Joint and Bone Disease in Mucopolysaccharidoses VI and VII: Identification of New Therapeutic Targets and BioMarkers Using Animal Models

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ABSTRACT

The mucopolysaccharidoses (MPS) are inherited metabolic disorders resulting from the defective catabolism of glycosaminoglycans. In this report, we find that the stimulation of MPS connective tissue cells by the inflammatory cytokines causes enhanced secretion of several matrix-degrading metalloproteinases (MMPs). In addition, expression of tissue inhibitor of metalloproteinase-1 was elevated, consistent with the enhanced MMP activity. These findings were not restricted to one particular MPS disorder or species, and are consistent with previous observations in animal models with chemically induced arthritis. Bromodeoxyuridine incorporation studies also revealed that MPS chondrocytes proliferated up to 5-fold faster than normal chondrocytes, and released elevated levels of transforming growth factor-beta, presumably to counteract the marked chondrocyte apoptosis and matrix degradation associated with MMP expression. Despite this compensatory mechanism, studies of endochondral ossification revealed a reduction in chondro-differentiation in the growth plates. Thus, although MPS chondrocytes grew faster, most of the newly formed cells were immature and could not mineralize into bone. Our studies suggest that altered MMP expression, most likely stimulated by inflammatory cytokines and nitric oxide, is an important feature of the MPS disorders. These data also identify several proinflammatory cytokines, nitric oxide, and MMPs as novel therapeutic targets and/or biomarkers of MPS joint and bone disease. This information should aid in the evaluation of existing therapies for these disorders, such as enzyme replacement therapy and bone marrow transplantation, and may lead to the development of new therapeutic approaches. (*Pediatr Res* **57:** 701–707, 2005)

Abbreviations
BMT, bone marrow transplantation
BrdU, bromodeoxyuridine
ECM, extracellular matrix
ERT, enzyme replacement therapy
GAGs, glycosaminoglycans
IL1-β, interleukin 1-beta
MMPs, matrix metalloproteinases
MPS, mucopolysaccharidoses
NO, nitric oxide
OA, osteoarthritis
RA, rheumatoid arthritis
TGF-β, transforming growth factor-beta
TIMP, tissue inhibitor of metalloproteinase
TNF-α, tumor necrosis factor-alpha

The accumulation of partially degraded glycosaminoglycans (GAGs) in late endosomes and lysosomes of connective tissue cells is characteristic of a family of heritable lysosomal storage diseases known as the mucopolysaccharidoses (MPS) (1). The MPS family consists of 12 chronic and progressive syndromes, each with a specific enzyme deficiency leading to a character-istic pattern of accumulating GAGs within lysosomes. Among the different MPS disorders, there are several clinical characteristics present in most affected individuals. These include anterior hypoplasia of lumbar vertebrae, enlarged diaphyses of the long bones, underdeveloped epiphyseal centers, marked dwarfism, degenerative joint disease, dysostosis multiplex, facial dysmorphia, organomegaly, corneal clouding, and in most syndromes mental retardation (1). Cartilage is a major site of pathology in these diseases, leading to painful joints, poor joint mobility, poor bone growth, and an abnormal larynx and trachea producing breathing abnormalities often requiring tracheotomies.

There are numerous MPS animal models available for study, either naturally occurring or generated through targeted disruption of the corresponding mouse gene (*e.g.*, 2–5). These animal models have biochemical and pathologic features that are similar to their human counterparts, and their availability has facilitated the evaluation of various therapeutic approaches and a better understanding of the disease pathogenesis.

Using these animals, several therapies have been investigated for the treatment of the MPS disorders, including bone marrow transplantation (BMT) (6–8), enzyme replacement therapy (ERT) (9–12), and gene therapy (13). Varying results have been obtained from these endeavors, in part due to differences in the procedures used and/or specific issues related to the particular disease and animal model studied. Currently, for human patients BMT is undertaken for several of the MPS disorders, and ERT is available for MPS I and under evaluation for MPS II and VI (14). However, these therapies have proven to be very limited in their ability to prevent or reverse most of the serious bone and cartilage complications. In addition, few biomarkers exist to monitor the severity of bone and joint involvement in these disorders, or the effects of therapies.

