α_2-Macroglobulin
Autologous Protease Inhibition Technology

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KEYWORDS

• α_2-macroglobulin (A2M) • Inflammation • Anti-inflammatory • Cytokines • Arthritis • Osteoarthritis • Discogenic back pain • Autologous

KEY POINTS

- A2M has emerged as a potential treatment of cartilage-based pathology and inflammatory arthritis because of its ability to bait and trap inflammatory mediators.
- A2M has been successfully applied to musculoskeletal pathology to decrease pain and modulate cartilage degeneration.
- Autologous A2M can be concentrated from plasma using a unique filtration process.
- New recombinant formulations of A2M can even more precisely target molecular pathways of intra-articular and extra-articular and intervertebral disk disease.

INTRODUCTION

Musculoskeletal conditions causing pain are ubiquitous, making up a large percentage of physician visits each year. Etiology and treatment range widely, and it is not necessarily appropriate to discuss the application of a particular treatment of all aspects of musculoskeletal pain. Musculoskeletal pathology, however, can be generally divided into the following categories:

1. Intra-articular joint pain
2. Extra-articular pain
3. Spinal intervertebral (discogenic) pain

Intra-articular joint pain is most commonly attributed to idiopathic osteoarthritis (OA), posttraumatic OA (PTOA), and other systemic inflammatory arthropathies,

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such as rheumatoid arthritis (RA). In recent decades the development of tumor necrosis factor α (TNF-α) inhibitors have been a significant clinical impact on the treatment of RA, providing many patients with significant pain relief and disease progression modification. Unfortunately, no such treatment has been adopted for OA or PTOA. OA is a common problem affecting a large proportion of the population and can affect many joints, including but not limited to the spine, knee, shoulder, hip, fingers, and ankle. It is characterized by progressive cartilage degeneration and loss. Current treatment of OA is limited to physical therapy in attempts to improve joint stabilization, weight loss to reduce joint reactive forces, systemic anti-inflammatory medications (ie, nonsteroidal anti-inflammatory drugs [NSAIDS]) and intra-articular injections of substances, such as steroids or so-called joint lubricants like hyaluronic acid. Physical therapy often has limited benefit, especially as disease progresses, and patients often have difficulty losing weight if physical activity is painful. Systemic NSAIDS can have serious side effects, including gastrointestinal bleeding, and have recently been implicated in cardiac side effects, thus limiting their use. Intra-articular injections of steroids have been demonstrated to have no additional benefit compared with an exercise program alone in a randomized controlled trial[1] and may double the infection rate after total knee or hip replacement.[2] Furthermore, multiple studies have recently brought into question the efficacy of hyaluronic acid injections, leading the American Academy of Orthopaedic Surgeons (AAOS) to withdraw their recommendation of its clinical use.[3] Perhaps most importantly, none of these potential therapies can successfully prevent cartilage degeneration and osteoarthritis.

Distinct from cartilage pathology and osteoarthritis, extra-articular joint pain most commonly involves inflammation of tendons inserting at or near a joint. The most common examples of such enthesopathies are Achilles tendonitis, subacromial bursitis of the rotator cuff, and lateral epicondylitis of the elbow (tennis elbow). These often resolve with activity modification or with a short course of NSAIDS. Persistent enthesopathies can be difficult to treat, however, and have led to many attempts to treat with various types of platelet-rich plasma injections. Several studies have failed to demonstrate a significant benefit, however, with the possible exception of Achilles tendonitis,[4] although this too has been called into question in randomized studies.[5]

Spinal intervertebral discogenic pain is possibly the most controversial musculoskeletal pain etiology — invoking some physicians to question even its existence as a pain generator, whereas others advocate invasive surgical procedures, such as spinal fusion or total disk replacement surgery. The authors believe that discogenic pain does exist but that determining with certainty which particular disk(s) is(are) the source of back pain can be challenging. Nonsurgical treatments for this clinical entity have been limited until recently.[6] There is growing evidence that discogenic pain may be an inflammatory process, without any discrete mechanical pathology.[7] In contrast, spinal radiculopathic pain is caused by compression and/or inflammation of a spinal exiting nerve root, usually by a herniated intervertebral disc. Although this clinical entity most often resolves without surgical treatment approximately 80% to 90% of the time, the remaining cases can be treated successfully by surgical decompression.[8] This pathophysiology of radiculopathic pain should be differentiated from spinal intervertebral discogenic pain, because they are 2 distinct clinical problems.

