

# Hyaluronan Suppresses IL-1 $\beta$ -induced Metalloproteinase Activity from Synovial Tissue

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**Intraarticular injection of hyaluronan (viscosupplementation) is commonly used to treat knee pain from osteoarthritis. The therapeutic benefit might derive from hyaluronan inhibition of the activity of the cytokine-regulated catabolic enzymes that attack joint cartilage (matrix metalloproteinases). We tested the hypothesis that hyaluronan inhibited interleukin-1 $\beta$ -induced matrix metalloproteinase activity secreted by explants of synovial tissue from patients with osteoarthritis and investigated the mechanism of the effect. Hyaluronan with a molecular mass of 12.8 MDa (number average) antagonized induced metalloproteinase activity in proportion to hyaluronan concentration in the clinically relevant range of 2 to 8 mg/mL. The effect was not attributable solely to molecular mass because 1.2-MDa hyaluronan produced comparable inhibition. Based on measurements involving hyaluronans of different average molecular masses, polydispersity and viscosity were similarly ruled out as primary responsible factors. The effect of hyaluronan on induced metalloproteinase activity was mediated partially by CD44, the principal cell surface receptor for hyaluronan. Hyaluronan inhibited interleukin-1 $\beta$ -induced metalloproteinase production from osteoarthritic synovial tissue by a process that was not solely dependent on hyaluronan molecular mass but that was partly mediated by hyaluronan binding to CD44. The efficacy of viscosupplementation could be explained if hyaluronan also blocked catabolic enzyme activity in the joint.**

Knee osteoarthritis (OA) is a disabling condition that affects approximately 13% of the population older than 65 years.<sup>5</sup> The disease no longer is conceptualized only in terms of cartilage degeneration but now is seen in relation to cytokine signaling pathways and dysregulation of regulatory mechanisms that govern remodeling of joint tissues.<sup>20,25</sup> The key effector agents in the remodeling are matrix metalloproteinases (MMPs), a class of calcium-dependent enzymes that degrade extracellular matrix.<sup>23</sup> Proinflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ) stimulate the expression of MMPs by synovial cells, and there is a consensus that these enzymes, particularly MMP-1 and MMP-3, are principally responsible for the cartilage destruction that is characteristic of OA.<sup>22,25</sup>

Hyaluronan (HA) is a multifunctional molecule composed of repeating disaccharides of D-glucuronic acid and N-acetyl-glucosamine. In cartilage, HA acts as a structural element by creating space and organizing the tissue.<sup>16</sup> In synovial fluid, HA's unique viscoelastic properties provide joint lubrication and shock absorption.<sup>7</sup> Hyaluronan also can act as a signaling molecule.<sup>17,26,31</sup> All HA functions are dependent on the length of the molecule and therefore on its molecular mass. High-molecular-mass HA is associated with protective or antiinflammatory effects, but at lower molecular masses, it can initiate proinflammatory changes in various cell types, including synovial cells.<sup>17,26,31</sup>

Intraarticular injection of exogenous HA (viscosupplementation) is commonly used to treat knee pain resulting from OA.<sup>32</sup> Exogenous HA mostly is eliminated from the knee within 3 days of injection,<sup>19</sup> which is far less than the period of pain relief provided by the treatment,<sup>32</sup> suggesting its benefit probably is not attributable solely to increased lubricity of joint fluid as originally proposed.<sup>1</sup> One possibility is the biologic signaling properties of HA result in an antiMMP effect.<sup>27,30</sup> Several different molecules on the cell surface can bind HA, including CD44, its principal receptor.<sup>17</sup>

We tested the hypothesis that HA at clinically relevant concentrations inhibited IL-1 $\beta$ -induced MMP activity se-

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creted by explants of synovial tissue from patients with OA. After confirming this hypothesis, we asked two specific questions related to the mechanism of action of HA: (1) Is the mass of the HA molecule a determining factor in the inhibitory effect?; and (2) Does CD44 mediate the inhibition?

## MATERIALS AND METHODS

To test the hypothesis, we measured the effect of HA having a specific molecular mass on IL-1 $\beta$ -induced MMP activity secreted by synovial biopsies from patients with advanced OA. For each patient, we established the tissue was capable of exhibiting an IL-1 $\beta$ -induced MMP response and then compared that response in the presence and absence of a specific concentration of HA. All measurements (basal, +IL-1 $\beta$ , +IL-1 $\beta$ /HA) were made in triplicate and the mean was used in the subsequent statistical analyses. We could not obtain large enough biopsy specimens from individual patients to resolve the role of HA concentration in one experiment. We therefore performed three independent experiments to test the hypothesis: the first two experiments involved one HA concentration but in the third experiment, we obtained a sufficient number of biopsy specimens to allow an evaluation of two HA concentrations (Fig 1). In two additional experiments, the effect of HA on MMP activity was measured in synovial biopsy specimens from patients who had moderate OA or no OA. To answer the question of the effect of HA mass on inhibition, we measured the effect of HA of different molecular masses but the same concentration (8 mg/mL) in three independent experiments (Fig 1). To answer the question of CD44 mediating the inhibition, we compared MMP activity from synovial biopsy specimens treated with IL-1 $\beta$  and HA in the presence or absence of antibody against CD44 (Fig 1).