In part, the lack of effective bone-directed therapies is due to the poorly characterized underlying pathophysiology. Morphologic analysis of MPS growth plates has revealed clusters of enlarged, GAG-containing cells that disrupt the normal columnar architecture of the growth plate cartilage, presumably leading, in part, to the bone growth abnormalities (15,16). This has led to the suggestion that the main tissue site of pathology in these disorders is the cartilage, rather than the bone itself. The articular cartilage also is thicker in MPS VI and VII animals with a hyperplastic synovium, suggesting that there are also abnormalities in articular cartilage matrix formation and function (17).

Our previous studies have demonstrated increased apoptosis of MPS chondrocytes, leading to a depletion of proteoglycans and total collagen in the cartilage of MPS animals (18). During the course of this work, we also observed that many analogies could be made between the cartilage of MPS individuals and those with osteo- or rheumatoid arthritis (OA and RA, respectively). Similarities exist in the secretion of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and IL-1 beta (IL-1β), nitric oxide (NO) production, apoptosis of chondrocytes, and reduced proteoglycan content in the extracellular matrix (ECM). Matrix metalloproteinases (MMPs) are key enzymes involved in ECM degradation, and enhanced MMP activity is an important feature of arthritis. MMP transcription is regulated by growth factors and cytokines, while specific tissue inhibitors of metalloproteinase (TIMPs) regulate their translation and proenzyme activation (19,20). In arthritis, tissue destruction often correlates with the imbalance of MMPs over TIMPs. For example, in RA, synovial fluid contains elevated levels of MMP-2 and MMP-9, both of which degrade the joint matrix because of their gelatinase activities (21). In addition to proinflammatory cytokines, NO production has also been shown to act as a proinflammatory mediator leading to enhanced MMP production (22).

Herein, we report the further characterization of joint and bone disease in MPS animal models. Enhanced expression of MMP-2 and MMP-9 was observed in the MPS animals, and was associated with abnormal expression of TIMP-1. In addition, we show that increased expression of TGF-β in MPS cartilage leads to enhanced proliferation of chondrocytes in these tissues, but that a majority of these newly formed cells remain immature and cannot mineralize into bone. Based on these findings, we suggest that further research on the treatment of MPS joint and bone degeneration should be based on the emerging picture of inflammatory disease in these disorders, and its particular effects on cartilage. In addition, we suggest that many of the molecules we have identified in the course of these studies may serve as new biomarkers to monitor MPS disease progression and treatment, as they have in arthritis, and may also be important therapeutic targets. Lastly, we suggest that the MPS animal models may be useful to the arthritis research community since they are naturally occurring and relatively homogeneous, and could serve as adjuncts to the chemically induced arthritis models that are currently used.

METHODS

Animals. The MPS VI cat and rat models, and MPS VII dog model have been previously described (2–4). Affected and control animals were raised under National Institutes of Health and USDA guidelines for the care and use of animals in research. Rats were maintained at the Mount Sinai School of Medicine, while the larger animal models were maintained at the University of Pennsylvania School of Veterinary Medicine. The animals were housed with *ad libitum* food and water, 12-h light cycles at 21°C, with 12–15 air changes per hour. Euthanasia was performed on cats and dogs using 80 mg/kg of sodium pentobarbital (Veterinary Laboratories, Lenexa, KS) in accordance with the American Veterinary Medical Association guidelines. Euthanasia of rats was performed using carbon dioxide inhalation. Breeding colonies were maintained under IACUC approved protocols and genotyped by clinical/pathologic screening of WBC and by PCR-based methods (23,24).

Sample isolation and chondrocyte cultures. Proximal humeri, femurs, and proximal tibias were collected from age-matched normal and MPS animals and placed in either PBS (PBS) for chondrocyte isolation or fixed in neutral buffered 10% formalin (Sigma Chemical Co. Chemical, St. Louis, MO) for immunohistochemical assessment (see below). For immunohistochemistry, the formalin-fixed bones were decalcified in 8% formic acid (Sigma Chemical Co. Chemical) for 5 d, embedded in paraffin, and sectioned (5 μm). The tissues were obtained from four animals in each age group, ranging from 2 wk to 12 mo in the rats, 3 to 60 mo in the cats, and 2 to 6 mo in dogs.