In the setting of advanced cartilage disease and osteoarthritis, there is little that can be offered patients short of joint replacement. Even in early stages of disease, however, historically there have not been disease-modifying agents that are clinically effective. Discogenic back pain and enthesopathies present a similar challenge, because there has not been any clear effective clinical intervention to mediate the course of

disease or symptoms. The concept of biologic rather than surgical treatment of musculoskeletal pathologies has led to the emergence of regenerative interventional therapies. With respect to cartilage degeneration in particular, another goal of emerging biologic therapies is to prevent the onset or progression of arthritides.

Osteoarthritis, for example, is mediated by numerous biomechanical and biochemical processes involved in its pathophysiology. Inflammatory cytokines have been demonstrated to increase production of metalloproteases that degrade cartilage, and the catabolic cartilage products further stimulate production of additional proinflammatory cytokines.[9–13] Recently, increased attention is given to inhibiting metalloproteases involved in cartilage catabolism, with the intention of modulating cartilage breakdown and preventing the cascade of inflammatory mediators involved in disease progression.

α_2-Macroglobulin (A2M) has emerged as a unique potential treatment of cartilage-based pathology and inflammatory arthritides. This article describes the unique method by which A2M can not only inhibit the associated inflammatory cascade but also disrupt the catabolic process of cartilage degeneration. Autologous concentrated A2M from plasma is currently in use by some providers to successfully treat various painful arthritides, including mild to moderate OA, PTOA, enthesopathies. and spinal discogenic back pain.

α_2-MACROGLOBULIN MODULATES THE INFLAMMATORY CASCADE AND CARTILAGE DEGRADATION

Two important biochemical networks that contribute to OA pathology include proinflammatory cytokines and matrix metalloproteinases (MMPs).[7] A2M is a major plasma glycoprotein best known for its ability to inhibit a broad spectrum of serine, threonine, and metalloproteases by a unique bait and trap method (**Fig. 1**).

A2M uses a 39–amino acid bait region that, when cleaved by a protease, induces a large irreversible conformational change that physically traps the protease within a steric cage. As part of the entrapment, the protease forms a covalent bond with A2M, exposing a receptor recognition site, triggering the endocytosis and eventual

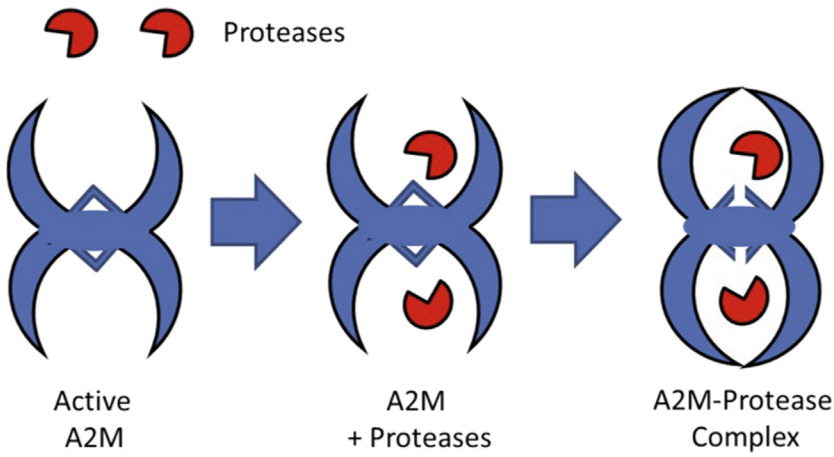

Fig. 1. Pictorial representation of the mechanism by which A2M traps proteases. Each dimer traps a single protease. After the second trapped protease, the molecule then undergoes active transport for its elimination.

clearance of the A2M-protease complex. A2M has also been demonstrated to bind proinflammatory cytokines, such as TNF-α and IL-1β, and reduces the cytokine-induced up-regulation of collagenases in chondrocytes (Fig. 2).

