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Therapeutic Effects of an anti-ADAMTS-5 Antibody on Joint Damage and Mechanical Allodynia in a Murine Model of Osteoarthritis

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Abstract

Objective—The primary goal of this study was to test the disease-modifying effect of blocking a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-5 with a neutralizing monoclonal antibody (mAb) starting 4 weeks after destabilization of the medial meniscus (DMM) in the mouse. We also investigated whether ADAMTS-5 blockade reversed mechanical allodynia and decreased monocyte chemoattractant protein (MCP)-1 production by dorsal root ganglia (DRG) cells.

Methods—Ten-week old male C57BL/6 mice underwent DMM surgery and were either left untreated or treated with anti-ADAMTS-5 mAb or IgG2c isotype control mAb starting 4 weeks after surgery. Knees were collected for histopathology 4 or 12 weeks later. Mechanical allodynia was monitored biweekly in the ipsilateral hind paw through 16 weeks. DRG were collected and cultured 8 weeks after DMM for analysis of MCP-1 production.

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Conflict of interest: JL is a current employee of, and shareholder in, GlaxoSmithKline which holds patent application WO2011002968A2.

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AMM: Conception and design, acquisition of data, analysis and interpretation of data, drafting of the article, final approval of the article
Anne-Marie Malfait takes responsibility for the integrity of the work as a whole, from inception to finished article.

Results—By 4 weeks after DMM, mild cartilage degeneration was evident in the medial compartment, small osteophytes were present, and subchondral bone sclerosis was established. By 16 weeks after surgery, significant cartilage deterioration was apparent on the medial tibial plateaux and medial femoral condyles, osteophyte size had increased, and subchondral bone sclerosis was maintained.

Treatment with ADAMTS-5 mAb from week 4-16 after surgery slowed cartilage degeneration and osteophyte growth but did not affect subchondral bone sclerosis. Moreover, ADAMTS-5 blockade resulted in temporary reversal of mechanical allodynia, which correlated with decreased MCP-1 production by cultured DRG cells.

Conclusions—This study suggests therapeutic efficacy of an ADAMTS-5 mAb in the DMM model, when therapy starts early in disease.

Keywords

Osteoarthritis; Pain; ADAMTS-5; Mouse model

Introduction

Osteoarthritis (OA) is characterized by increased turnover of joint tissues, most notably articular cartilage and subchondral bone, and extracellular proteases play a key role in this pathological process. Based on extensive research in human tissues and in animal models, including genetically modified mice, a range of proteases has been identified as promising targets for the development of disease-modifying OA drugs (DMOADs), including ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs)-4 and ADAMTS-5, matrix metalloproteinase (MMP)-13, and cathepsin K [1-3].

The key enzymes responsible for aggrecan degradation in OA cartilage are the aggrecanases, ADAMTS-4 and ADAMTS-5 [4]. Inhibition of either ADAMTS-4 or ADAMTS-5 by siRNA reduced aggrecan loss from human OA cartilage explants [5]. *Adamts5*$^{-/-}$ mice demonstrate long-term protection from cartilage degeneration in experimental OA induced by destabilization of the medial meniscus (DMM) [6] and in antigen-induced arthritis (AIA) [7].

Mechanical allodynia, defined as pain in response to a normally innocuous stimulus, is a behavioral measure of nervous system sensitization. We have previously shown that following DMM, but not sham surgery, mice develop secondary mechanical allodynia in the ipsilateral hind paw [8, 9]. This mechanical allodynia can be alleviated with morphine or acetaminophen [8], indicating that it is a pain-related behavior. *Adamts5*$^{-/-}$ mice are protected from secondary mechanical allodynia through 8 weeks after DMM [8].

Recently, a fully selective anti-ADAMTS-5 monoclonal antibody (mAb) was developed and characterized by GlaxoSmithKline [10]. *In vivo* target engagement was confirmed by intraperitoneal administration of a one-time dose of an IR800 dye-labeled antibody, 6 weeks after DMM surgery. Four days later, the antibody was detected in the superficial cartilage zone and pericellular region of articular chondrocytes. *In vivo* efficacy was tested in a prophylactic protocol, where mice were pre-dosed 3 days before DMM and once weekly

through 8 weeks after surgery. Mice treated preventatively with ADAMTS-5 mAb had attenuated joint damage and were protected from mechanical allodynia through 8 weeks following DMM [10], essentially mimicking findings in *Adamts5*$^{-/-}$ mice [6, 8]. These findings demonstrate the potential beneficial effect of continued ADAMTS-5 blockade on both structural damage and pain-related behavior in experimental OA. This was corroborated by recent studies in the rat medial meniscal tear (MMT) model, which showed that prophylactic administration of small molecule aggrecanase inhibitors that inhibit both ADAMTS-4 and ADAMTS-5 resulted in protection against development of cartilage damage [11, 12] and weight-bearing changes (an indicator of pain) [12].