To establish primary chondrocyte cultures, the articular cartilage was sliced from the underlying bone, and washed in minimal essential medium (MEM, Invitrogen, Carlsbad, CA). The cartilage was minced and digested with 1% hyaluronidase for 1 h at 37°C, washed three times with PBS containing 1 mM EDTA, and sequentially incubated with 2.5% trypsin (Invitrogen) and 0.01 M EDTA for 1 h at 37°C, followed by further digestion at 37°C for 8 h with 0.2% collagenase (Sigma Chemical Co.) prepared in MEM containing 10% FCS. Cells were isolated by centrifugation (450 g), washed twice, and plated in 96 well plates at a concentration of 7×10^4 cells in MEM containing 10% fetal bovine serum, 1% glutamine, penicillin (150 units/mL), and streptomycin (50 μg/mL). After 24 h, the media was changed to serum-free, and then collected after 48 h and either immediately analyzed or stored at −20°C.

Samples of synovial fluid also were obtained from the joints of normal and MPS VII dogs. Dogs were euthanized and the joints were examined to confirm the lack or presence of gross pathologic changes. Samples of synovial fluid were collected from the following joints: stifle, elbow, shoulder and coxofemoral. After isolation, synovial samples were divided into aliquots and stored frozen at −70°C until analyzed. Synovial membranes from MPS VII and normal dogs also were collected from stifle following euthanasia, embedded in OCT media (Sakura Finetek, Torrance, CA), frozen, and stored at −70°C until analyzed.

Immunohistochemical staining. Bone sections were deparaffinized, rehydrated in a series of descending ethanol concentrations, and digested for 1 h with testicular hyaluronidase (2 mg/mL, Sigma Chemical Co.) in PBS. Immunohistochemistry for MMP-2 was performed using an anti-mouse MMP-2 (72 kD collagenase IV) MAb (NeoMarkers, Fremont, CA), while MMP-9 was detected using an anti-rat MMP-9 (95 kD collagenase/gelatinase B) MAb

(Chemicon International, Temecula, CA). Immunohistochemical detection of osteonectin was carried out using an anti-human osteonectin antibody (Chemicon International). Dilutions of the specific antisera were applied in PBS containing 1% BSA, and the sections were incubated overnight at 4°C. After several rinses with PBS, visualization was performed using the streptavidin-biotin complex method (Histostain-Plus kit, Zymed, San Francisco, CA). Biotin conjugated secondary antibodies were applied and incubated for 10 min at room temperature. The slides were washed twice with PBS, incubated with streptavidin-conjugated horseradish peroxidase for 10 min, stained with diaminobenzidine, and counterstained with hematoxylin.

Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from cultured chondrocytes using the Rneasy Mini Kit (Qiagen, Valencia, CA) and processed according to the manufacturers instructions. Total RNA was reverse transcribed into first-strand cDNA (cDNA) using the SuperScript II RT kit (Invitrogen) utilizing random hexanucleotide primers. For cDNA amplification, 1.0 μg of cDNA, 10$\times$ PCR buffer (100 mM Tris-HCl, 500 mM KCl, 25 mM MgCl$_2$), 2.5 mM dNTPs, 20 pmol of sense and anti-sense primers, and recombinant *Taq* polymerase (Invitrogen) was mixed in a total reaction volume of 25 μL. PCR amplification was performed using oligonucleotides corresponding to cDNA sequences for MMP-2 (25), MMP-9 (25), and TIMP-1 (26).

In situ *gelatinase activity in synovial membranes.* A method of fluorescent substrate overlay was used to detect *in situ* proteinase activity in sections of intact frozen tissues (27). Synovial membranes from MPS VII and normal dogs were snap-frozen and embedded in OCT media (Sakura Finetek). Transverse sections (6 μm) were immediately overlaid with 20 μL of FITC-labeled gelatin (2 μg/mL in 50 mM Tris-HCl, pH 7.6) (Molecular Probes, Eugene, OR). Coverslips were applied, and samples were incubated in a humidified chamber at 37°C for 3 h. Areas of proteolytic activity observed during microscopic examination were compared with areas from serial sections in which MMP activity was inhibited with 1,10-phenanthroline monohydrate. Images were captured by a Nikon E600 fluorescent microscope (Nikon Inc, Melville, NY) and associated Phase III Image Pro analyzer software (Phase III, PA).