Inflammation is an early stage in many painful pathologies, in particular OA, and is mediated primarily by TNF-α, interleukin (IL)-1β, and IL-6 but also involves several other cytokines and chemokines.[11] Along with their roles in mediating the inflammation, TNF-α and IL-1β down-regulate the production of extracellular matrix proteins in chondrocytes[14–16] and induce the up-regulation of MMPs, including those that degrade collagen, such as MMP-1, MMP-3, and MMP-13.[7–9] ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs) is also up-regulated or activated by IL-1β; however, this is not the case for ADAMTS-5.[17–19] Transgenic mouse models with a modified ADAMTS-5 gene have attenuated OA pathology, suggesting that inhibitors of ADAMTS-5 should be included in any therapy that targets proteases.

The degradative products of cartilage catabolism can in turn stimulate production of inflammatory proteases, in addition to cytokines, which further contributes to increases in inflammatory proteases, specifically elastase and cathepsins.[20] Fragments of fibronectin and collagen are reported to stimulate the production of inflammatory cytokines, chemokines, and MMPs.[21,22] Consequently, the fragments of extracellular matrix proteins, and other degradative products in OA, produced by catabolic proteases might contribute to the inflammatory response that stimulates further protease

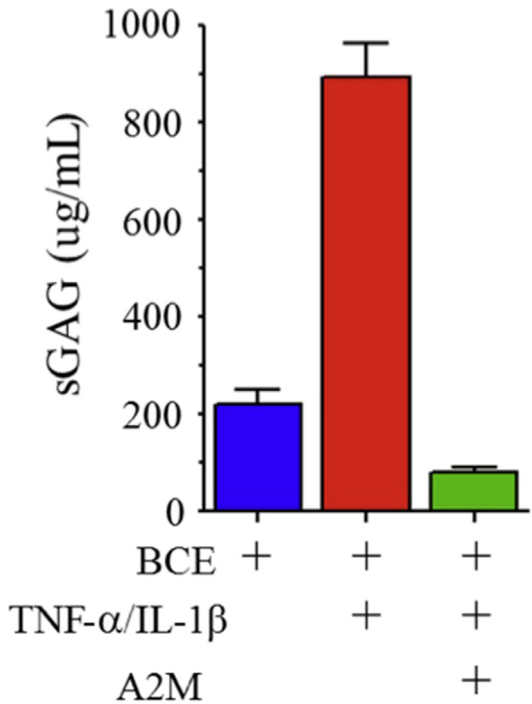

Fig. 2. A2M is chondroprotective against inflammatory cytokines. Treatment of bovine cartilage explants (*blue column*) with proinflammatory cytokines TNF-α and IL-1β (*red column*) induce chondrocytes within the cartilage to produce or activate proteases resulting in increased production of sulfated glycosaminoglycan (sGAG). Treatment with purified human A2M (*green column*) potently inhibited cartilage catabolism. BCE, bovine cartilage explant.

production. Moreover, proteases that cleave aggrecan to release the G3 domain (MMP-2, MMP-7, MMP-9, and MMP-13) are responsible for the formation of the fibronectin-aggrecan complex (FAC).[23]

Fibronectin (an extracellular matrix protein) and its fragments can stimulate cytokine production and activation of MMPs.[22] Aggrecan (a proteoglycan component of articular cartilage) undergoes extensive degeneration during aging and triggers signaling cascades, which augment joint and cartilage damage.[24] This cartilage degradation product, the FAC, has been shown to be associated with joint pathology as well as predict response to lumbar epidural steroid injection in patients with radiculopathy intervertebral disc herniation.[25,26]

