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Microvascular Pericytes Express Aggrecan Message Which Is Regulated by BMP-2

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Multipotential mesenchymal stem cells capable of chondro-osseous induction contribute to the endochondral callus of healing fractured bone. Microvascular pericytes serving the role of multipotential mesenchymal stem cells are considered osteoprogenitors because they express type I collagen, alkaline phosphatase enzyme activity, osteocalcin immunoreactivity, and bone sialoprotein mRNA. Previous electron microscopic studies indicate that this cell type has a contribution to the fracture callus. Limited data suggest that pericytes may also assume a chondrogenic phenotype. We undertook *in vitro* studies to understand how the chondro-osseous phenotype of the pericyte might be regulated. Using Northern analysis and semiquantitative reverse transcriptase–polymerase chain reaction (RT-PCR), we found that cultured pericytes produce aggrecan and type II collagen mRNA indicating their chondrogenic potential. Aggrecan message is elevated by BMP-2 as analyzed by both Northern hybridization and RT-PCR. This finding suggests a regulatory role for this morphogen on this phenotype in pericytes. RT-PCR amplified versican product was also associated with pericyte cultures but was not affected by BMP-2. Our data strongly support a chondrogenic role for the pericyte and that the phenotype is regulated at least in part by BMP. © 2000 Academic Press

In the postnatal vertebrate injured bone possesses the remarkable capacity to heal by the recapitulation of its phenotype. This unique outcome is essential to reestablishing functional rigidity. The continuity of healing bone is conferred by the fracture callus. Bone formation within the callus occurs by both endochondral and direct processes of ossification (1). In endochondral ossification a cartilage intermediate precedes bone formation within the hematoma generally beyond the limits of the torn periosteum. Ossification of the cartilage is associated with invading microvessels originating from the periosteum and surrounding soft tissue. This portion of the callus is termed the interfragmentary or inductive callus. The term inductive in this context refers to the population of multipotential mesenchymal stem cells of extraosseous origin which is capable of locally-induced chondro-osteogenesis.

The ability of cells of nonosseous origin to form bone is established. Bone morphogenetic protein a noncollagenous protein of the bone matrix can reproducibly induce endochondral bone in the ectopic implantation assay. BMP designates a multigene family of structurally homologous proteins most of which possess this *in vivo* inductive property. Since the bone matrix is the most abundant source of BMP in the postnatal vertebrate, it is evident, that bone possesses the biochemical means to induce new bone formation subsequent to injury. However, complete understanding of fracture callus phenotype regulation may not be completely understood until inducible cells are identified and the mechanisms of their phenotypic regulation are studied.

Urist characterized the BMP-induced cell in his work as a "fixed histiocyte or perivascular young connective tissue cell" (2, 3). A strong association exists between bone formation and vascularization which lends compelling support for a role for vascular elements in osteogenesis. Bone formation associated with vascular invasion of the cartilagenous callus was mentioned above. Ossification of the cartilage anlage in the embryonic limb is initiated at the site of penetration of the nutrient artery. Ossification proceeds on the calcified cartilage of the active physes of lengthening limb bones in association with advancing capillaries. The constant remodeling of bone throughout the life of the postnatal animal is a process of osteoclasis followed by osteogensis associated with advancing capillaries in the newly forming osteons.

The microvascular pericyte has come under increasing scrutiny as a possible osteogenic precursor cell. Pericytes are typically described as a smooth muscle-like cell based upon their expression of smooth muscle

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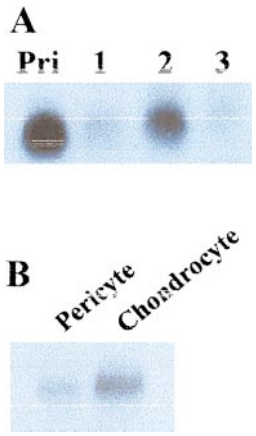

FIG. 1. (A) Aggrecan mRNA was detected by Northern hybridization in primary pericyte cultures (Pri) as well as first, second, and third passage cultures (1, 2, 3, respectively). (B) Aggrecan mRNA from pericyte and articular chondrocyte cultures detected by Northern hybridization had the same migration pattern on formaldehyde denaturing agarose gel electrophoresis.