Proliferation assays. Chondrocytes from normal and MPS age-matched animals were seeded at $1-4 \times 10^5$ cells/mL into 96 well culture plates and grown for 72 h in the presence of bromodeoxyuridine (BrdU). A cell proliferation assay kit (Calbiochem, SanDiego, CA) was used to detect BrdU incorporation into newly synthesized DNA of proliferating cells.

Immunoassays. Il-1β, TIMP-1, TGF-β, and TNF-α protein expression was quantified by immunoassays using normal and MPS plasma, conditioned chondrocyte media, and/or synovial fluid using rat and human Quantikine® immunoassay kits (R&D Systems, Minn. MN) according to the manufacturer's protocols. MMP-2 and -9 enzymatic activities also were measured using commercial assay kits (Chemicon International) according to the manufacturer's protocol. Before use, samples for the MMP assays were activated with 5 mM p-aminophenylmercuric acetate (Sigma Chemical Co. Chemical) at 37°C for 30 min. All immuno- and enzymatic assay experiments were performed in duplicates and for each experiment at least three samples were studied per animal. The student's 2-tailed *t* test was used to calculate the statistical significance of the difference between the two groups.

RESULTS

Gelatinase expression. Abnormal regulation of gelatinase activity is an important factor in arthritic bone disease. To assess total gelatinase activity in tissues from MPS animals (MPS VI rats and cats, and MPS VII dogs), cartilage and synovial membranes were subjected to *in situ* gelatinase assays. As shown for the synovial membranes of MPS VII dogs (Fig. 1), markedly enhanced gelatinase activity was observed in tissues from each of these MPS models. High levels of MMP activity were present in the synoviocytes, as well as in the extracellular spaces of the underlying synovial membranes (Fig. 1*B*). The majority of this enhanced activity was attributable to MMPs because co-incubation with 1,10 phenanthrolene, a metalloprotease inhibitor, reduced the *in situ* gelatinase staining (Fig. 1*C*).

MMP associated gelatinase activity also was evaluated in serum free-conditioned media obtained from primary chondrocytes of each MPS animal model. The MPS conditioned media had from 67.8% to 330% more MMP gelatinase activity when compared with media from normal cells. To investigate these findings further, immunohistochemical staining for MMP-2 and -9 was carried out using tissues from the MPS animals. As shown in Figs. 2 and 3, enhanced MMP immunostaining was seen in the cartilage and bone marrow of the MPS animals, confirming the *in situ* and *in vitro* gelatinase assays. Enhanced MMP expression was primarily detected in the superficial and intermediate zones, and bone marrow osteoclasts and osteoblasts. Neutrophils and macrophages in the bone marrow also stained positive for the two MMPs. In most cases, the MMP immunostaining was intracellular and/or pericellular.

To confirm the protein expression results, MMP gene expression also was examined in the MPS animals by RT-PCR analysis. As shown in Fig. 4, expression of the MMP-2 and -9 genes was significantly enhanced in articular chondrocytes, consistent with enhanced MMP gelatinase activity.