A therapeutic agent that prevents the formation of the G3 domain of aggrecan reduces the FAC G3 domain and accordingly may be an efficacious treatment in painful pathophysiology. Because the production of G3 domain of aggrecan is catalyzed by different known classes of proteases, a common inhibitor of all of these proteases may represent an ideal therapeutic agent. This again suggests the potential and proposed mechanism of efficacy of A2M as a multipurpose protease inhibitor and anti-inflammatory mediator.

It has been shown ex vivo that A2M decreases cartilage catabolism, inhibiting the protease activity of ADAMTS-5, metalloproteinases, and other known mediators of the pathologic cartilage catabolism process[27] (see **Fig. 2**). Therefore, the inhibition of ADAMTS-5 and other metalloproteinases and mediators is the probable mechanism of action of A2M as an anticatabolic agent (**Fig. 3**).

Genetically engineered A2M variants with superior inhibition of ADAMTS-5 and other proteinases could further result in enhanced chondroprotection.

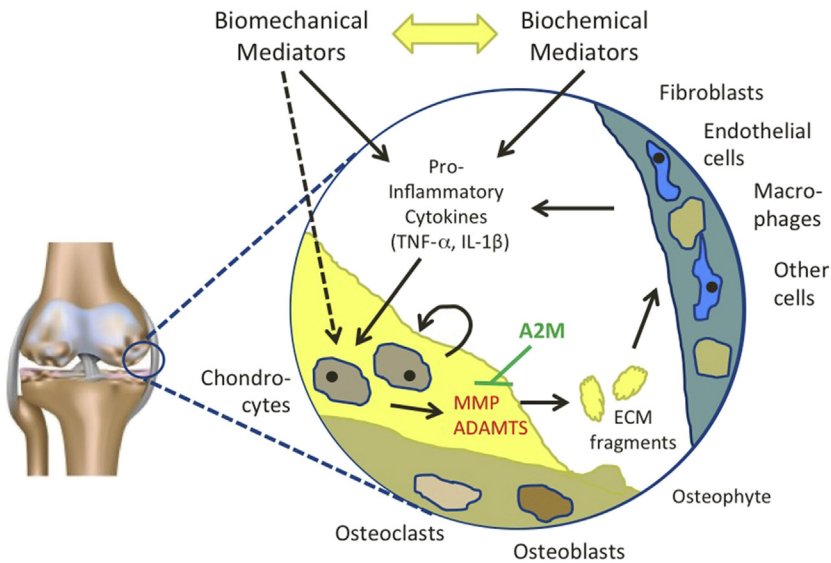

Fig. 3. Schematic of the inflammatory cascade, which occurs in OA — cartilage breakdown leads to extracellular matrix (ECM) breakdown products, such as FAC, which then stimulate the release of inflammatory cytokines, leading to a vicious cycle of further cartilage breakdown (positive feedback loop).

CLINICAL APPLICATION OF α_2-MACROGLOBULIN

A method of A2M concentration from autologous blood has recently been developed (Autologous Platelet Integrated Concentration [APIC]–Cell-Free [Cytonics, West Palm Beach, Florida]) and is currently undergoing a Food and Drug Administration clinical trial for the treatment of mild to moderate knee OA (Fig. 4).

A similar autologous formulation of A2M is achieved by APIC protein-rich plasma (Cytonics), which is approved by the Food and Drug Administration and has been used for various musculoskeletal conditions. The system uses a unique filtration step using a tangential flow filter (Fig. 5).