The majority of preclinical OA studies aiming to test disease-modifying effects have focused on the prophylactic efficacy of drugs (*i.e.*, treatment begins prior to induction of disease) [13]. While these types of studies are useful for establishing efficacy, they do not mimic the clinical situation, where treatment would presumably begin after onset of joint pathological changes and associated symptoms. Moreover, while OA patients prioritize pain and function, most preclinical studies monitor joint damage alone [13].

Therefore, the goal of the current study was two-fold. First, we sought to test whether ADAMTS-5 blockade starting 4 weeks after DMM surgery affected progression of structural joint damage through 16 weeks. Secondly, we investigated whether ADAMTS-5 blockade altered mechanical allodynia and associated production of the pro-algesic chemokine, monocyte chemoattractant protein (MCP)-1, by dorsal root ganglia (DRG) neurons [9]. We chose to start treatment 4 weeks after DMM surgery since, by this time, mild structural damage is evident [14-16] and mechanical allodynia has fully developed [8, 9], reflecting an early stage of disease.

Materials and Methods

Animals and surgery

For these studies, a total of 76 mice was used. All animal experiments were approved by the Institutional Animal Care and Use Committee at Rush University Medical Center. Animals were housed in a specific pathogen free facility, with food and water *ad libitum* and kept on 12-hour light cycles. All experiments were performed during the light cycle. Wild-type C57BL/6 mice bred at Rush or ordered from Charles River Laboratories were used in these studies. DMM surgery was performed as previously described [9, 14] in the right knee of 10-week old male mice while mice were anesthetized using xylazine (5 mg/kg) and ketamine (100 mg/kg). Briefly, after medial parapatellar arthrotomy, the anterior fat pad was dissected to expose the anterior medial meniscotibial ligament, which was severed. The knee was flushed with saline and the incision closed.

Antibody

An ADAMTS-5-specific mAb (12F4.1H7) was developed and characterized by GlaxoSmithKline, as described in detail in [10], with a KD = 0.035 nM and IC_{50} = 1.46 nM. An IgG2c isotype control mAb was also obtained from GlaxoSmithKline.

Treatment groups

Three treatment groups were included in this study: untreated, ADAMTS-5 mAb-treated, or IgG2c isotype control mAb-treated mice (Fig. 1). Ab treatment commenced 4 weeks after DMM surgery. Mice were injected once per week (10 mg/kg in 100 μL i.p.) until the time of sacrifice (either 8 or 16 weeks after surgery). This dosage regimen has been shown previously to provide sufficient antibody in circulation [10]. Animals were randomized to treatment groups based on their week 4 withdrawal thresholds in order to ensure that all three groups had developed similar levels of allodynia prior to start of treatment. Four independent studies were conducted over a course of two years. Treatment from 4 to 8 weeks after surgery was repeated in 2 independent experiments (study 1: n=4 untreated mice, n=6 IgG2c, n=6 anti-ADAMTS-5; study 2: n=3 untreated, n=3 IgG2c, n=3 anti-ADAMTS-5). Treatment from 4 to 16 weeks after DMM was repeated in 2 independent experiments (study 3: n=6 untreated, n=4 IgG2c, n=5 anti-ADAMTS-5; study 4: n=6 untreated, n=7 IgG2c, n=7 anti-ADAMTS-5) (Fig 1). The number of mice required for study 4 was based on the histopathology results of study 3 in order to ensure 80% power with α = 0.05 comparing untreated mice to mice treated with anti-ADAMTS-5. Mice were weighed weekly, and no weight loss or other ill effects were observed in mice receiving either treatment.

Histopathology of the knee

Four (n=11 mice), eight (n=7-9 mice/treatment group), or sixteen (n=11-12 mice/treatment group) weeks after DMM surgery, histopathology of the knee was evaluated based on a modified OARSI score [17] (Alison Bendele, Bolder BioPATH, Inc., Boulder CO). Joints were fixed in 10% formalin for 48 h, and decalcified for 2 days in 10% formic acid. Knee joints were trimmed of extraneous tissue, embedded in the frontal plane and sectioned. One 8-μm section was taken from each joint at the approximate mid-point of the frontal plane and stained with Toluidine blue. Scoring was performed by an evaluator blinded to the treatment groups, using the following criteria:

Cartilage degeneration—Four joint surfaces, medial and lateral femoral condyles and tibial plateaux, were scored for severity of cartilage degeneration. For each cartilage surface, scores were assigned individually to each of 3 zones (inner, middle, outer) on a scale of 0-5, with 5 representing the most damage. Maximal score for femoral + tibial cartilage degeneration on either the medial or lateral side = 30. Therefore, the maximum possible total cartilage degeneration score for the whole joint is 60.

Osteophyte measurement—The largest osteophyte (medial tibia or femur) was measured using an ocular micrometer.