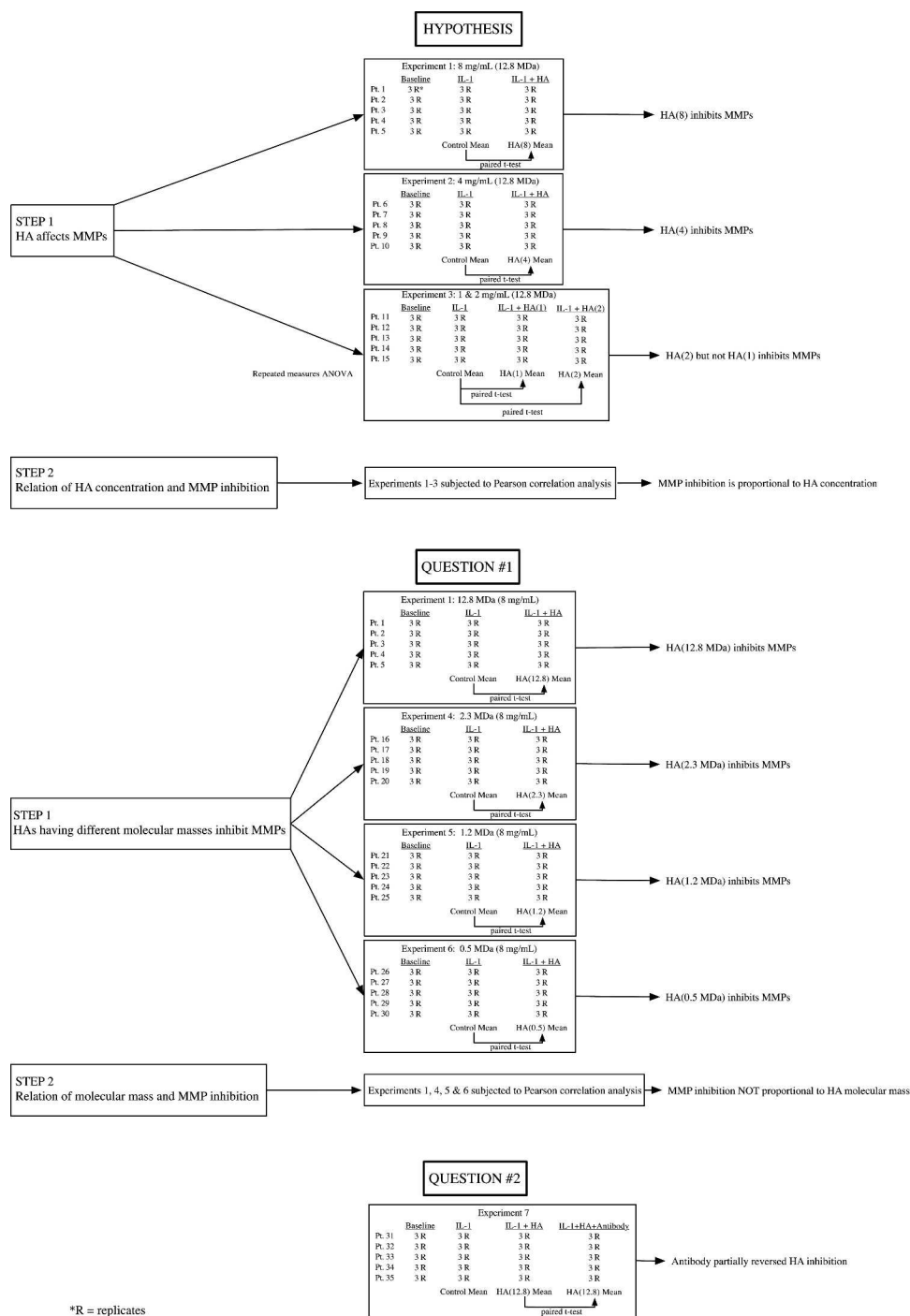
We obtained synovial biopsy specimens from 45 patients during surgery performed by one of the authors (DDW) between April 2003 and April 2005. Thirty-five patients had advanced OA (Kellgren-Lawrence Grade IV)<sup>13</sup> and were undergoing TKAs. Five patients had moderate OA (Kellgren-Lawrence Grade I or II) and were undergoing meniscectomy or ligament reconstruction; five additional patients were operated on for the same reasons but had no radiographic evidence of OA and no arthroscopic evidence of inflammation of the synovial lining (control subjects). The patients in each group were selected randomly. The tissues were taken from the suprapatellar pouch and the fat pad using a full bite of an arthroscopic basket punch (Number 012013; ACUFEX<sup>TM</sup>; Smith and Nephew, Inc, Andover, MA); only relatively flat areas were biopsied to minimize variations in the number of synovial lining cells attributable to irregularities of the synovial surface. The biopsy specimens averaged 20 mg (0.12 cm<sup>2</sup>, 0.1 cm thick)  $\pm$  15%; nine to 12 biopsy specimens were obtained from each patient. All experimental procedures were approved by the Institutional Review Board for Human Research at our institution.