TIMP-1 expression. TIMP-1 is an important component of the complex regulatory pathway that controls gelatinase activity, and a balance of gelatinase and TIMP-1 is required for normal bone homeostasis. In arthritis, elevated gelatinase activity is associated with elevated TIMP-1, however the net ratio of gelatinase activity to TIMP-1 is significantly increased, leading to enhanced bone degradation (21,28,29). TIMP-1 expression was measured in serum free conditioned media obtained from primary MPS VI rat chondrocyte cultures, and was found to be elevated $\sim$35% compared with cultures established from age-matched normal animals (Fig. 5*A*). Similarly, RT-PCR analysis revealed slightly
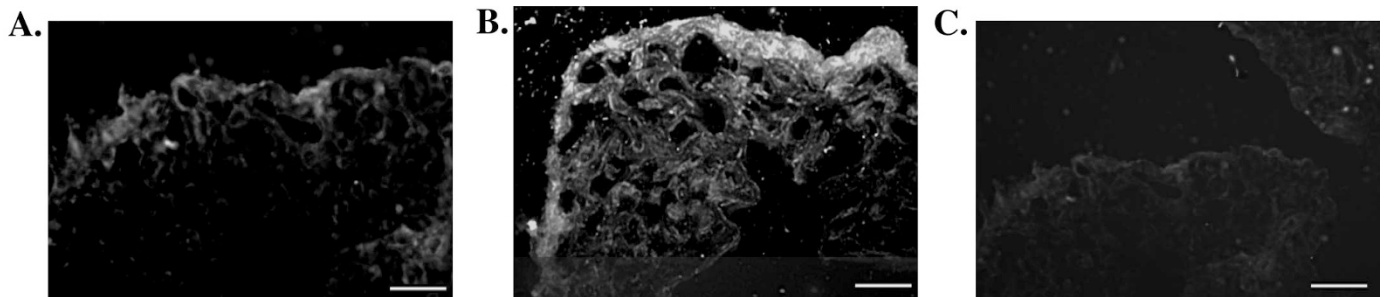
Figure 1. *In situ* gelatinase activity in normal and MPS VII dog synovial membranes. (*A*) Gelatin overlay of synovium from normal one-year old dog. (*B*) Gelatin overlay of age-matched MPS VII dog synovium showing enhanced MMP activity concentrated in the synoviocytes and the underlying extracellular matrix of the hyperplastic synovium. (*C*) Gelatin overlay of MPS VII dog synovium treated with an MMP inhibitor (1,10-phenanthroline monohydrate), resulting in quenching of the activity. Magnification 60$\times$.
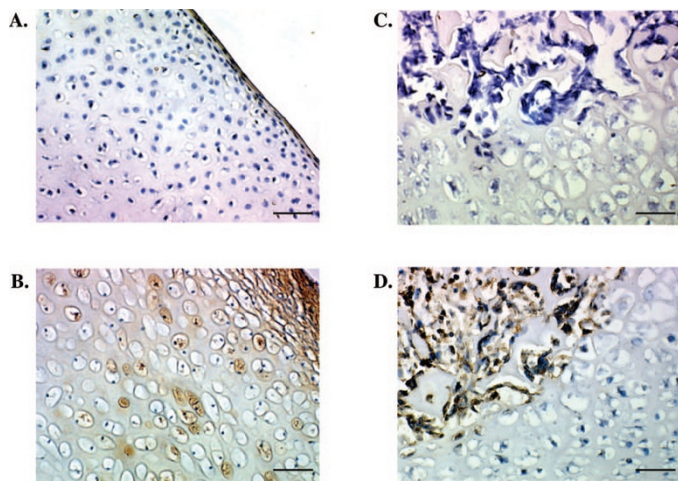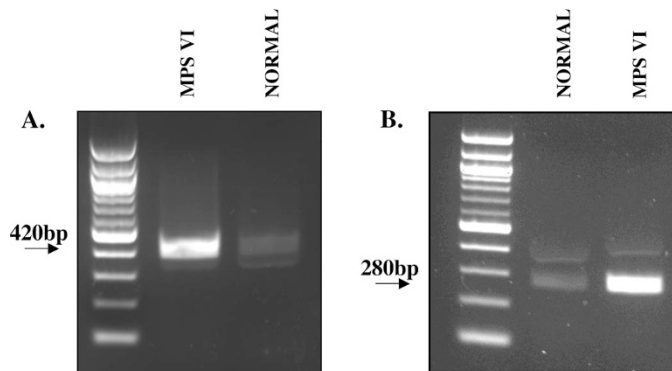
Figure 2. Immunostaining of MMP-2 in rat tibia. (*A*) MMP-2 staining in superficial and intermediate cartilage of normal 6-month old rats. (*B*) MMP-2 staining in the superficial and intermediate cartilage from age-matched MPS VI rats. Note that most of the MMP-2 staining is intracellular with slight staining of the extracellular matrix. (*C*) MMP-2 staining in subchondral bone of normal 6-month old rats. (*D*) MMP-2 staining in age-matched MPS VI rat subchondral bone. Note that most of the enhanced immunostaining is observed in the bone marrow and within osteoblasts (*arrow*). Magnification 60×.

Figure 4. MMP-2 (*A*) and MMP-9 (*B*) mRNA expression in rat articular cartilage. RT-PCR analysis was performed from mRNA obtained from tibial plateau cartilage. Equivalent amounts of DNA (1 μg) from 6-month old normal and MPS VI rats were used as templates for the PCR.

elevated TIMP-1 gene expression in chondrocytes (Fig. 5*B*). These results are similar to those found in arthritis, and are consistent with elevations of proinflammatory cytokines that we have previously shown in these animals (18). A 3-fold TIMP-1 elevation also was observed in synovial fluid from MPS VII animals (data not shown).