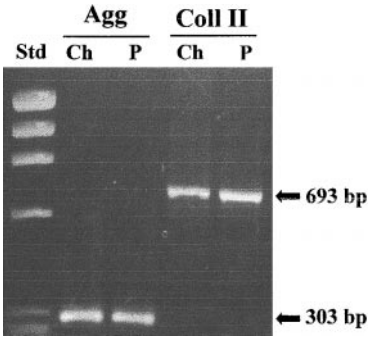

FIG. 2. RT-PCR generated amplified products for both aggrecan (Agg) and type II collagen (Col II) were detected from total RNA isolated from pericyte (P) and chondrocyte (Ch) cultures. The amplified products appeared as single bands and comigrated at the expected base pair length based on the standard (Std) restriction fragments from ϕX 174 plasmid *Hae*III digest.

actin (4, 5). From among several putative functions, pericytes are considered resting mesenchymal stem cells suggesting participation in tissue renewal processes such as neoangiogenesis and wound healing (5). A specific osteogenic capacity ascribed to pericytes is due to their ability to express markers collectively associated with the osteogenic phenotype. Production of type I collagen, alkaline phosphatase activity and osteocalcin immunoreactivity have been identified in pericyte cultures (6). In long-term culture pericytes form distinct multilayered colonies that are positive for calcium on von Kossa staining (6, 7). Retinal derived pericytes were shown to possess immunoreactivity to STRO-1 an antigenic marker originally associated with osteogenic marrow stromal cells (8). Northern hybridization of total RNA from cultured pericytes demonstrated messages for bone sialoprotein, osteocalcin, osteonectin, and osteopontin (8).

Within the scope of skeletal research, relevant studies of microvascular pericytes have focused predominately on their osteogenic potential. However, there is limited data to suggest a chondrogenic identity for this cell type. Type II collagen and type X collagen immunoreactivity have been reported in retinal pericyte cultures (9–11). The chondrogenic potential of pericytes is a relevant area of inquiry with particular respect to fracture callus biology. As described above the inductive callus arising from the differentiation of multipotential mesenchymal stem cells elaborates bone primarily via endochondral ossification. Our work with microvascular pericytes reflects the hypothesis that these cells serve as inducible mesenchymal cells contributing to the fracture callus. We undertook studies to determine the regulation of the chondro-osseous phenotype of pericytes by factors associated with fracture healing. One of these factors is BMP-2. Using Northern analysis and reverse transcriptase–polymerase chain reaction (RT-PCR) we found mRNA for aggrecan and type II collagen from cultured pericytes. Further, we found that aggrecan mRNA is up regulated by BMP-2. This information is important because it is to our knowledge the first report describing aggrecan production by pericytes and an indication that this phenotype in the pericyte is regulated by BMP.

EXPERIMENTAL PROCEDURES

Isolation and passage of microvascular pericytes. Microvascular pericytes were obtained by serial passage from primary cultures of microvessels isolated from fetal bovine brain tissue (Brighton). The brain tissue was placed in cold Hanks' balanced salt solution (HBSS) and dissected free of meninges; minced with lab spoons; transferred to 50-ml smooth glass homogenizing vessels and homogenized with a motor-driven Teflon pestle. At this point the remaining steps were carried out at room temperature unless otherwise noted.