Synvisc<sup>®</sup> (Genzyme Biosurgery, Ridgefield, NJ), Hyalgan<sup>®</sup> (Fidia, Turin, Italy), Orthovisc<sup>®</sup> (Ortho Biotech Products, Bridgewater, NJ), and Supartz<sup>®</sup> (Artz; Smith and Nephew, Inc,

Memphis, TN) were purchased and dialyzed (4°C, 7 days) from phosphate-buffered saline into Neuman and Tytell medium (GIBCO<sup>®</sup>, Carlsbad, CA); the HA concentration was standardized (8 mg/mL). Molecular mass markers for HA were obtained from Hyalose LLC (Oklahoma City, OK). Monoclonal antihuman CD44 (BU75) was obtained from Ancell Corp (Bayport, MN); the antibody, which recognizes a receptor epitope involved in the binding of HA, was used at 5  $\mu$ g/mL (recommended by the manufacturer) and 10  $\mu$ g/mL. All other reagents were obtained from Sigma-Aldrich Corp (St Louis, MO).

The assay for MMPs was described previously.<sup>15</sup> Briefly, all of the biopsy specimens were incubated in serumless Neuman and Tytell medium at 37°C for 24 hours in the presence and absence of human recombinant IL-1 $\beta$  (100 pg/mL), HAs (1–8 mg/mL), and antibody against the HA receptor CD44 (5–10  $\mu$ g/mL). The viability of the tissue was assessed at the end of each assay using the trypan blue exclusion test<sup>29</sup>; tissue viability was greater than 95%. The supernatants were filtered, 1 mmol/L p-aminophenyl-mercuric acetate (APMA) was added to activate latent MMPs, and total enzymatic activity was measured (naturally active enzymes plus those activated by APMA). The enzyme subtypes were not characterized in this study, but numerous studies have shown they consisted principally of collagenase (MMP-1) and stromelysin (MMP-3),<sup>4,18,27,30</sup> both of which are strongly associated with the presence of OA.<sup>11,16</sup> We did not investigate the importance of nonactivated MMPs or the role of tissue inhibitors of MMPs. In preliminary experiments, we determined basal and IL-1 $\beta$ -stimulated enzymatic activity in the supernatants were equally effective against collagen I and collagen II substrates; a collagen I substrate was used in this study. The percent reduction of MMPs was calculated for each patient as  $100[1 - (A - C)/(B - C)]$ , where A, B, and C were the MMP activities measured in the presence of IL-1 $\beta$  and HA, presence of IL-1 $\beta$ , and the absence of IL-1 $\beta$  and HA, respectively. The calculation facilitated analysis of results from different experiments.

The HAs studied, as is the case with all HAs, consisted of molecules of differing lengths and therefore differing molecular masses (polydispersity). To determine HA polydispersity and average molecular mass, the HA solutions were electrophoresed at room temperature using 0.5% agarose gels. Each HA was run in duplicate on the same gel with an adjacent empty lane to permit measurement of the local gel background. The gel was stained overnight with 0.005% Stains-All<sup>®</sup> in 50% ethanol and then washed and photographed using a digital camera. After verifying the optical density response of the camera was linearly related to dye concentration, the optical density of each lane was measured (ImageQuant<sup>®</sup>, Molecular Dynamics; Amersham Biosciences Inc, Piscataway, NJ) as a function of position on the gel, resulting in a molecular mass distribution curve. The duplicate curves were averaged and corrected for background, and the ordinates were calibrated by setting the area under the curve equal to the amount of HA loaded on the gel (7  $\mu$ g). The positions of the HA standards were fitted to a polynomial to produce a calibration curve that was used to label the abscissas. The number-average (HA<sub>N</sub>) and weight-average (HA<sub>W</sub>) molecular masses were calculated from the curves as follows:



**Fig 1.** A diagram shows the experiments and analyses for the experimental hypothesis and two questions regarding the influence of HA mass and CD44 in inhibition of MMPs.

$$HA_N = \sum_i N_i M_i / \sum_i N_i$$

$$HA_W = \sum_i N_i M_i^2 / \sum_i N_i M_i$$

where  $N_i$  is the number of molecules of mass  $M_i$ . The polydispersity index was calculated as  $HA_W/HA_N$ ; the polydispersity index ( $\geq 1$ ) is a measure of the distribution of molecular mass.

