 9% polyacrylamide gels. The bands of interest were cut out of the gels, equilibrated in electrode buffer, and placed in the wells of a 12% gel. The gel pieces were overlaid with sample buffer containing 25 μg of V8 protease from *Staphylococcus aureus* (Miles Scientific Div.). The samples were electrophoresed into the stacking gel, and the current was turned off. After 30 min, electrophoresis was resumed (10, 25). The gels were then used for fluorography (see below).

Preparation of Rabbit Antiserum to Link Proteins: Two female New Zealand white rabbits were immunized with 3 mg of bovine cartilage A1D4 fraction dissolved in 0.5 ml of complete Freund's adjuvant and 0.5 ml of phosphate buffered saline (PBS), containing 0.01 M phosphate and 0.15 M NaCl, pH 7.4. Booster injections of 1.5 mg of A1D4 fraction in incomplete Freund's adjuvant were given 3–4 wk later and then every 2 wk (26, 27). Blood was drawn before the first immunization and 7–10 d after each injection to identify antibodies by electrophoretic transfer, as described below.

Preparation of Mouse Monoclonal Antibodies to Link Protein(s): Monoclonal antibodies to link protein(s) were raised using purified Swarm rat chondrosarcoma LP 2 as antigen. Procedures for producing monoclonal antibodies to proteoglycan and proteoglycan structures have been described previously (28). Detailed characterization of several monoclonal antibodies to link protein have been described elsewhere (29–31). One of these monoclonal antibodies, 9/30/8-A-4, recognizes an antigenic determinant present on link proteins isolated from rat, human, bovine, and chicken hyaline cartilages. Table I summarizes the specificity of this monoclonal antibody as determined by enzyme-linked immunosorbent assay (26, 28) and electrophoretic transfer with immunolocation analysis (30, 32). This monoclonal antibody does not recognize determinants present on proteoglycan monomer or proteoglycan fragments isolated from rat, bovine, or human hyaline cartilages. The epitope recognized by the 9/30/8-A-4 monoclonal antibody is not destroyed by trypsin digestion or reduction and alkylation of link protein(s). Peptide mapping analyses, using Staphylococcal V8 protease (10), have indicated that the epitope recognized by the 9/30/8-A-4 monoclonal antibody is present on a small peptide (~7,000 mol wt) that is common to several animal species (30). The 9/30/8-A-4 monoclonal antibody does not recognize link protein in cartilage proteoglycan aggregates, indicating that the epitope recognized by the antibody is masked in the aggregate structure. This monoclonal antibody has an IgG_{2b} heavy chain and a kappa light chain.

Electrophoretic Transfers and Immunolocation Analyses: The presence of antibodies to link proteins was identified by electrophoretic transfer. Samples of bovine cartilage A1 and A1D4 fractions treated with 2-ME and separated on 9% polyacrylamide slab gels were transferred to a nitrocellulose membrane (Millipore Corp., Bedford, MA) in an electro-blot apparatus (E-C Apparatus Corp., St. Petersburg, FL) containing deaerated 50 mM sodium phosphate buffer, pH 7.5 (33, 34). The nitrocellulose membrane was incubated with PBS containing 0.05% (vol/vol) Tween 20 (Sigma Chemical Co.) to prevent nonspecific binding, and was cut into 0.5-cm strips. The strips were incubated with rabbit polyclonal antiserum or with mouse monoclonal antibody, followed by 3–3.5 × 10^6 cpm of ^{125}I-labeled Staphylococcal protein A (35). Some membranes incubated with the mouse monoclonal antibody to link protein were exposed to ^{125}I-labeled goat anti-mouse immunoglobulin antiserum, a generous gift from Dr. W. J. Newhall, V (Indiana University School of Medicine), instead of Staphylococcal protein A. The strips were then sandwiched with Kodak X-Omat AR film and a Cronex lightning plus intensifying screen (DuPont Co., Wilmington, DE) for 48–72 h at −70°C.