Bone score—The extent of subchondral bone sclerosis/reduction in bone marrow area was scored from 0 to 5, where 0=no increase; 1=minimal (1-10% increase in bone mass/ trabecular widths); 2=mild (11-25% increase in bone mass/trabecular widths); 3=moderate (26-50% increase in bone mass/trabecular widths); 4=marked (51-75% increase in bone mass/trabecular widths); and 5=severe (>75% increase in bone mass/trabecular widths).

Bioavailability of antibody testing

Sixteen weeks after DMM surgery, serum was collected from a subset of mice (n=2-3/ treatment group) in order to test for bioavailability of antibody using two assays on an Octet platform. Assay 1 - Free drug assessment: Streptavidin-coated sensors were loaded with 5 μg/mL biotinylated ADAMTS-5 protein, sera was applied (diluted 1:100), followed by a challenge with goat anti-mouse IgG H+L antibody (10 μg/mL). Assay 2 -Immunogenicity/ neutralization: Streptavidin-coated sensors were loaded with 10 μg/mL biotinylated ADAMTS-5 mAb (12F4.1H7), sera were applied (diluted 1:100), followed by a challenge with purified human ADAMTS-5 (5 μg/mL) and a subsequent challenge with goat anti-mouse IgG H+L antibody (10 μg/mL). Real-time binding is calculated as relative intensity units (nm shift) using ForteBio Data Analysis Software v8.0.

von Frey testing

Mice were tested using the up-down staircase method of Dixon [18, 19] by an observer blinded to treatment. The threshold force required to elicit withdrawal of the paw (median 50% withdrawal) was determined twice on each hind paw (and averaged) on each testing day, with sequential measurements separated by at least 5 min. Baseline thresholds were assessed prior to surgery, and thresholds were assessed at weeks 4 (prior to beginning therapy), 5, 7, 9, 11, 13, and 16 after DMM surgery.

DRG cell culture following DMM surgery

Eight weeks after surgery, DRGs (L3-L5) were harvested and cells were isolated and pooled from 3-4 mice per treatment group via collagenase 4 (1 mg/mL) and papain (30 U/mL, Worthington Biochemical Corp, Lakewood, NJ) digestion. Cells were plated on poly-L-lysine and laminin (20 μg/mL) coated glass coverslips (25-mm diameter in 6-well plates), and cultured at 37°C with 5% CO_2 in adult neurogenic medium: F12 with L-glutamine, 0.5% FBS, l × N2 (Life Technologies), penicillin and streptomycin (100 μg/ml and 100 U/mL) [9]. On day 2, medium was changed, and on day 4, supernatants were collected and concentrated using 3-kDa molecular weight cutoff centrifugal filters (EMD Millipore) for determination of protein levels. Two independent experiments were performed. In one experiment, DRG cells were pooled from 3 mice per treatment group. The second experiment pooled DRG cells from 4 mice per treatment group.

Protein analysis of supernatant

Total protein levels were determined by BCA assay (Thermo Fisher Scientific, Inc., Rockford, IL), and levels of monocyte chemoattractant-1 (MCP-1) protein were determined via ELISA (R&D Systems Inc, Minneapolis, MN), following manufacturer recommendations.

Statistics

For knee histopathology, data were analyzed using the Kruskal-Wallis one-way analysis of variance (ANOVA). Within each treatment group, the 4, 8, and 16-week time points were compared. At the 16-week time point, the 3 treatment groups were compared to one another. When one-way ANOVA results were significant ($p<0.05$), post-hoc analysis was performed

via Dunn's multiple comparison test. For von Frey testing, a two-way ANOVA with Bonferroni post-tests was used to compare mice treated with ADAMTS-5 mAb to either untreated mice or to mice treated with IgG2c isotype control mAb at each time point. For MCP-1 analyses, a one-way ANOVA was performed with Bonferroni post-tests to compare all groups. All analyses were carried out using GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA).

Results

Progression of joint histopathology after DMM surgery

None of the 76 mice included in these studies showed cartilage damage or other histological changes on the lateral femoral or lateral tibial surfaces (not shown). Therefore, only histological scoring of the medial compartment is shown. Mild cartilage degeneration was evident on the medial femoral and tibial surfaces by 4 weeks after DMM surgery (Fig 2A-C). Additional degeneration from 4 to 8 weeks after surgery was minimal. By 16 weeks after DMM, significant cartilage deterioration was apparent on the medial tibial plateaux as well as on the medial femoral condyles (Fig 2A-C,F).

Small osteophytes were present 4 weeks after DMM surgery (Figs 2D) and remained a similar size through 8 weeks after DMM. Osteophyte size increased from 4 to 16 weeks (Fig 2D).

Subchondral bone sclerosis was established by the four-week time point and remained relatively unchanged through 16 weeks (Fig 2E).