The viscosity of Hyalgan®, Orthovisc®, and Supartz® were measured (DV-II viscometer; Brookfield Engineering Laborato-

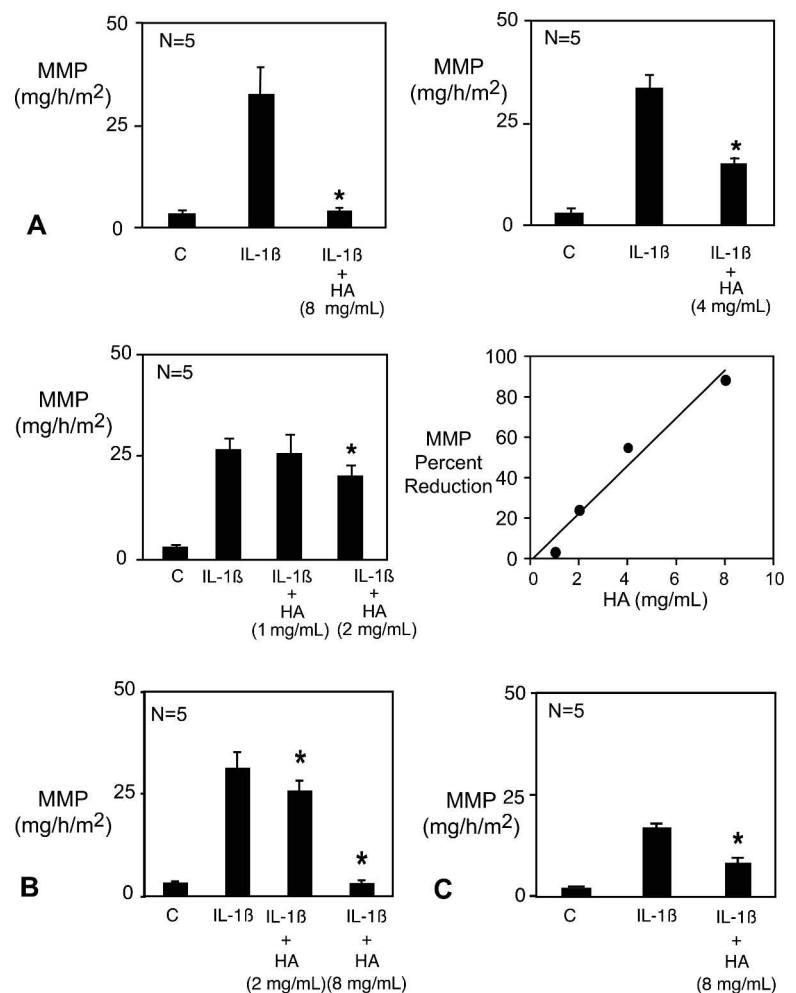
ries, Stoughton, MA) at a concentration of 8 mg/mL using shear rates of 0.3 to 19.2 s<sup>-1</sup>; the data reported were obtained at a shear rate of 2.5 s<sup>-1</sup>. The viscosity of Synvisc® was not measured because it exceeded the range of our viscometer.

To test the experimental hypothesis, we performed three independent experiments (Fig 1). In the first two with one HA concentration we compared untreated and treated tissue from the same patient using the paired t test; in the third with two HA concentrations we used a one-way repeated-measures analysis of variance (ANOVA) followed by Newman-Keuls post hoc tests (Fig 1). The overall effect of HA concentration was evaluated by computing the Pearson correlation coefficient between percent reduction in MMPs (see below) and HA concentration. To answer the first question of mass effect in three independent experiments we used the paired t test; the overall significance of molecular mass was assessed by computing the Pearson correlation coefficient between percent reduction in MMPs and HA mass (Fig 1). To answer the second question involving the role of CD44, we used the paired t test (Fig 1). The assumptions of data normality and uniformity of variance that are technically required for reliability of the statistical tests used were met by the data in each of our experiments. All tests were performed at

$p < 0.05$ . Assuming a rate reduction of 0.7, a sample size of 10 (five with HA treatment and five without) provided a 70% power to detect the treatment difference using the paired t test.