Results obtained with the monoclonal antibody after incubation with ^{125}I-labeled Staphylococcal protein A were the same as those obtained after incubation with ^{125}I-labeled goat anti-mouse immunoglobulin antiserum. The rabbit polyclonal antiserum and the mouse monoclonal antibody identified two link protein bands in reduced bovine articular A1 and A1D4 fractions at ~48,000 and 44,000 mol wt, corresponding to LP 1 and LP 2, respectively. Electrophoretic transfers of Type II collagen and proteoglycan subunit (D1D1), generous gifts from Dr. G. N. Smith, Jr. (Indiana University School of Medicine), were performed as controls in studies of the rabbit polyclonal antiserum, and demonstrated no cross-reactivity.

The rabbit polyclonal antiserum and the mouse monoclonal antibody were used to identify link proteins after electrophoretic transfers of 9% polyacrylamide gels of canine articular cartilage A1D4 fractions and extracts of canine synovial cell cultures to nitrocellulose membranes.

In all experiments, some strips were incubated with nonimmune rabbit serum or with monoclonal antibody to dinitrophenol, a generous gift from Dr. Betty Tsao (Indiana University School of Medicine), as negative controls.

TABLE I

Specificities of the 9/30/8-A-4 Monoclonal Antibody

Species	Reactivity with intact link protein*	Reactivity with peptide‡
Rat§	+	+
Dog§‖	+	ND
Pig§¶	+	ND
Cow§	+	+
Human**	+	+
Chicken§	+	+

+, Positive; ND, not determined.
*Link protein isolated from proteoglycan aggregates (A1).
‡Common peptide obtained after digestion of link protein with Staphylococcal V8 protease.
§Hyaline cartilage.
‖This paper.
¶Fife, R. S., and B. Caterson, unpublished observations.
**Hyaline and intervertebral disk cartilage.

Immunofluorescence Studies: Primary synovial cell cultures, subcultured overnight on microwell slides, were fixed in methanol, air-dried, and incubated with rabbit anti-link protein antiserum or nonimmune rabbit serum in a moist chamber at 37°C for 30 min. After the slides were washed with PBS, pH 7.0, containing 1% nonimmune goat immunoglobulin (Sigma Chemical Co.) for 30 min (36), they were incubated with fluorescein isothiocyanate-labeled goat anti-rabbit immunoglobulin antiserum (Sigma Chemical Co.) for 30 min, washed three times in PBS for 30 min per wash, mounted in buffered glycerine, and examined under a fluorescence microscope (4).

Fluorography: Gels of [^{3}H]leucine-labeled synovial cell extracts and of Staphylococcal V8 protease digests were treated with EN^{3}HANCE (New England Nuclear), per the manufacturer's instructions, and were sandwiched with Kodak X-Omat AR film for 1–2 wk at −70°C.

RESULTS

Electrophoresis of the A1D4 fraction from canine articular cartilage extracts was performed to establish the electrophoretic mobility of canine cartilage link proteins. Examination of Coomassie Blue–stained gels containing unreduced samples revealed three bands with apparent molecular weights of 45,000, 40,000 and 38,000 (Fig. 1*A*). These proteins correspond in electrophoretic mobilities to LP 1, LP 2, and LP 3, respectively, from bovine and human hyaline cartilage. The band with the slowest electrophoretic mobility (LP 1) was the most intense. After reduction with 2-ME, the electrophoretic mobility of all the bands in this fraction decreased slightly, and only two bands at ~48,000 and 44,000 mol wt were clearly visible (LP 1 and LP 2, respectively). This change in electrophoretic mobility is characteristic of link proteins. Again, the band with the slower mobility stained more intensely (Fig. 1*B*).

Electrophoresis of 4 M GuCl extracts prepared from serially passaged confluent synovial cell cultures (~3 × 10^7 cells) was performed on 9% SDS-polyacrylamide slab gels (Fig. 2). High molecular weight material that did not enter the gel was observed both before and after reduction of the sample with 2-ME. Two faint bands with apparent molecular weights of ~43,000 and 42,000 were present in unreduced synovial cell extract samples (not shown). After reduction with 2-ME, a single intense band with a slower electrophoretic mobility at ~48,000 mol wt was noted (Fig. 2). This band corresponds in migration to LP 1. During exhaustive dialysis of the synovial cell extracts against distilled water at 4°C, a white precipitate formed that was found to be enriched in link proteins by electrophoresis on SDS-polyacrylamide gels stained with Coomassie Blue (not shown). The electrophoretic mobility of these synovial cell proteins, their behavior after reduction, and their insolubility in aqueous solutions were comparable to that of canine cartilage link proteins and link proteins isolated from other sources that were similarly analyzed. Synovial cell extracts also contained a second prominent band at ~70,000 mol wt (Fig. 2) before and after reduction with 2-ME.