Effect of ADAMTS-5 mAb on joint histopathology

Treatment from 4-8 weeks (Fig 1) with ADAMTS-5 mAb or with IgG2c isotype control mAb had no effect on cartilage degeneration (Fig 2A-C), osteophyte width (Fig 2D), or subchondral bone sclerosis (Fig 2E) assessed at the 8-week time point.

Treatment with ADAMTS-5 mAb from 4-16 weeks slowed progression of cartilage degeneration on both the medial femoral and tibial surfaces (Fig 2B,C,H) as well as osteophyte formation (Fig 2D), compared to untreated mice or to mice treated with IgG2c isotype control mAb. As a result, mice treated with ADAMTS-5 mAb had significantly less total cartilage degeneration (Fig 2A) and smaller osteophytes (Fig 2D) compared to untreated mice at the 16-week time point. In contrast, mice treated with IgG2c isotype control mAb developed similar cartilage degeneration (Fig 2A,G) and osteophyte formation (Fig 2D) compared to untreated mice by the 16-week time point. Subchondral bone sclerosis was similar among all groups 16 weeks after surgery (Fig 2E).

Mechanical allodynia

We have previously shown that mice develop mechanical allodynia in the ipsilateral hind paw by 4 weeks after DMM, and this allodynia is maintained through 16 weeks after surgery, unlike in sham-operated mice [8, 9]. Here, we confirmed these results, demonstrating again that in untreated mice, mechanical allodynia had fully developed by 4 weeks after DMM surgery and was maintained through week 16 (Fig 3A-DMM). Following

the 4-week test for mechanical allodynia, weekly treatment with either ADAMTS-5 mAb or with IgG2c mAb was started. A subset of mice was tested 24 hours after the first injection, and no acute effect of treatment was observed (Fig 3B). Treatment with ADAMTS-5 mAb resulted in decreased mechanical allodynia from 7 through 11 weeks after DMM surgery (Fig 3A-DMM+anti-ADAMTS-5), compared to mice receiving IgG2c mAb (Fig 3A-DMM +IgG2c) or to untreated mice. However, the antiallodynic effect was not sustained, and by 16 weeks after surgery, mice receiving ADAMTS-5 mAb had mechanical allodynia equivalent to the other treatment groups.

MCP-1 production by DRG cells

We have previously shown that DRG cells harvested from mice 8 weeks after DMM surgery produce increased amounts of MCP-1 compared to DRG cultures from sham and from age-matched naïve mice [9]. Here, DRG cells were harvested 8 weeks after DMM surgery from the 3 treatment groups (Fig 1), and MCP-1 produced in culture was compared among the groups. DRG cultures from mice treated with ADAMTS-5 mAb produced significantly less MCP-1 compared to mice treated with IgG2c mAb and to untreated mice (Fig 4). This was repeated in two independent experiments.

Bioavailability of antibody 16 weeks after surgery

Since the protective effect of ADAMTS-5 mAb treatment on mechanical allodynia had faded by 16 weeks *post* surgery, serum was collected at this time point in order to test for the presence of free ADAMTS-5 mAb and for the presence of neutralizing antibodies against ADAMTS-5 mAb. Using an Octet platform, sera from mice treated with ADAMTS-5 mAb bound immobilized ADAMTS-5, while sera from untreated mice or from mice treated with IgG2c mAb did not bind, indicating that free ADAMTS-5 mAb was still present at the 16-week time point (Table 1; Supplemental Fig 1A). In contrast, serum samples from all groups did not differentially bind to immobilized ADAMTS-5 mAb or impede subsequent binding of ADAMTS-5 to the immobilized mAb, suggesting that mice treated with ADAMTS-5 mAb had not developed ADAMTS-5 mAb neutralizing antibodies by the 16-week time point (Table 2; Supplemental Fig 1B).

Discussion

We have demonstrated that early therapeutic intervention with an ADAMTS-5 mAb slows progression of joint damage over 16 weeks following DMM surgery. Specifically, ADAMTS-5 blockade attenuated cartilage degeneration and osteophyte growth, but it did not affect subchondral bone sclerosis. Moreover, treatment with ADAMTS-5 mAb resulted in a temporary reversal of mechanical allodynia, which correlated with decreased MCP-1 production by cultured DRG cells. Together, these findings are the result of four independent studies.

The protection against cartilage degradation reported here is concordant with a recent study, which used a different ADAMTS-5 mAb in STR/Ort mice, a strain that develops spontaneous OA [20]. In that study, two intra-articular injections of ADAMTS-5 mAb administered at 5 months (at which time mice have mild-to-moderate OA [21]) and 6.5

months of age were shown to slow progression of cartilage degradation by 8 months of age [20]. Effects on other joint tissues or on behavioral changes were not assessed as part of that study. Together with our data, this suggests that blocking ADAMTS-5 with Abs starting in an early stage of OA may represent a promising method of slowing cartilage destruction associated with OA.