## RESULTS

Hyaluronan (Synvisc®) at concentrations in the range of 2 to 8 mg/mL inhibited IL-1 $\beta$ -induced MMP activity secreted by explants of synovial tissue from patients with OA (Fig 2A–B). When synovial tissue from five patients with advanced OA was cultured for 24 hours in the presence of IL-1 $\beta$ , the total MMP enzymatic activity secreted into the supernatant was more than five times greater ( $p < 0.05$ ) than the basal level (Fig 2A, top left panel). The response to IL-1 $\beta$  was reduced ( $p < 0.05$ ) when HA was added at 8 mg/mL. This effect occurred even when the tissue had first been incubated in HA (8 mg/mL) for 24 hours before the addition of the IL-1 $\beta$ , indicating the HA did not adversely affect the ability of the tissue to respond to IL-1 $\beta$  (data not shown). In two additional experiments,



**Fig 2A–C.** (A) In three independent experiments, when synovial biopsy specimens from patients with advanced OA were cultured in the presence of IL-1 $\beta$  (100 pg/mL) for 24 hours, the amount of MMP activity in the supernatant was decreased by the addition of HA (Synvisc®) in proportion to its concentration (Pearson  $r = 0.99$ ,  $p < 0.05$ ); C = control specimens (no IL-1 $\beta$ ). (B) HA also inhibited IL-1 $\beta$ -induced MMP secretion by synovial tissue from patients with moderate OA. (C) IL-1 $\beta$ -induced MMP secretion by synovial tissue was inhibited by HA from patients without OA. The results of five patients are shown in each of the five data panels (five experiments). Bar and whiskers represent mean and standard deviation. \* =  $p < 0.05$  compared with IL-1 $\beta$ .

MMP activity also was reduced at 4 and 2 mg/mL (Fig 2A, middle and right panels). The reduction of MMP activity was directly proportional to HA concentration (Fig 2A, bottom right panel). Hyaluronan alone (no IL-1 $\beta$ ) had no effect on tissue basal secretion of MMPs (data not shown). Hyaluronan also produced a concentration-dependent effect on MMP activity in patients with moderate OA (Fig 2B) and an effect in patients without OA (Fig 2C). The percent reduction of MMPs at 8 mg/mL was 96%  $\pm$  4% (mean  $\pm$  standard deviation) and 98%  $\pm$  1% for advanced and moderate OA, respectively; both reductions were greater ( $p < 0.05$ ) than the corresponding value in patients with no OA, which was 64%  $\pm$  13% (Fig 2).

The mass of the HA molecule was not a determining factor in the inhibitory effect (Figs 2 and 3; Table 1). In principle, the distribution of HA molecular mass (the polydispersity) and the average value of the distribution (whether HA<sub>N</sub> or HA<sub>W</sub>) and viscosity of the HA all could have been involved in its effect on MMPs (Fig 2). We investigated these possibilities by determining the relative effect of different HAs after first measuring their mass-related properties (Table 1) to ensure they were suitable for exploring the role of mass in the effect on MMPs. We used other approved viscosupplements for this purpose because they were directly relevant to the rationale for the study. Also, because they were manufactured according to standardized laboratory practice, it was reasonable to expect they contained comparable levels of impurities such as endotoxins that could affect the MMP assay. Each of the HAs partially blocked IL-1 $\beta$ -induced MMP activity ( $p < 0.05$ ), but the size of the effects was not correlated with molecular mass (Fig 4A). Supartz<sup>®</sup> and Synvisc<sup>®</sup>

**TABLE 1. Average Molecular Mass, Polydispersity, and Viscosity of Viscosupplements**

Hyaluronan	HA <sub>N</sub> (MDa)	HA <sub>W</sub> (MDa)	Polydispersity Index	Viscosity (mPa · s)
Hyalgan <sup>®</sup>	0.6	0.8	1.36	72 $\pm$ 8
Supartz <sup>®</sup>	1.2	1.9	1.61	737 $\pm$ 6
Orthovisc <sup>®</sup>	2.3	4.4	1.89	3385 $\pm$ 23
Synvisc <sup>®</sup>	12.8	18.8	1.47	25,000 $\pm$ 2000*

