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Resistin is elevated following traumatic joint injury and causes matrix degradation and release of inflammatory cytokines from articular cartilage *in vitro*

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Summary

Objective: Resistin is a secreted factor that is elevated in rheumatoid arthritis (RA) and believed to drive joint inflammation *in vivo*. This study was undertaken to determine if resistin is present in the joint following joint injury and to elucidate the role of resistin in cartilage degradation.

Methods: The level of resistin was measured in paired synovial fluid (SF) and serum samples from patients following joint injury (anterior cruciate ligament, ACL or meniscus tear). Localization of resistin was visualized by immunohistochemistry of synovial tissue and cartilage from healthy and OA donors. Mouse and human cartilage cultures were used to assess the effect of resistin on cartilage metabolism.

Results: In trauma patients, resistin levels declined with increasing time post injury. The resistin levels were highest in samples collected up to 1 week following traumatic injury (SF: 2980 pg/ml, serum: 7901 pg/ml) and lowest in samples collected 6–26 years post injury (SF: 686 pg/ml, serum: 5682 pg/ml). Resistin was shown to be expressed in macrophage-like cells in both healthy and OA synovial tissue. Treatment of mouse cartilage cultures with recombinant resistin led to a dose dependent loss of proteoglycan and induction of inflammatory cytokine and PGE_2 production. Recombinant resistin inhibited proteoglycan synthesis in human cartilage explants.

Conclusion: Resistin is elevated both systemically and locally in the weeks immediately following joint injury and has a direct effect on cartilage matrix turnover and cytokine production. Resistin may play a role in the early stages of trauma-induced OA and may represent a new therapeutic target to slow joint destruction in OA.

Key words: Resistin, Cartilage, Osteoarthritis, Injury, Cytokine.

Abbreviations: SF synovial fluid, OA osteoarthritis, RA rheumatoid arthritis, ACL anterior cruciate ligament, IL-1α interleukin 1, alpha, IL-1β interleukin 1, beta, IL-1Rα interleukin 1 receptor antagonist, IL-6 interleukin 6, IL-7 interleukin 7, CCL4/MIP-1α chemokine (C-C motif) ligand 4, CCL3/MIP-1β chemokine (C-C motif) ligand 3, CXCL8/IL-8 interleukin 8, IL-10 interleukin 10, CCL2/MCP-1 chemokine (C-C motif) ligand 2, G-SCF colony stimulating factor 3, TNF-α tumor necrosis factor, alpha, EGF epidermal growth factor (beta-urogastrone), GMSCF colony stimulating factor 2, CCL5/Rantes chemokine (C-C motif) ligand 5, CCL11/Eotaxin chemokine (C-C motif) ligand 11, CXCL10/IP-10 chemokine (C-X-C motif) ligand 10, IL-2 interleukin 2, IL-5 interleukin 5, IL-4 interleukin 4, TGFα transforming growth factor, alpha, CXCL1/fractalkine chemokine (C-X3-C motif) ligand 1, IL-12p40 interleukin 12b, IL-13 interleukin 13, IL-15 interleukin 15, IL-17 interleukin 17, sCD40L CD40 ligand, VEGF vascular endothelial growth factor A, PGE_2 prostaglandin E synthase 2, IFNγ interferon gamma, NFkB nuclear factor of kappa light polypeptide gene enhancer in B-cells, ESR erythrocyte sedimentation rate, IHC immunohistochemistry, CRP C-reactive protein, CXCL8/KC mouse orthologue of human interleukin 8, CCL2/JE mouse orthologue of human chemokine (C-C motif) ligand 2, DAS28 disease activity score 28.

Introduction

Human resistin is a macrophage/monocyte-derived[1] proinflammatory mediator that belongs to the found in inflammatory zone (FIZZ) protein family containing a conserved C-terminal Cys-rich domain[2]. Resistin stimulates the release of several proinflammatory cytokines including interleukin 1 (IL-1), interleukin 6 (IL-6), IL-12, and tumor necrosis factor, alpha (TNF-α) mediated by nuclear factor of kappa light polypeptide gene enhancer in B-cells (NFκB) [3,4]. It induces the expression of intracellular adhesion molecule-1, vascular cell-adhesion molecule-1 and chemokine (C-C motif) ligand 2 (CCL2/MCP-1), all of which are in chemotactic pathways involved in leukocyte recruitment to sites of inflammation[5–7]. Resistin itself can be up-regulated by interleukins and also by microbial antigens such as lipopolysaccharide[8].