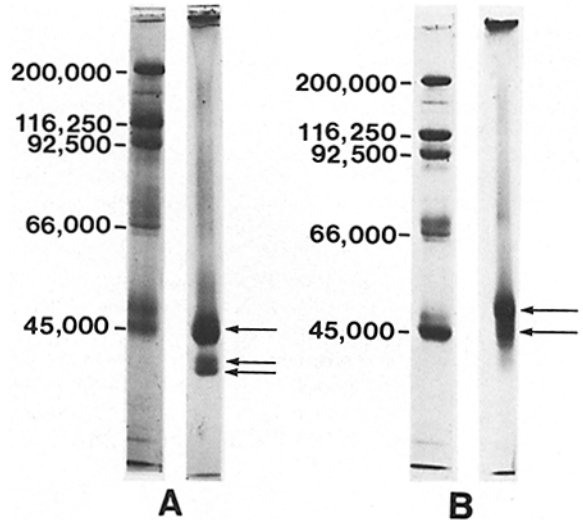

FIGURE 1 9% SDS-polyacrylamide gels of A1D4 fraction from canine articular cartilage before (*A*) and after (*B*) reduction with 2-ME. Note the three distinct bands (arrows) in the unreduced sample and the two bands (arrows) after reduction. Molecular weight markers run simultaneously in left-hand lanes of *A* and *B* were: 200,000, myosin; 116,250, β-galactosidase; 92,500, phosphorylase B; 66,000, BSA; 45,000, ovalbumin. Gels were stained with Coomassie Blue.

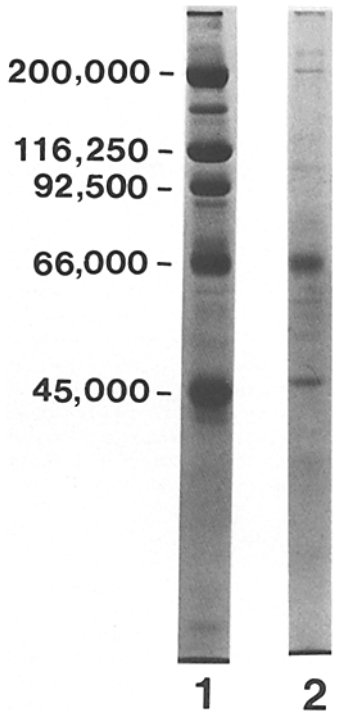

FIGURE 2 Lane *1*, molecular weight markers as in Fig. 1; lane *2*, 9% SDS-polyacrylamide gel of synovial cell extract after reduction with 2-ME. Note the band at ~48,000 mol wt that is consistent with link protein. Note also the band at ~70,000 mol wt. The gel was stained with Coomassie Blue.

Gels containing samples of the dialyzed culture medium (Fig. 3) showed a pattern similar to that of the 4 M GuCl extracts of synovial cells, with prominent bands at ~48,000 and 70,000 mol wt after reduction with 2-ME.

Additional characterization of the canine cartilage and synovial link proteins was performed using immunolocation studies. Autoradiographs of samples of reduced A1D4 fraction from canine articular cartilage after electrophoretic transfer and incubation with the polyclonal rabbit anti-link protein antiserum revealed two radioactive bands of equal intensity at ~48,000 and 44,000 mol wt, consistent with link proteins (not shown). Electrophoretic transfers using the monoclonal antibody to link protein (see Materials and Methods) showed a similar pattern (Fig. 4*A*), confirming the presence of LP 1 and LP 2 in canine articular cartilage. Transfers using nonimmune rabbit serum or monoclonal antibody to dinitrophenol were negative.