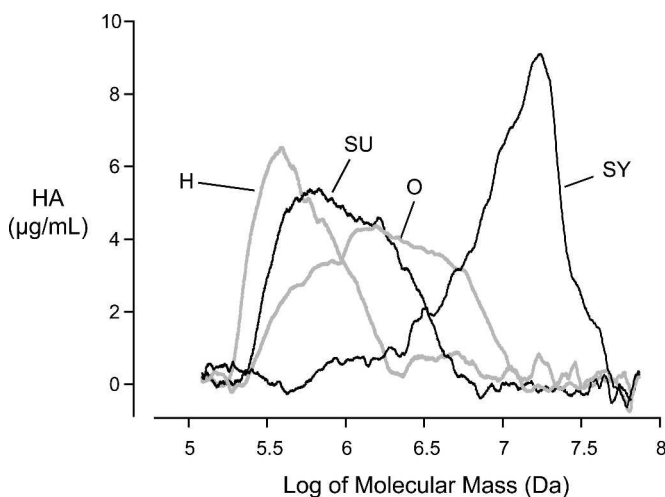
\*Reported in product information; HA<sub>N</sub> = number-average molecular mass; HA<sub>W</sub> = weight-average molecular mass

blocked approximately 90% of the IL-1 $\beta$ -stimulated MMP activity, and Hyalgan<sup>®</sup> and Orthovisc<sup>®</sup> were approximately 20% effective (Fig 4B) (percent reduction in MMPs not correlated with molecular mass). The HAs alone (absence of IL-1 $\beta$ ) had no effect on tissue basal secretion of MMPs (Orthovisc<sup>®</sup> was not studied).

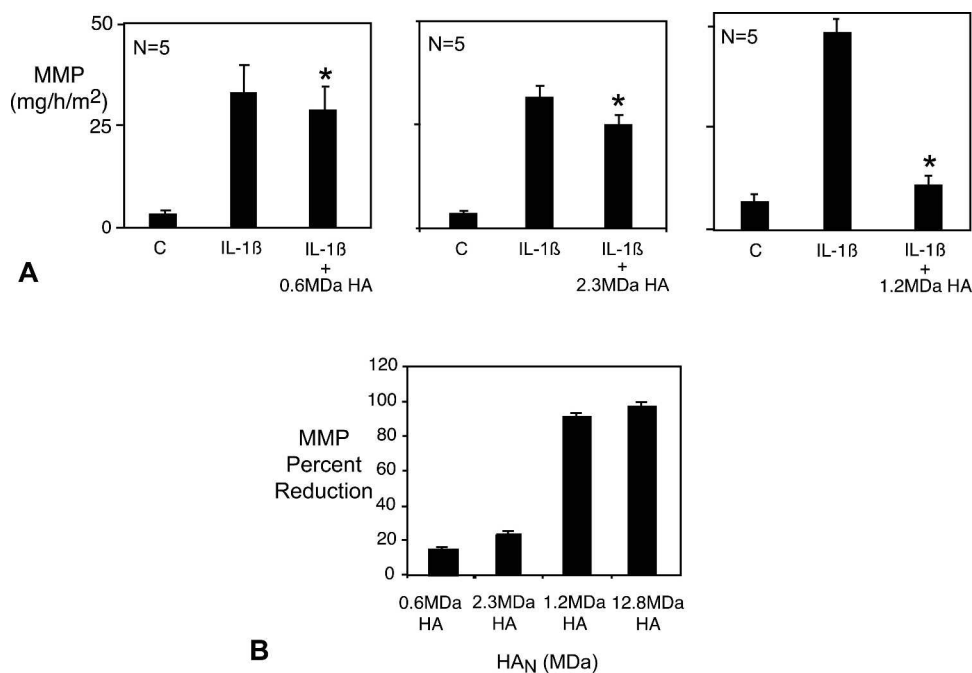
The ability of HA to block the effect of HA on IL-1 $\beta$ -induced MMP activity was partly mediated by CD44 (Fig 5). When antibody (5  $\mu$ g/mL) against a CD44 epitope involved in binding HA<sup>10</sup> was added to cultures containing IL-1 $\beta$  and HA (8 mg/mL), the blocking effect of the HA on MMP activity was reduced ( $p < 0.05$ ) (Fig 5). Increasing the antibody concentration to 10  $\mu$ g/mL did not alter the results (data not shown). In control experiments (no IL-1 $\beta$ ) involving the addition of antibody with or without HA, there was no effect on the constitutive secretion of MMP activity (data not shown).

## DISCUSSION

The effectiveness of viscosupplementation initially was believed to stem from resulting improvements in the viscoelastic properties of synovial fluid produced by the exogenous HA.<sup>1</sup> However, that theory did not explain the persistence of the clinical effect,<sup>32</sup> which extended well beyond the time that the injected material was metabolized and removed from the joint.<sup>19</sup> Osteoarthritis is mediated by MMP destruction of joint cartilage.<sup>22,25</sup> In principle, a reduction in MMP secretion would reduce the chronic, low-level inflammatory process in the joint thereby lessening the frequency or intensity of joint pain. We tested the hypothesis that HA inhibited the catabolic enzyme activity of MMPs secreted by synovial tissue explants in response to stimulation by IL-1 $\beta$ . Our rationale was that evidence of such an inhibition would support the theory that a similar mode of action could explain the clinical efficacy of intraarticular injection of HA. We also inquired into the mechanisms that might be responsible for HA inhibition of MMP activity. First, we asked whether the extent of the inhibition increased linearly with an increase



**Fig 3.** The molecular mass distributions of the four HAs used in the study, Hyalgan<sup>®</sup> (H), Supartz<sup>®</sup> (SU), Orthovisc<sup>®</sup> (O), and Synvisc<sup>®</sup> (SY), differed (logarithmic abscissa).