Serum resistin levels are positively correlated with inflammatory markers in patients with metabolic diseases[9,10]. Recent studies have shown that resistin is increased in rheumatoid joint fluid[4] and is associated with increased TNF-α, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and disease

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activity score 28 (DAS28)[11,12]. In contrast, one study of 31 rheumatoid arthritis (RA) and 18 healthy individuals failed to detect an increase in resistin levels in RA synovial fluids (SFs)[13].

Osteoarthritis (OA) is a disease of the whole joint and its pathology is characterized by loss of hyaline articular cartilage, osteophyte formation, bony sclerosis, synovial inflammation, increased laxity of the ligaments, and weakening of the muscles surrounding the joint[14]. Though OA has historically been thought of as a non-inflammatory arthritis driven by joint malalignment and biomechanical factors, there is increasing evidence that synovial tissue inflammation is a characteristic in at least a subset of cases. Synovial abnormalities visible during arthroscopy were reported in 50% of patients with painful primary OA with inflammation of the synovial membrane visible in 21%[15]. Inflamed synovium was associated with more severe cartilage degradation over 1 year[15]. Histologic analysis of synovial tissue from OA patients revealed 31% with severe inflammation characterized by thickened intimal lining, angiogenesis, and macrophage infiltration[16]. Magnetic resonance imaging (MRI) has also been used to quantify synovial thickening and this correlates with synovial inflammation by histologic analysis of biopsies in OA patients[17].

Following joint injury in early adulthood, patients are at an increased risk for the early development of OA[18–21], irrespective of surgical intervention to stabilize the joint[22,23]. In men and women who tore their anterior cruciate ligament (ACL) in early adulthood, 41% and 51%, respectively, progressed to radiographic OA within 14 years[22,23]. Meniscal tears typically occur in middle age and 43% of patients with this type of injury progressed to OA by 16 years[24]. Joint injury appears to initiate a cascade of metabolic responses which, in some individuals, result in cartilage destruction, pain, compromised function, and eventually OA. Analysis of human SFs after ACL or meniscal injury has shown elevated levels of cartilage matrix molecules, matrix degrading enzymes, and inflammatory cytokines (interleukin 1, beta (IL-1β), TNF-α, IL-6)[25–31]. Taken together, these data suggest that inflammation is a part of OA pathology and may contribute to disease progression.

The primary objectives of this study were (1) to assess the levels of resistin in SF and serum of patients with injury-induced OA, and (2) to elucidate the direct effects of resistin on cartilage in culture. The data from such studies may provide evidence that blocking resistin activity following joint injury has the potential to decrease inflammation and cartilage degradation and thereby slow progression of joint deterioration toward OA in this patient population.

Materials and methods

RESISTIN LEVELS IN SERUM AND SF

Paired SF and serum samples were obtained from patients at various times following knee injury ($n = 216$). SF was obtained without lavage by joint aspiration to dryness. Injury patients included ACL tears, meniscal tears, or a combination of ACL and meniscus damage diagnosed by arthroscopy (median age 31 (range 13–70) years, 51/216 women). Resistin levels were assayed using an adipokine multiplex kit that included adiponectin, resistin, and active PAI-1 (Linco Research, St Charles, MO). Measurement of resistin by this kit was validated by spike and recovery experiments. The determined working ranges of the assay used were 2.9–1800 pg/ml and 0.6–9200 pg/ml for serum and SF, respectively, with a recovery range of 80–120%. The intra- and inter-assay variabilities were below 10% and 20%, respectively. The serum and SF samples were measured at a dilution 1/4000 and 1/5, respectively. When the measured concentration in either matrix was above the upper limit of quantification, the sample was further diluted to fall into the working range and re-measured. Results from injury patients were split into similar size groups ($n = 29–43$) that represented different lengths of time since injury. Summary statistics for paired serum and SF samples were calculated and boxplots over time were created.

IMMUNOHISTOCHEMISTRY (IHC) WITH ANTI-RESISTIN ANTIBODY IN JOINT TISSUES

Fixed, paraffin embedded synovial and cartilage tissues from three healthy and three OA patient donors were analyzed by IHC with an anti-resistin antibody (R&D Systems, Minneapolis, MN). Tissues were sectioned at 4 μm, dewaxed through 4- and 5-min changes of xylene, and then re-hydrated by a graded alcohol series to distilled water. Steam heat induced epitope recovery was used followed by 20 min blocking and 60 min primary antibody incubation. A biotinylated secondary antibody was used for detection. Tissues were counter stained with hematoxylin. Staining with an isotype control antibody was used to assess specificity of IHC with anti-resistin antibody.

