INTRODUCTION

ADAMTS5 cleaves the extracellular matrix (ECM) proteoglycans aggrecan and versican, which are major components of cartilage and soft-tissues respectively.1,2 The aggrecanase activity is believed to be essential in the destructive process of arthritis, since mice with a deletion of the catalytic domain of ADAMTS5 are resistant to both inflammatory and instability induced arthritis.3,4 Whether arthritis is due to overproduction of aggrecanase by the chondrocytes or other joint tissues or to post-translational activation of aggrecanase present in the cartilage matrix is currently unresolved.5,6 Adamts5 mutant mice and combinatorial mutants of Adams4 and Adamts5 are apparently normal and little is known about the normal function of ADAMTS5 expressed in the musculoskeletal system.1,3,5 The **goal** of these studies was to identify sites at which Adamts5 may function, by defining its expression in the normal murine musculoskeletal system and in an ex-vivo model of joint inflammation.

METHODS

Trangenic mice:

*Adamts5*°/° mice (Jackson Laboratories, Bar Harbor, ME) were outbred for over eight generations into the C57Bl/6 strain and used under an approved protocol. These mice contain an IRES-LacZ cassette inserted into exon 2 of *Adamts5* which generates a null allele.2 Tissues were obtained post-mortem at one week, six weeks and eight months of age after euthanasia done as per recommendations of the American Veterinary Association Panel on Anesthesia. *Adamts5°/°* animals were sacrificed at 8 weeks of age for the ex-vivo joint inflammation model, similar to femoral head cultures previously described.3 *Adamts5°/°* mice served as a negative control for this analysis.

β-Galactosidase (β-Gal) Staining, Tartrate Resistant Acic Phosphatase (TRAP) Staining, Histology and Immunohistochemistry (IHC):

For determination of β-Gal staining in normal tissues, a previously published protocol was used.8 Tissues treated with cytokines were stained at 37 °C for 2 hours. They were then decalified in EDTA, and paraffin-embedded for sectioning and histology. Immunostaining was done using the aggrecanase neo-epitope antibody anti-NITEGE (JSCNIT Affinity Bioreagents, Golden, CO), anti-PACE4 (Sigma, Saint Louis, MO) or anti-endomucin antibodies overnight at 4°C. TRAP staining was done using a leukocyte acid phosphatase kit (Sigma, Saint Louis, MO).

**Ex-vivo model of joint inflammation:**

Animals were euthanized, the knee joint was sectioned sagittally in the midline using a scalpel, washed three times, then incubated with DMEM containing 10% fetal bovine serum for 48 hours at 37°C with 5% CO2. After 48 hours the media were removed and the knees were incised using a scalpel, washed three times, then incubated with anterior cruciate ligament and fibrochondrocytes of its insertion (40X). Sections were counterstained with eosin (red) postchondrocytes and fibrochondrocytes of its insertion (40X). Sections were counterstained with eosin (red).

**RESULTS**

Tissues from *Adamts5°/°* mice showed no β-gal staining under the conditions used. Therefore, β-gal staining represents *Adamts5* expression sites. *Adamts5* was highly expressed at specific sites in the murine musculoskeletal system (Fig. 1). There was expression in osteoblasts, ligament fibroblasts, tenocytes, tenosynovium, developing, but not mature skeletal muscle, synovial fibroblasts, endothelial cells of the blood vessels in bone, as well as in the endothelium of bone marrow sinusoids. Surprisingly, there was only sparse, sporadic expression in periosteal osteoblasts, bone marrow endothelial cells, and skeletal muscle compared to their eight month old counterparts. TRAP staining and endomucin staining confirmed that *Adamts5* was not expressed by osteoclasts, but by endothelial cells closely associated with these cells.

When treated with inflammatory cytokines joint cartilage showed an increase in aggrecan breakdown as measured by anti-NITEGE immunoreactivity, but there was no corresponding increase in *Adamts5* β-gal staining. However, increased *Adamts5* expression was seen in tenocytes in response to treatment with inflammatory cytokines.

**REFERENCES**