In addition to inhibiting cartilage degradation, early therapeutic intervention with an ADAMTS-5 mAb also slowed osteophyte growth when evaluated by histology 16 weeks after DMM. Data on osteophytes in *Adamts5* null mice are scarce, but one study showed a trend of protection ($p=0.09$) against development of osteophytes when evaluated by μ-computed tomography (μ-CT) 8 weeks after DMM [22].

We found that by 4 weeks after DMM surgery, subchondral bone sclerosis – as assessed by histology - had been fully established, remaining relatively constant through 16 weeks. Jackson et al. recently reported similar subchondral bone histological findings in the DMM model up to 8 weeks after surgery [15]. *Adamts5* null mice were protected from subchondral bone changes, measured via μ-CT, through 8 weeks after DMM [22]. We demonstrate here that therapeutic treatment with ADAMTS-5 mAb had no effect on subchondral bone sclerosis, as determined histologically, although this method is less sensitive and less quantitative compared to μ-CT. Together, these studies suggest that treatment to prevent subchondral bone changes may be more effective prior to the 4-week time point in the DMM model.

Synovitis was not included as part of the histopathological scoring in this study due to the fact that a recent report in the DMM model shows that synovitis peaks 4 weeks after surgery and returns to sham levels by 16 weeks [15].

Taken together, these data suggest that treatments targeted at one specific tissue, in this case cartilage, can have effects on other aspects of the joint as well, since complex interrelationships exist among the various joint tissues in OA [23]. It should also be recognized that these effects may change depending on the time of intervention in the disease course.

Mechanical allodynia was temporarily alleviated with ADAMTS-5 mAb treatment. By 16 weeks after surgery, however, allodynia levels in the treated mice had returned to levels similar to untreated mice, even though antibody was still bioavailable at that time (Tables 1,2). One explanation for the temporary reversal of mechanical allodynia is that despite the slowing of joint damage in mice treated with ADAMTS-5 mAb, the antibody treatment did not fully prevent progression. This may suggest that a certain amount or rate of joint damage may be sufficient to promote mechanical allodynia. Mechanical allodynia in the ipsilateral hind paw is an evoked measure of sensitization of the nervous system. This mechanical allodynia can be alleviated with morphine or acetaminophen in the DMM model [8], indicating that it is a pain-related behavior, but additional behaviors should be analyzed in future studies in order to better understand the relationship between structural changes and pain.

In addition to behavioral changes, molecular alterations in the DRG have been associated with the development of pain. In particular, we have shown that 8 weeks after DMM surgery, increased production of MCP-1 by DRG neurons is associated with the persistence of mechanical allodynia [9]. Here, treatment with an ADAMTS-5 mAb was associated with a decrease in DRG MCP-1 production, which suggests that changes in the peripheral nervous system may be delayed by slowing joint deterioration.

An alternative explanation for the observed effects on mechanical allodynia and on MCP-1 production may be direct effects of ADAMTS-5 mAb on the peripheral nervous system. Indeed, ADAMTS-5 can be expressed by DRG neurons [24, 25] and chondroitin sulfate proteoglycans have been shown to interact with peripheral nerves [26]. We demonstrated that there was no acute effect of ADAMTS-5 treatment on mechanical allodynia (Fig 3B), but long-term effects cannot be ruled out and future work will seek to understand this in greater detail.

Whether halting structural progression results in pain alleviation remains unknown. In addition to the current study, a literature search identified just 4 other studies that have attempted to address this question. One study used an antibody against GM-CSF to target inflammation in the mouse collagenase-induced instability model of OA [28]. Treatment beginning at an intermediate point in the model was able to inhibit weight-bearing deficits (an indicator of pain) and cartilage damage over a three-week period. The bisphosphonate, zoledronate, was tested in two different rat models, the medial meniscal tear (MMT) and monoiodoacetate (MIA) models [29, 30]. In both models, early treatment was effective in inhibiting subchondral bone changes, cartilage degeneration, and weight-bearing deficits. In contrast, treatment started late in the model was ineffective in the MMT study and only partially effective in the MIA study. Another study targeted subchondral bone changes using osteoprotegerin (OPG) in rat MIA [31]. OPG treatment starting at an intermediate point in the model partially inhibited weight-bearing deficit and osteoclast number, but it had no effect on mechanical allodynia, cartilage damage, synovitis, or osteophyte score. Together with the current study, these experiments suggest that therapeutically treating bone (zoledronate and OPG), inflammation (GM-CSF Ab), or cartilage (ADAMTS-5 mAb) may be beneficial in slowing both structural progression and development of pain, when treatment is started early in the disease course. Future experiments must be performed to evaluate the therapeutic effect when ADAMTS-5 blockade begins in late-stage experimental OA, particularly after the 8-week time point since that is when disease progression rapidly increases in the DMM model. In addition, it may be interesting to test efficacy of combination therapies that target multiple tissues in order to evaluate whether it is possible to extend the period of time that mechanical allodynia is alleviated.