The homogenate was allotted among 50-ml polypropylene centrifuge tubes and washed with HBSS by resuspension of the homogenate, centrifugation for 10 minutes at 1000g and vacuum aspiration of the supernatant. This wash step was repeated twice. Following the

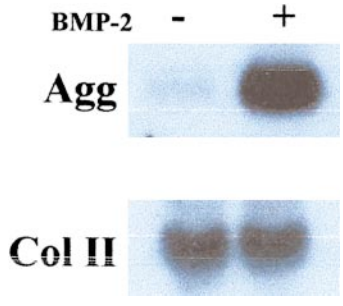

FIG. 3. BMP-2 markedly upregulates steady-state aggrecan mRNA (Agg) in pericyte cultures as detected by Northern hybridization. Type II collagen mRNA (Col II) was also detected in pericyte cultures but BMP-2 had no effect on the steady-state message level.

initial wash steps 20-ml fractions of the homogenate were resuspended in a 15% solution of dextran in Medium 199 for final volumes of 50 ml. The mixtures were centrifuged at 1000*g* for 25 minutes. The result was suspended glial and microvascular fractions which were collected and resuspended in 15% dextran. The suspension was transferred to polycarbonate tubes which were centrifuged at 5500*g* for 10 minutes. With this step microvascular-enriched fractions were sedimented while glial fractions remained suspended over the supernatant. The sedimented microvascular enriched fractions were pooled and washed two times in calcium and magnesium-free Hanks' (HCMF). The washed microvascular pellet was layered on a Percoll gradient which had previously been prepared by centrifuging a 50% Percoll solution in HCMF at 25,000*g* for 1 hour at 10°C. The microvascular fraction was centrifuged through the gradient for 10 minutes at 1000*g* after which the microvascular layer was collected from the interface and transferred to a 50-ml tube and washed 2 times with HBSS. The microvascular fraction was digested overnight in 2% collagenase (Sigma blend type L) in α-MEM containing 5% newborn calf serum (NBCS) and antibiotics in a humidified cell culture incubator in an atmosphere of 21% O_2 as injected ambient air and 5% CO_2 with gentle mixing on a rotating platform.

The cells in the overnight digest were collected by centrifugation, washed in HBSS and resuspended in culture medium (α-MEM/10%NBCS/penicillin and streptomycin and fungizone) and plated as primary cultures onto 10-cm polystyrene culture dishes. The cultures were incubated at 37°C in a humidified atmosphere of 21% O_2 and 5% CO_2. Microvessels adhere to the culture surface over the next 3 to 5 days at which time the first media change was done and then thereafter every third day. Pericytes rapidly grow out from the microvessels fragments, and the cultures were passaged just prior to confluence. Continued passage overwhelmingly favors the growth of pericytes over endothelial cells. Third or fourth passage cultures plated at a density of 25,000 cells/cm^2 were used for our experiments. Cultures used for our studies were supplemented with sodium ascorbate at 50 μg/ml 24 hours after plating and again at media change every third day. Supplementation with human recombinant BMP-2 (hrBMP-2; a generous gift of the Genetics Institute, Cambridge, MA) in designated cultures was done at 100 ng/ml at each media change. Slide cultures were stained with an α-smooth muscle actin antibody to confirm the pericyte phenotype.

Primary cultures of fetal bovine articular chondrocytes. Cultures of fetal bovine articular chondrocytes were used as a source of total RNA for comparing to chondrogenic messages similarly analyzed from pericyte cultures. Cartilage slices were shaved from articular surfaces of fetal bovine metacarpal and metatarsal bones with a scalpel blade into a polystyrene culture dish over ice. The pieces were minced and then washed in HCMF. The articular fragments were digested in succession in 0.04% hyaluronidase (bovine testicular; Sigma) in HCMF for 90 minutes; 0.2% trypsin in HCMF for 30 minutes and 0.1% collagenase (Sigma blend type L) in α-MEM/5%NBCS and antibiotics overnight. All digests were carried out at tissue culture conditions with respect to temperature and atmosphere. Cartilage slices were washed in HCMF as intervening steps between digests. The next morning the suspended cells are collected by centrifugation washed with HBSS and resuspended in culture medium. High density primary adherent cultures of 200,000 cells/cm^2 were established.