Cell proliferation and TGF-β expression. In our previous work we observed marked apoptotic cell death in MPS cartilage (18). However, despite increased chondrocyte death, no significant cell loss was observed. To address this apparent contradiction, we determined the proliferation rate of MPS chondrocytes in culture. As shown in Fig. 6 for MPS VI rats and cats, but also observed for MPS VII dogs, BrdU incorporation studies revealed a markedly increased proliferation rate of these cells (up to 5-fold), perhaps explaining why little cell loss was observed in the MPS cartilage despite massive cell death.

TGF-β is an important growth factor involved in regulating cell proliferation, and also plays an important role in the control of TIMP-1 gene expression (30). As shown in Fig. 7,

TGF-β levels in serum free conditioned media from primary MPS VI articular chondrocyte cultures was significantly elevated, consistent with the enhanced cell proliferation rate and elevated TIMP-1 and gelatinase expression. However, despite the fact that MPS chondrocytes were proliferating at a rapid rate (presumably to compensate for the enhanced apoptosis), the functional capacity and developmental program of these newly formed cells remained unknown. We also carried out immunostaining for osteonectin, a marker of maturing chondrocytes, on growth plate cartilage from MPS VI rats. As can be seen in Fig. 8, osteonectin staining was markedly reduced in the MPS animals, suggesting that the newly formed chondrocytes were not mature, and thus, could not undergo hypertrophy and develop into bone.

DISCUSSION

We previously reported enhanced chondrocyte apoptosis in the articular cartilage of MPS animal models, and found that the levels of IL-1β, TNF-α, and NO were markedly increased in the media of MPS chondrocyte cultures compared with normal cells. Furthermore, these levels were significantly greater in cells from older MPS animals when compared with young (18). To explain why, despite the very high apoptosis rate, the overall chondrocyte cell number was not markedly reduced in these animals, we postulated that TGF-β release
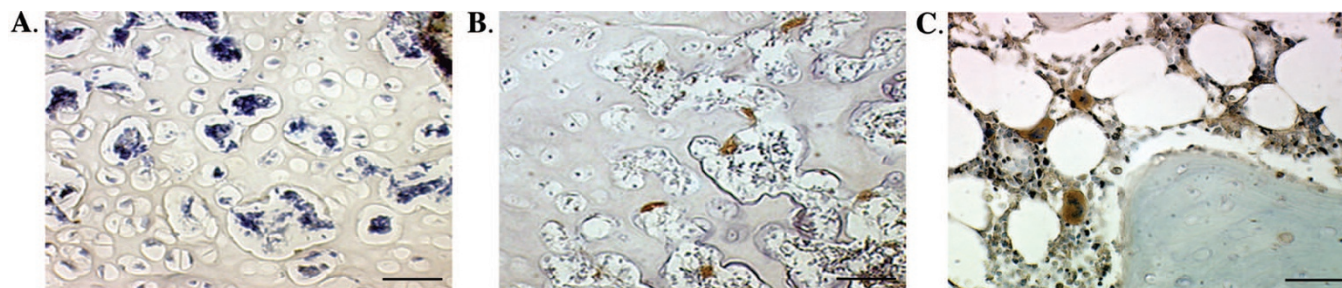