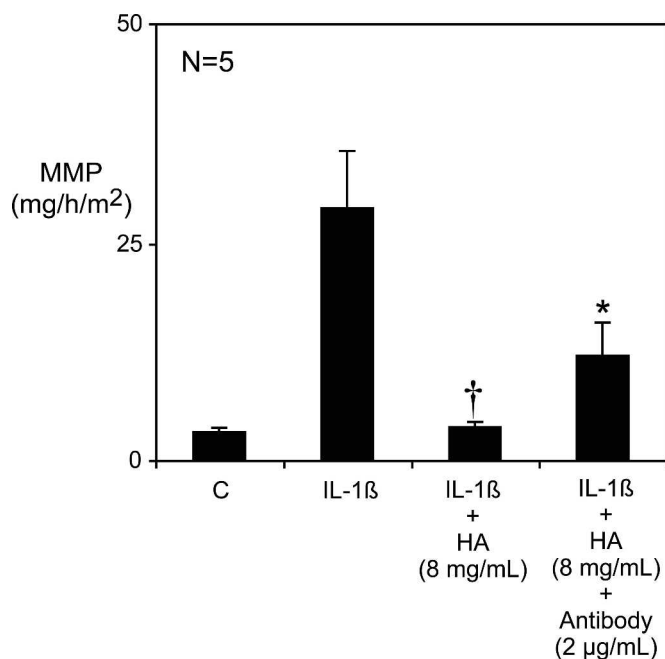
**Fig 4A–B.** (A) When synovial biopsy specimens from patients with advanced OA were cultured in the presence of IL-1 $\beta$  (100 pg/mL) for 24 hours, the decrease in MMP activity in the supernatant resulting from the addition of HA (8 mg/mL) was not proportional to HA average molecular mass (HA<sub>N</sub>) of 0.6, 2.3, and 1.2 MDa for Hyalgan<sup>®</sup>, Orthovisc<sup>®</sup>, and Supartz<sup>®</sup>, respectively; C = control specimens (no IL-1 $\beta$ ). The results of five patients are shown in each of the three panels (three experiments). (B) Supartz<sup>®</sup> (1.2 MDa) and Synvisc<sup>®</sup> (12.8 MDa) (data from Fig 2A) were greater than 90% effective and Hyalgan<sup>®</sup> (0.6 MDa) and Orthovisc<sup>®</sup> (2.3 MDa) were approximately 20% effective in blocking IL-1 $\beta$ -induced MMP activity. Bar and whiskers represent mean and standard deviation. \* =  $p < 0.05$  compared with IL-1 $\beta$  (paired t test)

in the mass of the HA molecule; we planned to interpret evidence for or against such a relationship as implying the HA molecular mass was or was not a determining factor in the inhibitory effect. Second, we asked whether the inhibition was mediated by the ligand-receptor interaction between HA and CD44. We planned to interpret evidence that an interaction-blocking antibody did or did not block the inhibition as evidence for or against the importance of the ligand-receptor interaction.

We note several limitations to our study. The HAs were not well-defined agents because they were polydispersed and may have contained endotoxins or other impurities; these factors could have influenced the results. Our decision to study clinically relevant products helped minimize the inferential limitations resulting from impurities because the HAs were manufactured under regulated conditions (Good Manufacturing Practices). Additionally, we characterized the polydispersity of the HAs we studied. The presence of adipocytes in the synovial biopsy specimens constituted another study limitation because the cells can synthesize MMPs in response to IL-1 $\beta$ <sup>21</sup> and can express CD44.<sup>33</sup> However, adipocytes are materially less responsive than synovial cells to IL-1 $\beta$ .<sup>21</sup> The potential role

of adipocytes was minimized by using the mean of three independent measurements to characterize the tissue response to each condition of interest. Finally, the MMP assay contained no mechanical forces. In this respect, it contrasted sharply with the time-dependent forces normally present in a joint, and the importance of this difference with respect to the relevance of the in vitro model is unknown.