Isolation of total RNA. Total RNA for Northern analysis was isolated from cells cultured on 10-cm plates as previously described. Pericyte cultures were harvested at days 12, 18, 24, 30, and 37 post-seeding. Chondrocyte cultures were harvested between days 5 and 10 postseeding. The isolation of RNA from 35-mm cultures for RT-PCR was performed using TRI REAGENT (Molecular Research, Inc., Cincinnati, OH) according to the manufacturer's protocol. 1 ml of the reagent was used to lyse the cells in each 35-mm dish and the lysates were transferred to 1.5-ml microfuge tubes in which the extraction procedure was completed. 1-bromo-3-chloropropane (Molecular Research, Inc.) was used as the organic solvent. RNA precipitation was achieved with isopropanol. The precipitated RNA was washed in 75% chilled ethanol, air-dried, and dissolved in DEPC-treated water. The RNA quality and quantity was determined spectrophotometrically and 2-μg aliquots were stored at −70°C for RT-PCR analysis.

Northern blotting and hybridization. 20 μg of total RNA were dissolved in 10 μl of sample buffer containing 50% deionized formamide; 6.3% formaldehyde; and 0.02 M Mops/EDTA buffer (pH 7.0; 0.02 M Mops, 0.005 M sodium acetate, 0.001 M EDTA). The samples were heat denatured at 65°C for 15 minutes then chilled on ice. 2 μl of running dye was added to each sample and the samples were applied to a 1% formaldehyde agarose gel. The samples are electrophoresed in a running buffer of 0.02 M Mops, 0.05 M sodium acetate and 0.1 M EDTA, pH 7.0. At the completion of electrophoresis the gel was soaked in deionized water for 10 minutes to remove residual formaldehyde and then soaked with gentle mixing in 50 mM NaOH for an additional 10 minutes. The gel was then briefly rinsed in deionized water and capillary transfer of the RNA was performed onto nylon membrane (GeneScreen Plus, Dupont) using 20× SSC as the transfer buffer. After overnight transfer the membrane was rinsed briefly in 2× SSC, blotted of excess SSC with filter paper, and stored at −70°C until hybridization.

Northern hybridization was performed with cDNA probes radiolabeled using a commercial random primer labeling kit (Prime-It II, Stratagene) according to manufacturers directions. Probes included bovine aggrecan (clone PG-LA5) a gift of Dr. A. Oldberg which is an 800-bp insert into the *Eco*RI site of the pUC19 vector. The type II collagen probe was a gift of Dr. K. Yamada and is a clone of the amino terminal portion of rat pro-alpha1 type II collagen. The clone is a 571-bp *Pst*I fragment in a pGEM 3Zf- vector. Prehybridization was performed for one hour at 42°C in a buffer composed of 50% deionized formamide, 3× SSPE, 1% SDS, 0.5% BLOTTO and 0.5 mg/ml denatured, sonicated salmon sperm DNA. Following prehybridization the labeled probe was added and hybridization was continued overnight at a temperature dependent upon the cDNA probe used. Posthybridization washes were performed initially at room temperature with 2× SSC; 0.1% SDS (two washes) and then with 0.1× SSC; 0.1% SDS at elevated temperatures with the specific temperature dependent upon the probe used. Following the washes the membrane was gently blotted to remove excess buffer, wrapped in Saran and exposed to X-ray film at −70°C.

RT-PCR and semiquantitative RT-PCR. cDNA synthesis was accomplished by adding 2 μg of total RNA to a final reaction volume of 20 μl. Components and respective final concentrations in the reaction were: 1× PCR buffer (Promega); 0.01 M dithiothreitol; 12.5 μg/ml of oligo dT primers; 1.0 mM each of dATP, dTTP, dGTP and dCTP; 5.0 mM $MgCl_2$; 1.0 U/μl RNAasin; and 5.0 U/μl (Superscript II, GibcoBRL). The first strand synthesis was performed in a thermocycler at 42°C for 60 minutes. The reaction was terminated by heating the mixture to 95°C for 10 minutes.