The concentration process is an office-based procedure that takes approximately 40 minutes and involves simple venipuncture and blood withdrawal for processing using tabletop centrifugation and ultrafiltration. The procedural steps are briefly as follows (see manufacturer guidelines for details):

1. Fill syringes provided with anticoagulant (acid citrate dextrose solution A [ACD-A]), 7 mL each.
2. Perform venipuncture on patient using standard precautions and fill each syringe with 45 mL blood.
3. Connect a blunt plastic cannula to each of the blood-filled syringes.
4. With cap secured, invert each syringe several times to achieve adequate mixing of blood and anticoagulant.
5. Remove the cap from the blunt plastic cannula and insert the cannula into the septum of the first APIC centrifuge tube.
6. Dispense 45 mL of anticoagulated blood from each syringe into the centrifuge tube. Note: do not invert or tilt the centrifuge tube because fluid may leak from the septum.
7. Load centrifuge tubes into the APIC centrifuge, using balanced technique, then close and lock the centrifuge lid.
8. Select the appropriate cycle and press “START.”

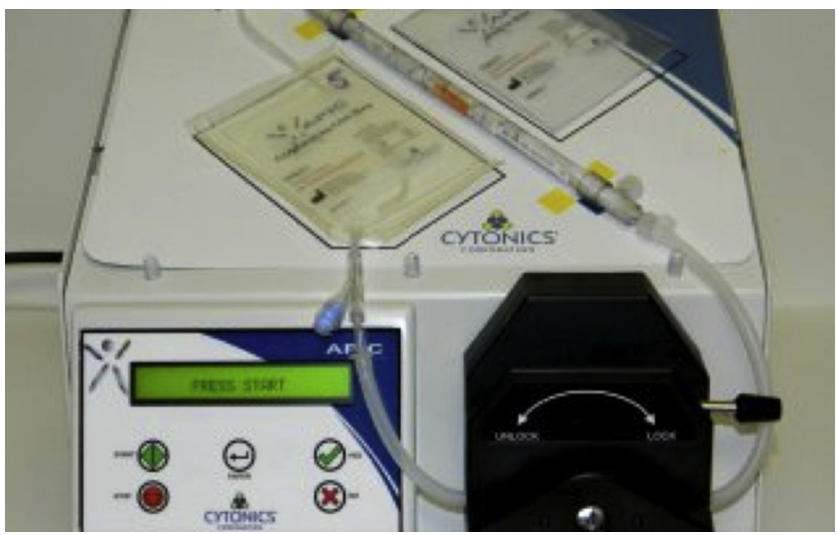

Fig. 4. System to process autologous blood, producing a solution that has approximately 6 times A2M concentration from blood.

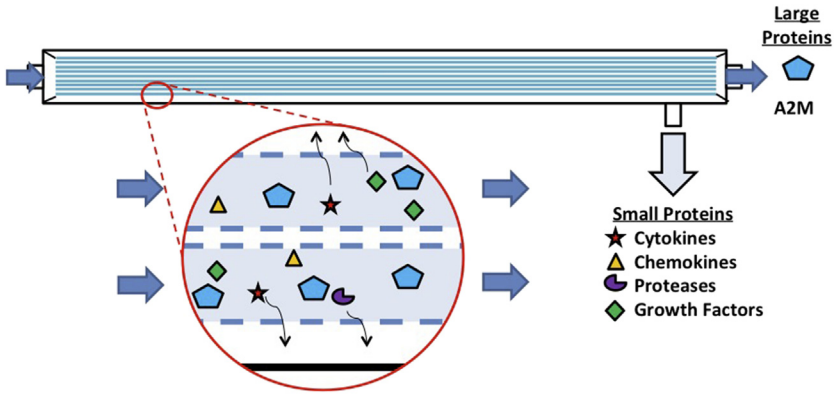

Fig. 5. Tangential flow filtration technology enables concentration of certain large proteins, such as A2M, whereas other smaller proteins, such as inflammatory cytokines, are diluted.