Interleukin-1 $\beta$ -stimulated production of MMP activity by osteoarthritic synovial tissue was reduced in the presence of HA having a number-average molecular mass of 12.8 MDa (Synvisc<sup>®</sup>) in proportion to concentration in the range of 2 to 8 mg/mL, which was comparable to the concentration normally injected into the joint (8 mg/mL); the extent of inhibition did not depend on OA grade (Fig 2A–B). Similar results were observed in rheumatoid synovial cells<sup>27</sup> and cartilage cells<sup>30</sup> but have not been described in intact tissue. Some studies have shown that low-molecular-mass HA is a biologic signaling agent that promotes proinflammatory changes.<sup>17,26,31</sup> The results here support and extend earlier observations<sup>27,30</sup> that high-molecular-mass HA has the opposite effect of promoting antiinflammatory changes. The effects induced by



**Fig 5.** Antihuman CD44 antibody partially reversed the blocking effect of HA (Synvisc®) on IL-1β– (100 pg/mL) induced secretion of MMP activity by synovial biopsy specimens from patients with advanced OA; C = control specimens (no IL-1β). The addition of antibody against HA receptor CD44 partially reversed the inhibition resulting from HA. The results of five patients are shown. Bar and whiskers represent mean and standard deviation. † =  $p < 0.05$  compared with IL-1β. \* =  $p < 0.05$  compared with IL-1β + HA

high-molecular-mass HA also differed in the salient respect that they occurred only at much greater HA concentrations.

High-molecular-mass HA products have been evaluated in studies in which several products were compared and differences in the assays were attributed to product differences, usually residual protein content or molecular mass.<sup>8,9,24</sup> In this study, four HA products were compared and the assay results divided the HA products into two groups with respect to the variable in the assay and hence with respect to the potential implications for the mechanism of action of HA. For example, we could conclude more than molecular size was involved in the HA signaling because the MMP inhibition resulting from 1.2-MDa HA (Supartz®) was comparable to that of 12.8-MDa HA (Synvisc®) (Fig 4B), both of which were greater than the inhibition caused by an HA having an intermediate HA<sub>N</sub> (2.3 MDa, Orthovisc®). Following the same reasoning, we could conclude the inhibition was not proportional to viscosity or polydispersity (Table 1), which supported the interpretation that molecular size was not the critical factor. The relative effect of the viscosupplements (Fig 4B)

was not directly pertinent to the issue of their relative clinical efficacy because none except Synvisc® was studied at its normal clinical concentration.

The question of how HA antagonized the IL-1β signaling pathway leading to MMP production currently cannot be resolved satisfactorily, but our results provide insight into the mechanisms that might be pertinent. First, MMP inhibition occurred only at HA concentrations that were orders of magnitude higher than those typically associated with ligand-binding interactions. For example, the concentration of IL-1β (100 pg/mL) was almost five orders of magnitude less than the HA concentration that antagonized the effect of the cytokine. Consequently, a simple model consisting of the interaction of HA with independent receptors on the cell membrane is probably inapplicable unless one assumes an extraordinarily low binding affinity of the HA with its receptor, and the weight of the evidence from HA-binding studies is against that assumption.<sup>2,28</sup> More likely, high concentrations of HA induced some form of cooperative interaction among membrane receptors such as receptor clustering,<sup>3</sup> leading to a transmembrane signal that modified the IL-1β signaling pathway. The large size of the HA molecules (Table 1) was conducive to the occurrence of cooperative behavior because they were capable of simultaneously binding to many receptors.<sup>3,17,31</sup>

Second, antibody against the portion of CD44 that binds HA partially blocked its inhibitory effect (Fig 5). One possibility was that signaling triggered by HA binding to CD44 down-regulated the nuclear transcription factor NF-κB, which mediates IL-1β-induced expression of MMPs by synovial cells.<sup>12</sup> We did not address the issue of the HA signaling pathway, but the point is that such pathways are known and could explain the effect of HA on MMPs. Marino et al reported synovial cells from patients with OA secreted more MMP activity in response to IL-1β than cells from patients without OA.<sup>21</sup> In the current study, we showed HA was more effective in blocking IL-1β-induced MMP activity in patients with OA (Fig 2). Therefore, there is increasing evidence suggesting synovial cells differ phenotypically in patients with OA. Moreover, we did not observe any difference in the effects of HA on tissue from patients with advanced OA compared with tissue from patients with moderate OA (Fig 2). This might mean the change in synovial physiology associated with the occurrence of OA, for example, an increase in expression of CD44,<sup>6</sup> is more fundamental than any differences associated with the degree of the disease. Another possibility is that the onset of OA is accompanied by a change in the expression or electrophysiologic characteristics of calcium or sodium channels, both of which are critical in the process of IL-1β transduction.<sup>14</sup>

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