PCR was performed in a final volume of 50 μl in a thermacycler. 1 to 2 μl of the RT reaction was generally a typical fraction of this final volume. A single PCR cycle included 3 one-minute steps each of denaturation at 94°C, annealing at 62°C and polymerization at 72°C. Analysis of PCR products was performed on ethidium bromide-stained agarose gels. Primers for amplifying bovine aggrecan have been published. The forward primer is 5′-CACTGTTACCGCCACTTCCC-3′ and the reverse primer is 5′-GACATCGTTCCACTCGCCCT-3′. This primer set amplifies a 303-bp sequence (nucleotides 6738 to 7041 of GenBank Accession No. M25616). Primers for bovine type II collagen were derived from the published sequence of a 3′ fragment of the bovine type II collagen pro-α 1 chain (clone Bc7). The forward primer 5′-CAACATGGAGACTGGCGAGA-3′ and the reverse primer 5′-TGCCCAGTTCAGGTCTCTTA-3′ amplify a 693-bp

sequence of the cDNA (nucleotides 27 to 270 of GenBank Accession No. X02420). Versican primers were generated from the published sequence from the V3 splice-variant message from the bovine gene (GenBank Accession No. AF 060459). The forward primer corresponds to the 1681 to 1700 bp segment (5′-GAGAATGGCCAG-TGGAATGA-3′) and the reverse primer corresponds to the 1914 to 1933 bp segment (5′-CGGCTCCAACGGTGATCATG-3′) of the cDNA. These primers amplify a 252 bp sequence that is expressed in all splice forms of the bovine versican message (V0, V1, V2, and V3).

For semiquantitative RT-PCR equal amounts of the MIMIC were added to each tube within a series of PCRs. We constructed a single MIMIC containing complementary sequences to primer pairs designed to amplify aggrecan, type II and type X collagens and link protein. These complementary sequences flank in tandem a 424-bp DNA fragment of the human BMP-2 genomic clone p70.3/*Sma*I (a kind gift of Dr. E. Shore). The sequences are arranged so that each message-specific primer pair gives the same size PCR product of 524 bp. Amplified products were radiolabeled by the addition of ^{32}P-dCTP. Reaction samples were electrophoresed on polyacrylamide gels which were dried and exposed to a phosphor screen (Molecular Dynamics, Sunnyvale, CA). The exposed phosphor screen was laser scanned on a commercial phosphorimager (Molecular Dynamics) and background corrected volumes were calculated for each aggrecan and MIMIC product using the ImagQuant software. Ratios of the aggrecan and MIMIC volume readings for each reaction were calculated. Statistical significance for BMP effects was determined using the Student *t* test with a significance of $P < 0.05$.

RESULTS

Pericyte cultures produce messages of the chondrogenic phenotype. Northern hybridization of total RNA isolated from pericyte cultures reveal transcripts for both aggrecan and type II collagen. Aggrecan mRNA has not been previously described in pericytes. The message was seen in primary cultures and persisted in third passage cultures which were used for further study (Fig. 1A). Total RNA isolated from fetal bovine articular chondrocytes was used as a standard for the aggrecan mRNA in Northern hybridizations. The aggrecan message from pericytes comigrated with the respective message from chondrocytes (Fig. 1B). This same relationship was seen using RT-PCR (Fig. 2). Amplified products from both pericyte and chondrocyte PCRs electrophoresed on agarose gels comigrated as single bands of expected molecular sizes determined from the published bovine-specific sequences. In addition, a sample of the RT-PCR-generated aggrecan product from pericyte RNA was isolated from the agarose gel using a commercial gel extraction kit and submitted for sequencing to the Core DNA Sequencing Facility at the School of Veterinary Medicine at the University of Pennsylvania. The sequences from samples from three different pericyte isolations each correlated with the published sequence for bovine aggrecan.