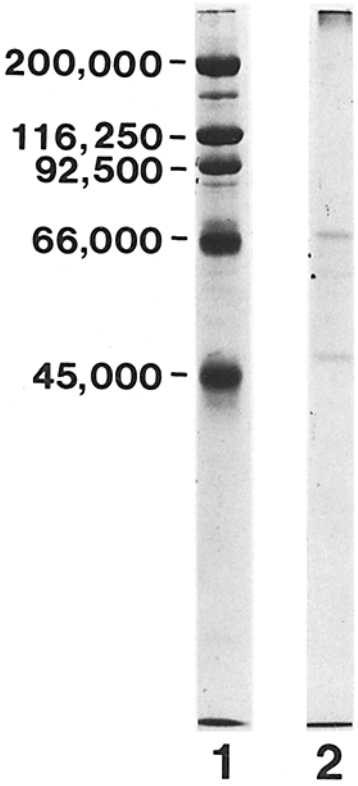

FIGURE 3 Lane *1*, molecular weight markers as in Fig. 1; lane *2*, 9% SDS-polyacrylamide gel of synovial cell culture medium reduced with 2-ME, showing a pattern similar to the synovial cell extract in Fig. 2. The gel was stained with Coomassie Blue.

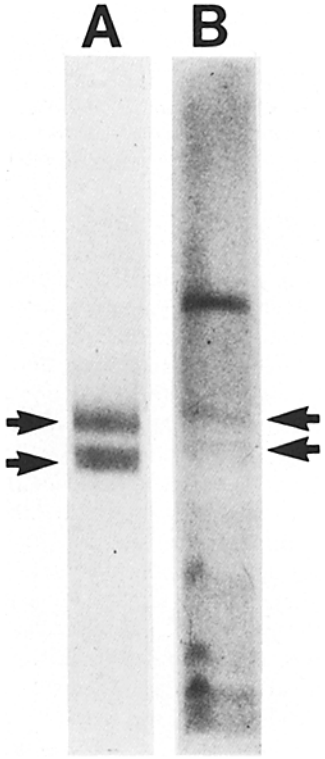

FIGURE 4 (*A*) Electrophoretic transfer of reduced canine articular-cartilage A1D4 fraction from a 9% SDS-polyacrylamide slab gel to nitrocellulose membrane. After incubation of strips of the membrane with the mouse monoclonal antibody and ^{125}I-labeled goat anti-mouse immunoglobulin antiserum or ^{125}I-labeled Staphylococcal protein A, followed by autoradiography, two bands are noted at ~48,000 and 44,000 mol wt (arrows). (*B*) Electrophoretic transfer of reduced synovial cell extract followed by incubation with the monoclonal antibody also reveals two bands at ~48,000 and 44,000 mol wt (arrows), representing link proteins. Note the band at ~70,000 mol wt and the minor species below 40,000 mol wt.

Electrophoretic transfers of gels prepared from samples of synovial cell extracts reduced with 2-ME were studied using the rabbit anti-link protein antiserum. These autoradiographs revealed two bands at 48,000 and 44,000 mol wt (LP 1 and LP 2, respectively), as well as several other bands including one at ~70,000 mol wt (not shown). Reduced extracts of synovial cell cultures transferred to nitrocellulose membranes and incubated with the monoclonal anti-link protein antibody and ^{125}I-labeled Staphylococcal protein A or ^{125}I-labeled goat anti-mouse immunoglobulin antiserum also demonstrated two bands of equal intensity at ~48,000 and 44,000 mol wt, representing LP 1 and LP 2, respectively (Fig. 4*B*). The mouse monoclonal antibody also reacted with the band at ~70,000 mol wt and with several minor bands below 40,000 mol wt, the latter probably representing degradation products of the link proteins (Fig. 4*B*), which have been reported by other investigators (37). Electrophoretic transfers of gels from reduced samples of the cell culture medium produced autoradiographs demonstrating the link protein bands at 48,000 and 44,000 mol wt after incubation with the monoclonal antibody (not shown). Transfers of all samples using nonimmune rabbit serum or monoclonal antibody to dinitrophenol were negative.