Figure 3. Immunostaining of MMP-9 in rat tibia. (*A*) Normal, 6-month old rat tibia. (*B*) Age-matched MPS VI rat tibia showing enhanced MMP-9 staining in multi-nuclear osteoclasts (*arrow*) Magnification 40×. (*C*) MPS VI rat tibia showing MMP-9 staining in neutrophils (*arrowhead*) and osteoblasts (*arrow*) Magnification 100×.
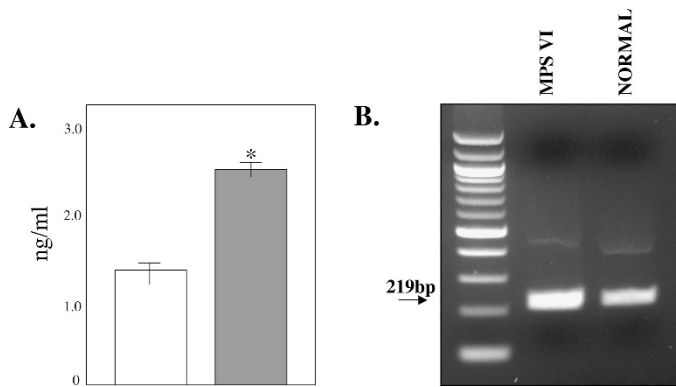
Figure 5. TIMP-1 expression in rats. (*A*) TIMP-1 concentration in supernatants from normal (*open box*) and MPS VI (*solid box*) rat chondrocytes. Data represent median values obtained from 4 individual animals. (*B*) TIMP-1 mRNA expression in tibial plateau cartilage. Equivalent amounts of DNA (1 μg) from 6-month old normal and MPS VI rats were used as templates for the PCR.

might also be enhanced. TGF-β is known to have a mitogenic effect on cells and to counteract the effects of apoptosis (31). Indeed, as described above, this was found to be the case, and proliferation studies performed on normal and MPS VI articular chondrocytes using BrdU immunolabeling revealed that the MPS cells were proliferating up to 5-fold faster than normal cells. Thus, MPS animals appear to be compensating for enhanced cell death in the cartilage by increasing cell proliferation.

However, despite this compensation mechanism, marked bone and joint abnormalities are still observed in the MPS animals. Nuttall *et al.* (32) has suggested that the osteopenia observed in MPS VI may be due to a delayed turnover of hypertrophic chondrocytes in the growth plate. Therefore, we used osteonectin as a marker for proliferating and differentiating chondrocytes and chondroid matrix formation, and observed a marked reduction in hypertrophic cells in the epiphyseal plate of MPS VI rats. While the mechanism for the reduced hypertrophy remains unknown, intriguingly, TGF-β inhibits terminal differentiation of immature chondrocytes (30,33), and enhanced expression of TGF-β in the MPS ani-
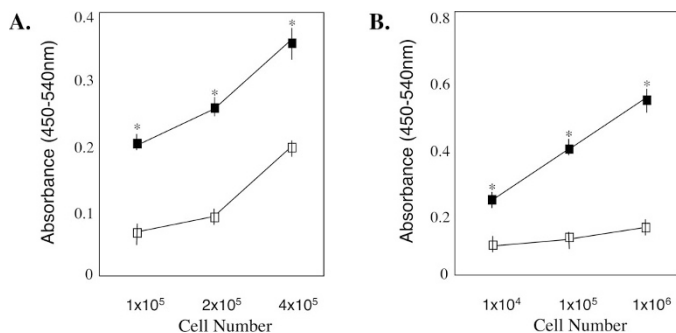
Figure 6. Chondrocyte proliferation studies in MPS VI animals. 6-month-old MPS VI rat (*A*) and one-year-old MPS VI cat (*B*) chondrocytes were grown in the presence of BrdU for 72 h, and proliferation rates were compared with chondrocytes grown from age-matched normal animals. Closed and open boxes represent MPS and normal animals, respectively. Data represents median values obtained from four independent experiments. MPS VI rat chondrocytes showed a 2-fold increase in proliferation rate, whereas MPS VI cat chondrocytes showed up to a 5-fold increase.
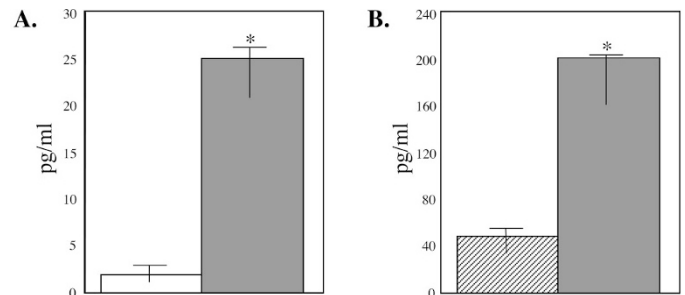

Figure 7. TGF-β activity in MPS VI chondrocytes. TGF-β concentrations were determined in serum-free culture supernatants after 48 h. Data represent median value of 4 individual animals. (*A*) Chondrocytes were obtained from 6-month old MPS VI rats (*solid box*) and normal age matched controls (*open box*). (*B*) Chondrocytes were obtained from one-year old MPS VI cats (*solid box*) and age-matched, MPS VI heterozygous cats (*hatched box*).

mals may, in part, be responsible for this observation. In addition, osteonectin, which is required for forming bone from cartilage, is a substrate for MMP-2 and -9, both of which are elevated in MPS bones and joints (see Figs. 1–4). Clearly, whatever the underlying mechanism, one component of the osteopenia in this MPS model appears to arise from a defect in bone production due to a reduction in hypertrophic chondrocytes available for mineralization into bone.