Aggrecan message from bovine pericytes is upregulated by rhBMP-2. The effects of BMP-2 on chondrogenic messages in ascorbate supplemented pericyte cultures were studied over a 37 day period. Initially using Northern hybridization the most significant finding was the upregulation of aggrecan message in pericyte cultures supplemented with rhBMP-2 compared to those without supplementation. There was no BMP-2 effect on type II collagen message (Fig. 3). Over the 37-day culture period, the magnitude of BMP-induced stimulation increased progressively. A competitive PCR technique comparing RT-generated cDNA from day 37 unsupplemented and BMP-2 supplemented cultures showed an approximate 10-fold stimulation of aggrecan amplified message by BMP-2 (Fig. 4). Semiquantitative RT-PCR on samples collected over this 37-day culture period shows the progressive BMP-induced increase of aggrecan message (Fig. 5). This increase over unsupplemented cultures was significant at almost all time points and the BMP-induced increase in aggrecan at day 37 was significant when compared to day 12 BMP-treated cultures (Fig. 6). In contrast, no significant differences in aggrecan message levels were noted among unsupplemented cultures. The type II collagen PCR product when similarly analyzed was not affected in a BMP-dependent fashion (not shown).

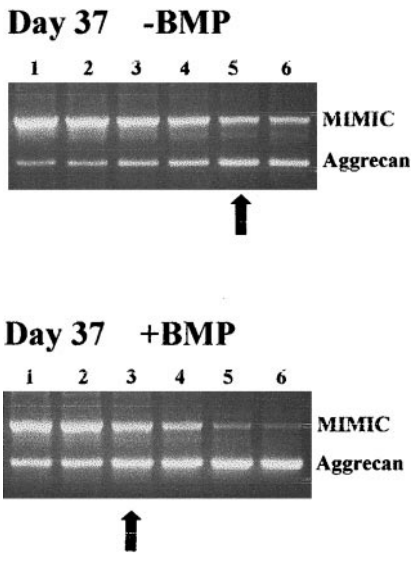

FIG. 4. Competitive RT-PCR of pericyte total RNA from untreated (−BMP) and treated (+BMP) day 37 cultures. Decreasing amounts of the competitive MIMIC were added to reactions 1 through 6. Reactions in which equal amplification of endogenous aggrecan product and MIMIC were compared between the untreated series (lane 5) and the treated series (lane 3). Reactions in lanes 3 had 10 times the amount of MIMIC compared to reactions in lanes 5. This shows a 10-fold upregulation of aggrecan mRNA by BMP in day 37 cultures.

Proteoglycan specificity of BMP-2 stimulatory effects in cultured pericytes. The BMP-dependent stimulation of aggrecan was compared with BMP-2 effects on versican using RT-PCR. In this procedure the aggrecan and versican products were coamplified in the same reaction by including both sets of primers in the reac-

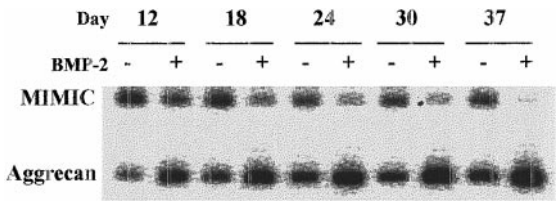

FIG. 5. A representative phosphorimage from a series of experiments looking at the effects of BMP-2 on aggrecan mRNA during the full 37-day culture period. An equal amount of competitive MIMIC is added to each reaction as well as an equal volume from the respective first strand synthesis reaction. As time in culture progresses, the BMP-2 effect becomes more pronounced with increasing signal of the endogenous aggrecan product compared to the signal of the competitive MIMIC.

tion mix. A sample of the reaction was withdrawn at specific cycles of PCR. In this manner the appearance and continued amplification of the respective products could give an assessment of their relative quantities as amplification proceeded. This analysis was carried out on samples from day 12 cultures with and without BMP supplementation and day 37 cultures with and without BMP-supplementation. The lack of a BMP effect on versican is evidenced by the uniform amplification of product between BMP-treated and untreated cultures at both time points. In contrast the BMP-dependent increase in aggrecan message may be seen