To study further the relationship of the 48,000-mol-wt link protein (LP 1) and the 70,000-mol-wt protein which cross-reacted with the specific antibodies to link proteins, peptide mapping with Staphylococcal V8 protease was performed on the 48,000-mol-wt and 70,000-mol-wt proteins from the [^{3}H]leucine-labeled synovial cell extract. As can be seen in Fig. 5, the peptide maps of these synovial cell proteins con-

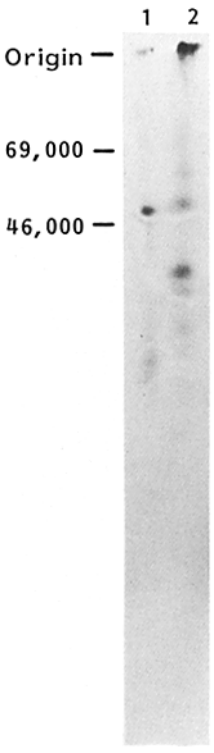

FIGURE 5 Fluorogram of 12% SDS-polyacrylamide gel of Staphylococcal V8 protease (25 μg) digests of the 70,000-mol wt band (lane *1*) and the 48,000-mol wt band (lane *2*) from 9% SDS-polyacrylamide gel of [^{3}H]leucine-labeled synovial cell extract. Molecular weight markers (indicated but not shown) are: 69,000, [^{14}C]albumin; 46,000, [^{14}C]ovalbumin.

tained some similar bands, but several additional labeled bands were present in the map of the 48,000-mol-wt protein.

Evidence for the presence of link proteins in synovial cell cultures was also obtained using rabbit anti-link protein antiserum in immunofluorescence studies. Microwell synovial cell cultures incubated with nonimmune rabbit antiserum showed no immunofluorescence. Incubation with rabbit anti-link protein antiserum revealed marked fluorescent staining of the cells (Fig. 6).

Synthesis of link proteins in [^{3}H]leucine-labeled synovial cell cultures was evaluated by preparing fluorograms from 9% SDS-polyacrylamide gels of the culture extracts. These experiments demonstrated two bands with apparent molecular weights just below 45,000 before reduction with 2-ME (not shown), which decreased slightly in electrophoretic mobility to a single band at ~48,000 mol wt (Fig. 7) after reduction with 2-ME. The electrophoretic behavior of these proteins was consistent with their presumptive identification as newly synthesized link proteins. A band at ~70,000 mol wt was noted before and after reduction, as was higher molecular weight labeled material that did not enter the gels. Fluorograms prepared from labeled culture medium showed a similar distribution of labeled materials. Exhaustive dialysis of labeled synovial cell extracts against distilled water revealed that most of the radioactivity was found in the precipitate.

DISCUSSION

The presence of link proteins in several connective tissues rich in glycosaminoglycans, and the indication in earlier studies that a synovial fluid component cross-reacts antigenically with link protein (19, 20), were the rationale for the present experiments.

We have demonstrated that canine articular cartilage extracts contain three link proteins before reduction, as does bovine nasal cartilage (10), human hyaline cartilage (11), and avian xiphoid cartilage (12). The monoclonal antibody used in these studies recognizes an antigenic determinant present in link proteins obtained from rat, bovine, human, and chicken hyaline cartilage (30). Only canine cartilage proteins with apparent molecular weights consistent with those of other link proteins reacted with this antibody, indicating the presence of an antigenic determinant on canine link proteins common to link proteins from other species.

Extracts of canine synovial cell cultures were found to contain proteins with molecular weights and electrophoretic behavior after reduction similar to hyaline cartilage link proteins. Electrophoretic transfer and immunolocation studies using both polyclonal and monoclonal antibodies to link proteins provided more specific evidence that these bands were in fact synovial cell link proteins. Proteins with characteristics similar to the link proteins were also identified in the synovial cell culture medium, indicating that they are exported from the cells. Electrophoretic analysis of culture extracts prepared from synovial cells labeled with [^{3}H]leucine indicated a synthesis of proteins corresponding in electrophoretic mobility to link proteins.