Despite the promising preclinical results obtained by ADAMTS-5 blockade, no inhibitors have made it to the clinic. One problem hindering the development of effective therapies may be that by the time of diagnosis, the majority of patients have pain and advanced structural damage [32]. Overall, our study suggests therapeutic efficacy of a potent and selective ADAMTS-5 mAb in the DMM model, when therapy starts at an early stage of disease. This is consistent with the recently proposed paradigm that improved identification

of early OA patients may allow for treatment before irreversible structural changes have occurred and before chronic pain develops, resulting in improved efficacy [32].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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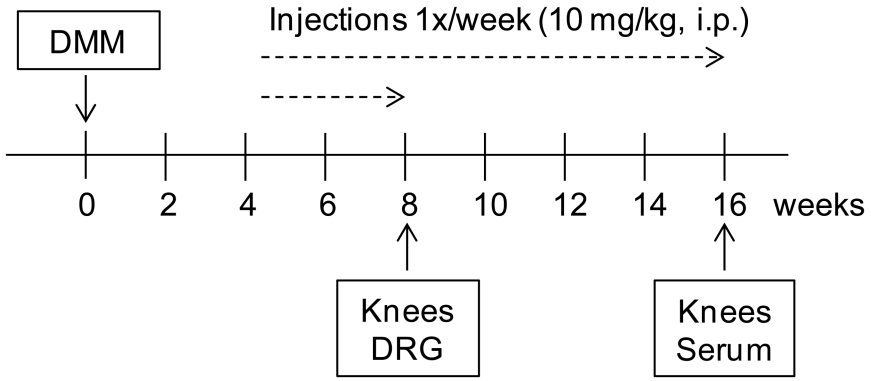

Figure 1.

Experimental design. Treatment groups: ADAMTS-5 mAb, IgG2c isotype control mAb, or no injection. Mice received injections 1×/week (10 mg/kg, i.p.) beginning at week 4 and continuing to week 8 or 16 after DMM surgery. DRG = dorsal root ganglion.

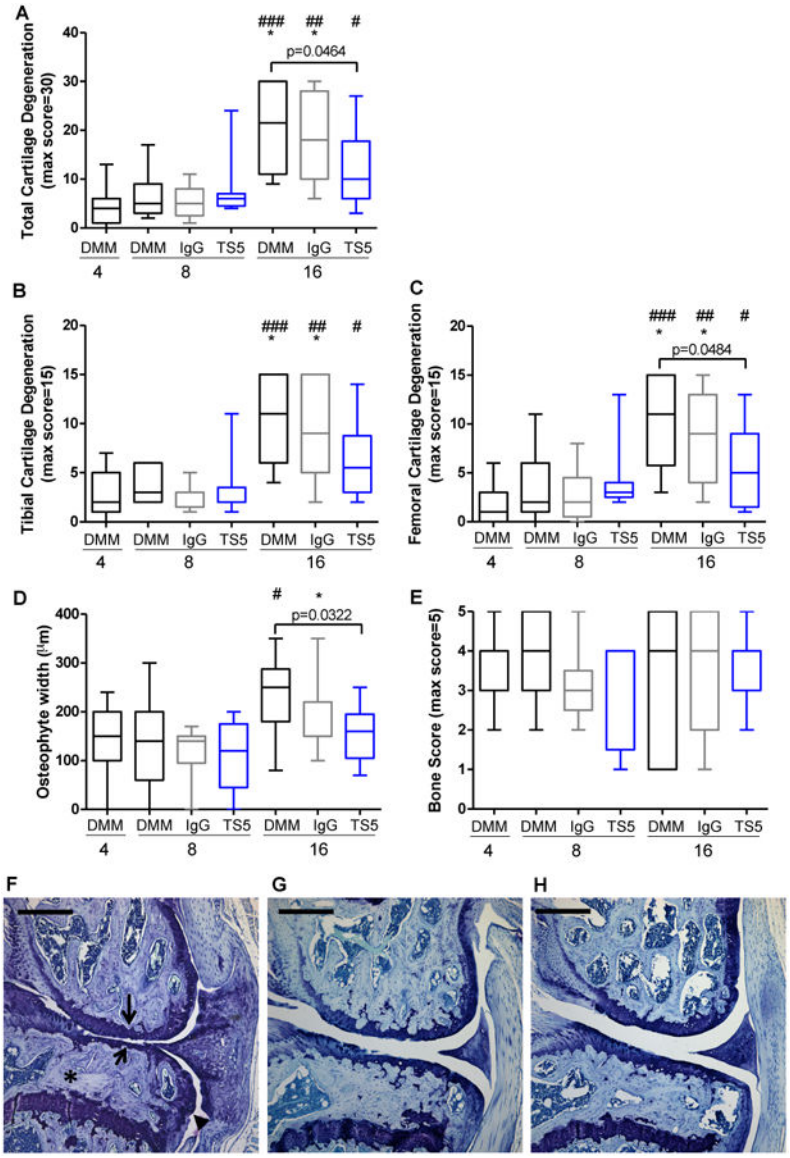

Figure 2.