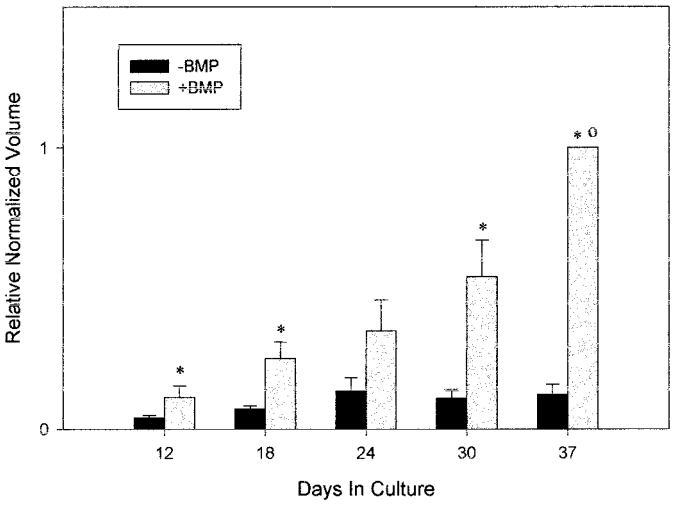

FIG. 6. Effect of BMP-2 on aggrecan mRNA determined by semiquantitative RT-PCR: Graph depicting the means of background corrected volumes from phosphorimage analysis of aggrecan message as a function of time in nontreated and BMP-2-treated pericyte cultures. The data are from three experiments. The volume readings in each experiment were normalized to the day 37, +BMP value of that experiment. The means and SEMs were then calculated from these normalized volume readings. The Student *t* test showed a significance of the BMP effect at $P < 0.05$ at almost all time points (*). The upregulation of aggrecan by BMP at day 37 was also significant compared the degree of upregulation by BMP at day 12 (o).

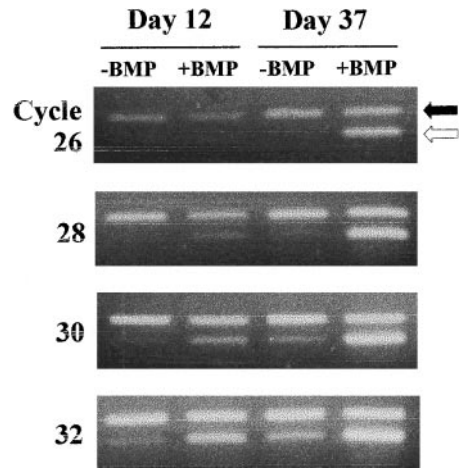

FIG. 7. Coamplification of versican (black arrow) and aggrecan (white arrow) from day 12 and day 37 nontreated (−BMP) and BMP-treated (+BMP) cultures. The aggrecan message from BMP-treated cultures is more rapidly amplified than the message from nontreated cultures as discussed. A BMP-related effect in amplification is not seen in the same samples with versican. This indicates some level of specificity of BMP stimulation among the proteoglycans elaborated by pericytes.

by the more rapid amplification of the product associated with BMP-supplementation as PCR cycling progresses (Fig. 7).

DISCUSSION

Its phenotypic plasticity and vascular association make the pericyte a prime candidate for fulfilling the role of a widely distributed, multipotential mesenchymal stem cell. Its collective expression of alkaline phosphatase enzyme activity, type I collagen protein and osteocalcin immunoreactivity indicates an osteogenic role for the pericyte (6, 7, 11) This is further supported by recent evidence linking STRO-1 immunoreactivity and bone sialoprotein message to cultured pericytes (8). Expression of type II collagen *in vitro* and *in vivo* as well as evidence of type X collagen immunoreactivity *in vitro* also suggests a chondrogenic role for this cell type (9–11). The work reported here further supports the chondrogenic nature of the microvascular pericyte. Northern analysis and RT-PCR analysis of total RNA isolated from pericyte cultures demonstrated the presence of aggrecan and type II collagen messages. This to our knowledge is the first report describing aggrecan associated with pericytes.