9. The spin cycle is complete after 4 minutes; carefully remove each centrifuge tube.
10. Connect the 60-mL syringe to the plasma collection needle.
11. Insert the plasma collection needle into the first centrifuge tube to a level 1 cm above the buffy coat.
12. Slowly collect 15 mL of plasma into the syringe, maintaining the position of the needle tip 1 cm above the buffy coat. Do not disturb the surface of the buffy coat. Repeat with other tubes.
13. Secure the syringe contents during transfer to the Concentration Kit.
14. Place the Concentration Kit onto the pump platform and press "Enter" on the pump keypad.
15. Remove the plasma collection needle from the syringe containing 45 mL of plasma and immediately connect the syringe to the blue port of the concentration bag.
16. Inject the plasma into the concentration bag and press "Enter" on the pump keypad.
17. Disconnect and discard the empty 60-mL syringe.
18. Load the tube into the pump head, lock the pump head lever, and press "START" on the pump keypad.
19. The process is complete in 20 minutes and will read "APIC Ready."
20. Immediately after the cycle completion, engage the white clamp on the waste bag tubing to prevent dilution.
21. Connect a syringe to the blue port of APIC Concentration Bag and draw the concentrated plasma into the syringe.
22. Disconnect the syringe containing the concentrated plasma.
23. Mix the concentrated plasma with autograft or allograft bone if indicated.
24. Apply concentrated A2M to appropriate clinical site.

There are several distinct clinical applications of autologous solutions rich in A2M, which have shown promising results to date. This includes treatment of intra-articular and extra-articular joint pain as well as spinal discogenic pain.[22,24] Mild to moderate OA of the knee, hip, and shoulder have been successfully treated with intra-articular injection of approximately 2 mL to 4 mL of A2M-rich concentrated plasma via APIC, with many patients experiencing pain relief for 6 months or more. Standard injection techniques familiar to orthopedists, rheumatologists, and physiatrists are used, such as the suprapatellar or anterolateral injection portal of the knee.

Intra-articular hip joint injection is typically performed using fluoroscopic guidance to ensure proper location. A phase 1/2 randomized control trial is currently under way to test the ability of a cell-free version of A2M-rich concentrate for the treatment of mild to moderate knee OA. The cell-free version contains similar A2M concentration while eliminating platelets and white blood cells.

Similarly, injection of autologous A2M-rich concentrate has been applied to painful extra-articular joint pathology, such as subacromial bursitis, lateral epicondylitiss, and Achilles tendonitis, with excellent clinical results in small studies (Gaetano Scuderi, 2016, personal communication). Large clinical trials of these pathologies have not yet been performed because these conditions are less commonly encountered than OA of the knee, hip, and shoulder.

When there is an inflammatory component to discogenic back pain, as evidenced by patients who test positive for FAC within the disc, A2M concentrate has also successfully treated spinal intervertebral discogenic pain. Patients report significant reduction in pain (4.9 mean improvement in Visual Analog Scale) and improved Oswestry Disability Index (ODI) scores (37-point mean ODI reduction) at the end of a 6-month study period.[28]

FUTURE DIRECTIONS

The early clinical success of A2M supplementation to a variety of musculoskeletal conditions is encouraging. Larger, prospective clinical trials are anticipated that may further support these preliminary results. The application of A2M is distinct from other autologous treatments, because its development is firmly grounded in a proposed mechanism of action based on understanding of cartilage pathology and inflammatory pain. Based on this science, the next step is to attempt to further improve on wild-type A2M mechanism, to perhaps more specifically address or augment its role in the pathophysiology at the site of disease. Genetic modifications of A2M are already under way, and several candidates demonstrate superior ability to modulate cartilage degeneration, for instance, compared with the wild type. Early evidence using reverse transcriptase–polymerase chain reaction shows up-regulation of collagen type 2 and aggrecan, precursors for cartilage regeneration. In some instances, these modifications specifically target metalloproteases known to be involved in OA and the development of joint pain. Further clinical characterization of these modified variants is anticipated, along with the ability to specifically target known pathologic mechanisms of disease progression. The development of a recombinant variant for clinical use will further enhance the clinical success of A2M in the treatment of musculoskeletal disease.

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