There are numerous analogies between our findings in the MPS animal models and arthritis. For example, MMPs are particularly important in the chondrolytic processes that contribute to degenerative changes in arthritis, and arthritic chondrocytes release elevated levels of MMP-2 and -9 (34). MMPs are secreted from cells as inactive zymogens, and the pro-forms are activated by serine proteinases and other classes of MMPs. In addition, MMP activities are reduced by tissue inhibitors of metalloproteinases (*e.g.*, TIMP-1) (35), while NO and proinflammatory cytokines induce their synthesis and/or activate them (36,37). Thus, the precise regulation of MMP activity is crucial for maintaining the proper balance of tissue remodeling and damage. In fact, abnormal articular extracellular matrix remodeling in RA is due, in part, to abnormalities in this precise MMP regulation pathway (22).

Similar to RA, in the MPS disorders synoviocytes and chondrocytes are the primary source of NO (38,39). Cytokines, such as IL-1β and TNF-α, also are produced by MPS chondrocytes, synoviocytes, and macrophages, and significantly up-regulate MMP gene expression (this manuscript), as they do in arthritis. Notably, we also found an increase in TIMP-1 gene and protein expression in cultured MPS cells, but we presume
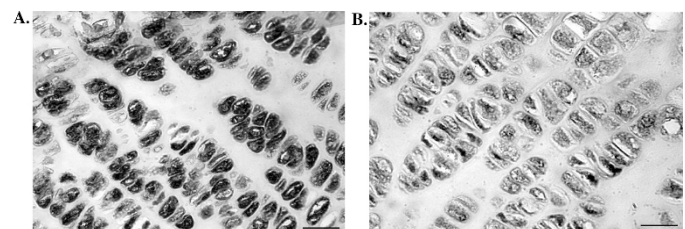
Figure 8. Osteonectin staining of rat hypertropic chondrocytes. (*A*) Staining in a normal 6-month old rat. (*B*) Reduced immunostaining in an age-matched, MPS VI rat indicating chondrocyte immaturity. Magnification 60×.

Table 1. *Potential, novel biomarkers for the MPS disorders**

Potential biomarker	Reference
IL-1β	40
MMP-2	41
MMP-9	42
NO	43
TGF-β	44
TIMP-1	21
TNF-α	40

* Note that each of these molecules can be measured in serum and/or synovial fluid (see references).

that this TIMP-1 cannot effectively compensate for the dramatic over-expression of MMP-2 and MMP-9, thus leading to enhanced matrix degradation. This observation was similar to the situation in OA, where TIMP-1 mRNA and protein expression is up-regulated, although its activity is reduced.

Our results suggest that strategies that use inhibition of cytokines, over-expression of TIMPs (or TIMP activation), and/or the prevention of apoptosis should be explored as treatments for the bone and joint disease in the MPS disorders. While these approaches will not address the underlying GAG storage abnormalities and may not be completely effective alone, in combination with ERT or gene therapy, they may be useful adjuncts. Our results also provide further documentation that the degenerative joint and bone disease in MPS animals is remarkably similar to that occurring in chemically induced animal models of arthritis. Thus, the MPS animals represent naturally occurring, somewhat inbred models that should be useful to the arthritis research community. Investigators in this area have been severely limited by their reliance on chemically induced models that are not homogenous and nonphysiologic. Lastly, this research has revealed several molecules (*e.g.*, NO, proinflammatory cytokines, MMPs, TIMP-1) that could be used as biomarkers to monitor disease severity and treatment in MPS patients (Table 1). Several of these markers can be easily measured in synovial fluid and/or serum, and may be a more direct measure of changes in the joints and bones than GAG release into urine, which is currently used.(40–44).

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