In fracture healing endochondral cartilage of the inductive callus develops within granulation tissue arising from the fracture hematoma (1). In fact, electron microscopy studies of microvessels at the site of fracture show proliferation of pericytes and their production of collagen (Hunt and Brighton). That BMP

may locally affect the phenotype of these proliferating mesenchymal cells is supported by studies demonstrating the association of BMPs and their receptors with the chondro-osteogenic cells of the developing fracture callus. *In situ* hybridization studies have shown BMP-2 associated with undifferentiated mesenchymal cells and chondroblasts in healing rabbit mandibular fractures (13). BMP-4 was demonstrated associated with proliferating cells at rib fractures in mice before new cartilage or bone was evident histologically (14). An immunohistochemical study employing antibodies to BMP receptors showed increased expression of the receptors in chondrocytes as well as fibroblast-like cells in the endochondral callus of a mouse femoral fracture model (15).

In this study BMP-2 was shown to have a regulatory influence on aggrecan expression by pericytes *in vitro.* The degree of this response is markedly enhanced by the length of time in culture. It is reasonable to speculate that the heightened response to BMP during the late culture period is a cell density effect. In the post confluent period ascorbate supplemented pericyte cultures begin to multilayer and form colonies which stain positively with Toluidine blue. The *in vitro* correlation of high cell density and the maintenance of the expression of the chondrogenic phenotype has been well documented in cultured articular chondrocytes and chondrogenic limb bud mesenchymal cells. The high cell density of postconfluent pericyte cultures may confer a BMP-responsive chondrogenic phenotype with specific respect to aggrecan production.

Members of the BMP family have been shown to increase proteoglycan production by chondrocytes both *in vivo* and *in vitro.* BMP-2 treatment of adherent monolayers of articular chondrocytes increased aggrecan message that was gradually lost over time in untreated cultures (16). Although BMP had a stimulatory effect on aggrecan message in our study we did not see the same effect with type II collagen. This scenario is like that seen with periosteal-derived cells which also expressed message for type II collagen in culture. Exposure to BMP-3 or BMP-4 had no effect on type II collagen steady-state message. Interestingly, transforming growth factor-beta supplementation did result in an increase in type II collagen message (17).

Finally, we also present evidence that the BMP effect seen with aggrecan has some level of specificity with respect to other proteoglycans elaborated by the pericyte. Based upon coamplification of aggrecan and versican messages in RT-PCRs sampled at specific cycles to detect progressive amplification of these coproducts, the results suggest BMP has no regulatory effect on RT-PCR-amplified versican product. Aggrecan is the major proteoglycan of cartilage and is a classic marker for that phenotype. Versican is also produced by articular chondrocytes but in much smaller amounts than aggrecan. Versican is more widely distributed than aggrecan being produced by connective tissue cells such as fibroblasts, kidney mesangial cells, and smooth muscle cells. Versican is the major proteoglycan associated with precartilagenous condensations of the bone of the developing limbs. The effects of BMPs on versican have not been formally studied although our data would suggest that these morphogens have no stimulatory effect on versican. It is not known whether BMPs may affect the amount of specific alternative splice forms. Studies do demonstrate that versican is upregulated by transforming growth factor-beta and platelet-derived growth factor (18, 19, 20).

In conclusion, limited data has emerged to support a chondrogenic phenotype for the microvascular pericyte. Our findings of type II collagen and aggrecan messages by Northern hybridization and RT-PCR from RNA isolated from bovine pericyte cultures support a chondrogenic capacity for this cell type. Additionally, rhBMP-2 stimulates levels of aggrecan measured as an amplified product in semiquantitative RT-PCR. The qualitative lack of a similar response with versican suggests some level of specificity of the BMP response of the proteoglycans elaborated by pericytes. This suggests a mechanism whereby BMP elaborated by injured bone can specifically induce chondrogenesis in a multipotential mesenchymal cell population thereby achieving recapitulation of bone via endochondral ossification. The characterization of both osteogenic and chondrogenic capacities for the microvascular pericyte provides a means to determine specific factors that favor respective phenotypic commitments within a single cell type.

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