A) Total cartilage degeneration score in the medial compartment, #p=0.015 DMM+4 vs DMM+TS5+16; ##p=0.0014 DMM+4 vs DMM+IGG+16; *p=0.0116 DMM+IGG+8 vs DMM+IGG+16; ###p=0.0002 DMM+4 vs DMM+16; *p=0.0149 DMM+8 vs DMM+16; **B)** Medial tibial cartilage degeneration score, #p=0.0319 DMM+4 vs DMM+TS5+16; ##p=0.0046 DMM+4 vs DMM+IGG+16; *p=0.0119 DMM+IGG+8 vs DMM+IGG+16; ###p=0.0007 DMM+4 vs DMM+16; *p=0.013 DMM+8 vs DMM+16; **C)** Medial femoral cartilage degeneration score, #p=0.0188 DMM+4 vs DMM+TS5+16; ##p=0.002 DMM+4 vs DMM+IGG+16; *p=0.0299 DMM+IGG+8 vs DMM+IGG+16; ###p=0.0003 DMM+4 vs DMM+16; *p=0.0333 DMM+8 vs DMM+16; **D)** Osteophyte width, *p=0.0208 DMM+IGG +8 vs DMM+IGG+16; #p=0.0496 DMM+4 vs DMM+16; and **E)** Subchondral bone score were assessed 4, 8, and 16 weeks *post* DMM surgery in mice that were untreated (DMM), treated with IgG2c isotype control mAb (IgG), or treated with ADAMTS-5 mAb (TS5).

Whiskers = 5-95 percentile; n=7-12 (details in Materials and Methods). Bar shows p-value for comparisons among treatment groups at a particular time point. **F-H)** Representative histology images of the medial knee joint compartment from mice 16 weeks after DMM surgery receiving **F)** no treatment, **G)** IgG isotype control Ab, or **H)** ADAMTS5 mAb starting 4 weeks after DMM. Images were chosen for mice representing the median histology scores from each treatment group based on the measures quantified in Figure 2A,D,E. In part **F**, arrows indicate medial femoral and tibial cartilage degeneration, an asterisk indicates tibial subchondral bone sclerosis, and the arrowhead indicates a tibial osteophyte. Scale bar = 0.5 mm.

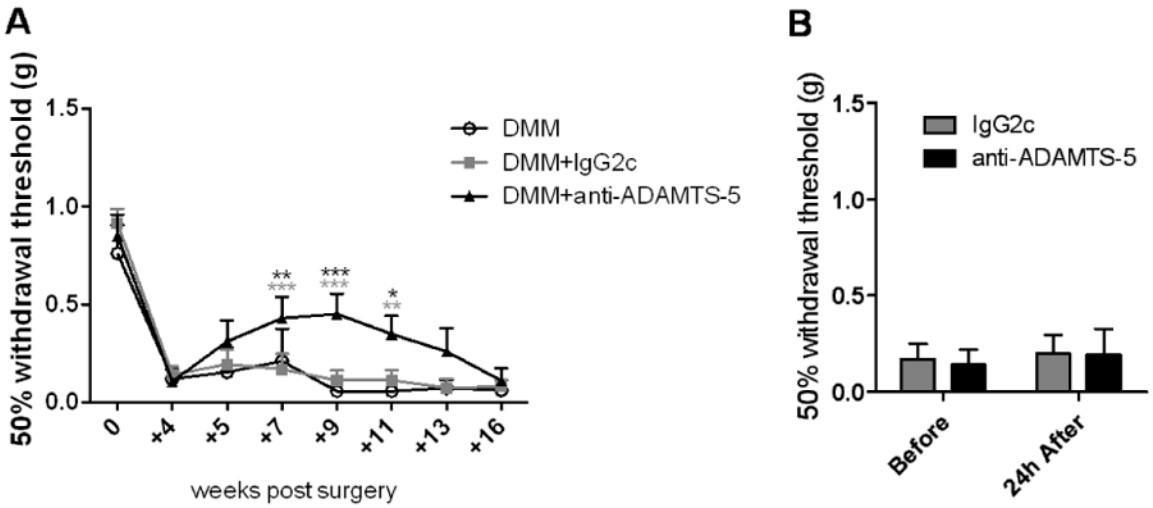

Figure 3.

Mechanical allodynia was assessed in mice that were untreated (DMM), treated with IgG2c isotype control mAb (+IgG2c), or treated with ADAMTS-5 mAb (+anti-ADAMTS-5) through 16 weeks after surgery (A). A decrease in withdrawal threshold from the baseline at time 0 indicates development of mechanical allodynia. A subset of mice (n=5) was tested 24 hours after the first injection to rule out an acute analgesic effect (B). mean+95%CI. DMM +anti-ADAMTS-5 vs DMM: +7 weeks, p=0.0051; +9 weeks, p=0.0001; +11 weeks, p=0.0109. DMM+anti-ADAMTS-5 vs DMM+IgG2c: +7 weeks, p<0.0001; +9 weeks, p<0.0001; +11 weeks, p=0.0024; black vs DMM; grey vs DMM+IgG2c.