As already indicated, three link proteins were present in normal canine articular cartilage extracts under nonreducing conditions in a discontinuous Laemmli system, but only two synovial cell link protein bands were identified under nonreducing conditions. The reason for this difference is as yet unknown. Two link protein bands of equal intensity were observed in reduced synovial cell extracts after electrophoretic transfer and exposure to monoclonal antibody, although only the slower-moving link protein band (LP 1) was noted in Coomassie Blue–stained gels of these samples. A similar study of canine articular cartilage A1D4 samples revealed two bands with comparable molecular weights after reduction, with the slower-moving band (LP 1) staining more intensely with Coomassie Blue. Both bands appeared equal in intensity using electrophoretic transfer techniques. This suggests that reduction with 2-ME may expose more of the epitope recognized by the monoclonal antibody.

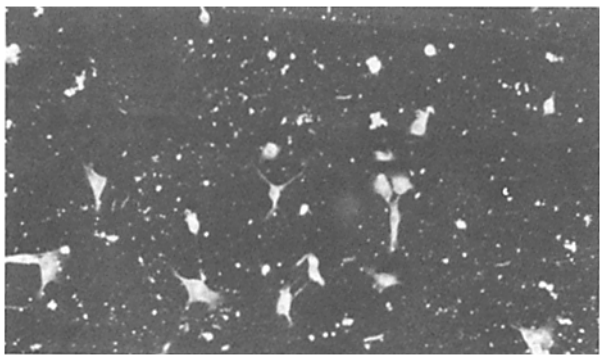

FIGURE 6 Microwells containing second-passage synovial cell culture monolayers incubated with rabbit antiserum to bovine link proteins reveal marked fluorescence. Control cultures incubated with nonimmune rabbit serum demonstrated no fluorescence (not shown).

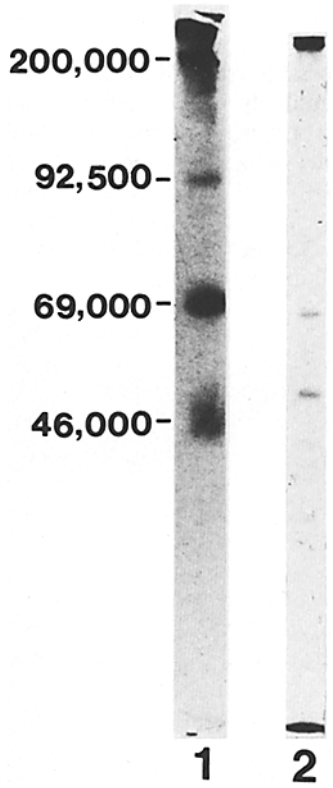

FIGURE 7 Lane *1*, fluorogram of molecular weight markers: 200,000, [^{14}C]myosin; 92,000, [^{14}C]phosphorylase B; 69,000, [^{14}C] albumin; 46,000, [^{14}C]ovalbumin. Lane *2*, fluorogram of a 9% SDS-polyacrylamide gel of [^{3}H]leucine-labeled synovial cell extract after reduction with 2-ME. Note the band at ~48,000 mol wt. Labeled high molecular weight material remains near the top of the gel, and a prominent band is present at ~70,000 mol wt.

An additional interesting observation was the presence in synovial cell extracts of a 70,000-mol-wt protein that reacted with both the rabbit polyclonal antiserum and the mouse monoclonal antibody to link protein, indicating that the 70,000-mol-wt protein shares an epitope with LP 1. Comparison of V8 protease digests of proteins labeled in vitro revealed several peptides in LP 1 that were not found in the 70,000-mol-wt protein, as well as several similar peptides. Further work is needed to clarify possible relationships between LP 1 and the 70,000-mol-wt protein.

The presence and synthesis of link proteins in synovial cell cultures is of importance because this is the first time, to our knowledge, that these proteins have been definitively demonstrated in synovial cells. Studies of proteoglycans and link proteins in developing chick limb bud cartilage suggest that link protein synthesis may have a regulatory function in the synthesis of proteoglycans capable of aggregation since such proteoglycans apparently are not synthesized until after two link proteins are produced (38). The role of link proteins in synovial tissue is as yet unknown. It is possible that they may be involved in biological roles other than the stabilization of proteoglycan aggregates. Further work to quantitate link proteins and to determine their function in synovial tissue is underway.

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