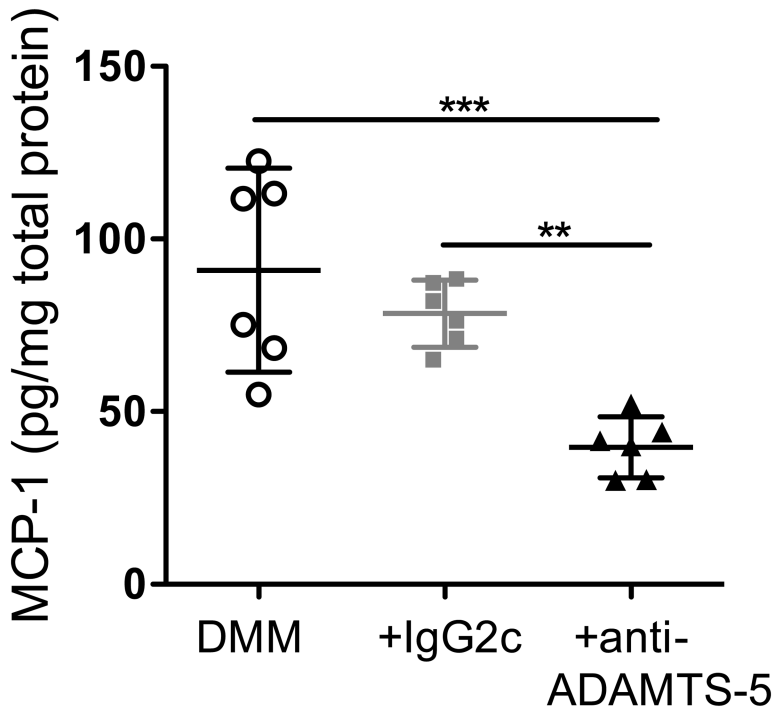

Figure 4.

Eight weeks after surgery, DRG cells were cultured from mice that were untreated (DMM), treated with IgG2c isotype control mAb (+IgG2c), or treated with ADAMTS-5 mAb (+anti-ADAMTS-5), and supernatants were analyzed for MCP-1 protein. mean±95%CI. **p=0.0056, ***p=0.0005. Dots represent individual wells. Plot shows the result of one out of two independent experiments (Expt 1).

Table 1
Free Drug Assessment in Serum 16 weeks after DMM

	Sensor Loading Step		Serum Binding Step	anti-Mouse Binding Step
Sensor	**Biotin ADAMTS-5 Binding (nm Shift)**	**Serum Sample ID (Mouse # / Treatment)**	**Serum Binding (nm Shift)**	**anti-Mouse IgG Binding (nm Shift)**
A1	5.71894	1425/IgG2c mAb	0.10265	0.05875
B1	5.81748	1426/ADAMTS-5 mAb	2.15856	2.39165
C1	5.67592	1464/No Injection	0.18539	0.04822
D1	5.60177	1465/No Injection	0.17637	0.04432
E1	5.0251	1466/ADAMTS-5 mAb	2.15137	2.35766
F1	5.46195	1467/IgG2c mAb	0.28938	0.03224
G1	5.70817	1505/ADAMTS-5 mAb	2.94853	1.88211
H1	5.61472	1507/IgG2c mAb	0.20515	0.04857

Table 2

Immunogenicity/Neutralization Assessment of Serum 16 weeks after DMM

	Sensor Loading Step		Serum Binding Step	ADAMTS-5 Binding Step	anti-Mouse Binding Step
Sensor	**mAb Binding (nm Shift)**	**Serum Sample ID (Mouse # / Treatment)**	**Serum Binding (nm Shift)**	**ADAMTS-5 Binding (nm Shift)**	**anti-Mouse IgG Binding (nm Shift)**
A1	4.56144	1425/IgG2c mAb	0.7295	2.07617	1.65979
B1	4.47963	1426/ADAMTS-5 mAb	0.48773	2.21794	1.67662
C1	4.71362	1464/No Injection	0.38654	2.3458	1.69864
D1	4.633	1465/No Injection	1.10738	2.03582	1.49423
E1	4.72779	1466/ADAMTS-5 mAb	0.47894	2.21242	1.64249
F1	4.67933	1467/IgG2c mAb	0.67129	2.18379	1.56537
G1	4.47408	1505/ADAMTS-5 mAb	0.89388	2.01044	1.60488
H1	4.54082	1507/IgG2c mAb	0.65863	2.09008	1.64701