RESISTIN TREATMENT OF MONOCYTES

Human primary monocytes isolated from three normal healthy volunteers were purchased from AllCells, LLC (Emeryville, CA). The cells were treated with recombinant human resistin (Peprotech, Inc, Rocky Hill, NJ; endotoxin level was measured as 0.9 EU/mg of protein). Following 2 days of culture in complete DMEM media containing 10% fetal bovine serum (FBS), conditioned media were collected and cytokines/chemokines assayed in duplicate by a human 30-plex cytokine kit (Linco Research, St Charles, MO). Statistical significance was calculated using Dunnett's test.

CARTILAGE CULTURE IN SUPERNATANTS FROM RESISTIN-TREATED MONOCYTES

Femoral head articular cartilage was isolated from 3-week old mice as described previously[32] and incubated for 2 days in DMEM with 10% FBS, antibiotics, HEPES buffer (Invitrogen, Carlsbad, CA), and ascorbic acid (Sigma, St. Louis, MO) to allow equilibration of the tissue to culture. Following this period, cartilage was cultured in conditioned media from human monocytes treated with recombinant human resistin (see above) diluted 1:3 with complete DMEM containing 10% FBS. Following 3 days of culture, media were collected and glycosaminoglycan (s-GAG) concentrations were measured by a Dimethylmethylene blue (DMMB) assay. Statistical significance was calculated using Dunnett's test.

RESISTIN-TREATED CARTILAGE

Femoral head articular cartilage was isolated from 3-week old mice and cultured as described above. Following equilibration, media were changed and varying amounts of recombinant human resistin (Peprotech, Inc, Rocky Hill, NJ) were added to the cultures. On day three of resistin treatment, culture media were collected and assayed for cytokines using the mouse 22-plex cytokine kit (Linco Research, St Charles, MO), prostaglandin E synthase 2 (PGE_2) by ELISA (R&D Systems, Minneapolis, MN), and s-GAG content by the DMMB assay. Cartilage tissue was fixed and prepared for histology. Briefly, 5 μm sections were deparaffinized, hydrated, and stained for s-GAG content by Toluidine Blue.

Human cartilage tissue samples were procured through Asterand, plc. (Detroit, MI). All tissues were collected pursuant to patient authorization regulated by the U.S. Health Insurance Portability & Accountability Act (HIPAA) and under approval of the Supplier's IRB and in accordance with all Centocor and Johnson & Johnson policies for ethical use and safety. Tissues were received within 24 h *post mortem*. Full thickness, 3 mm diameter biopsy punches were taken from femoral condyle cartilage of two non-OA donors and washed in PBS with antibiotics. These explants were then placed in 50:50 low glucose DMEM:F12 base media supplemented with 10% FBS, 1% antibiotic/antimycotic, 1% HEPES and 2% ascorbic acid and cultured at 37 °C in a humidified incubator with 5% CO_2 and allowed to equilibrate for 72 h. Following equilibration the explants were treated with recombinant resistin for 14 days (media change containing fresh resistin was added every 3–4 days). Cultured media were collected every 3–4 days during the treatment period and the concentration of s-GAG released was measured by DMMB assay. To assess proteoglycan synthesis the explants were incubated with [^{35}S]-sodium sulfate (PerkinElmer, Waltham, MS) for 16 h on the last treatment day. After labeling, the explants were washed in four changes of phosphate buffer and then digested with proteinase K (Roche, Mannheim, Germany). The [^{35}S]-sulfate content of the digests was then counted on scintillation counter. Statistical significance was calculated using Dunnett's test.

Results

RESISTIN LEVELS IN SERUM AND SF

Resistin protein was detected in all serum and synovial fluid samples tested. The level of resistin in both SF and

serum was high after meniscal and ligament injury and declined with increasing time post injury. In SF, the majority of resistin values $>50{,}000$ pg/mL were observed from 1 day to 10 weeks after injury. In serum, the highest values ($>25{,}000$ pg/mL) were distributed over a longer period post injury, one-third of these values were still observed in samples collected within 3 weeks of injury. The median resistin level in SF dropped from ~ 2980 pg/ml in samples collected up to 1 week after injury to ~ 686 pg/ml in samples collected 6–26 years post injury (an approximately fourfold decrease) [Fig. 1(A)]. Median serum resistin level changed from ~ 7901 pg/ml immediately after injury to ~ 5682 pg/ml in samples collected 6–26 years post injury [Fig. 1(B)].

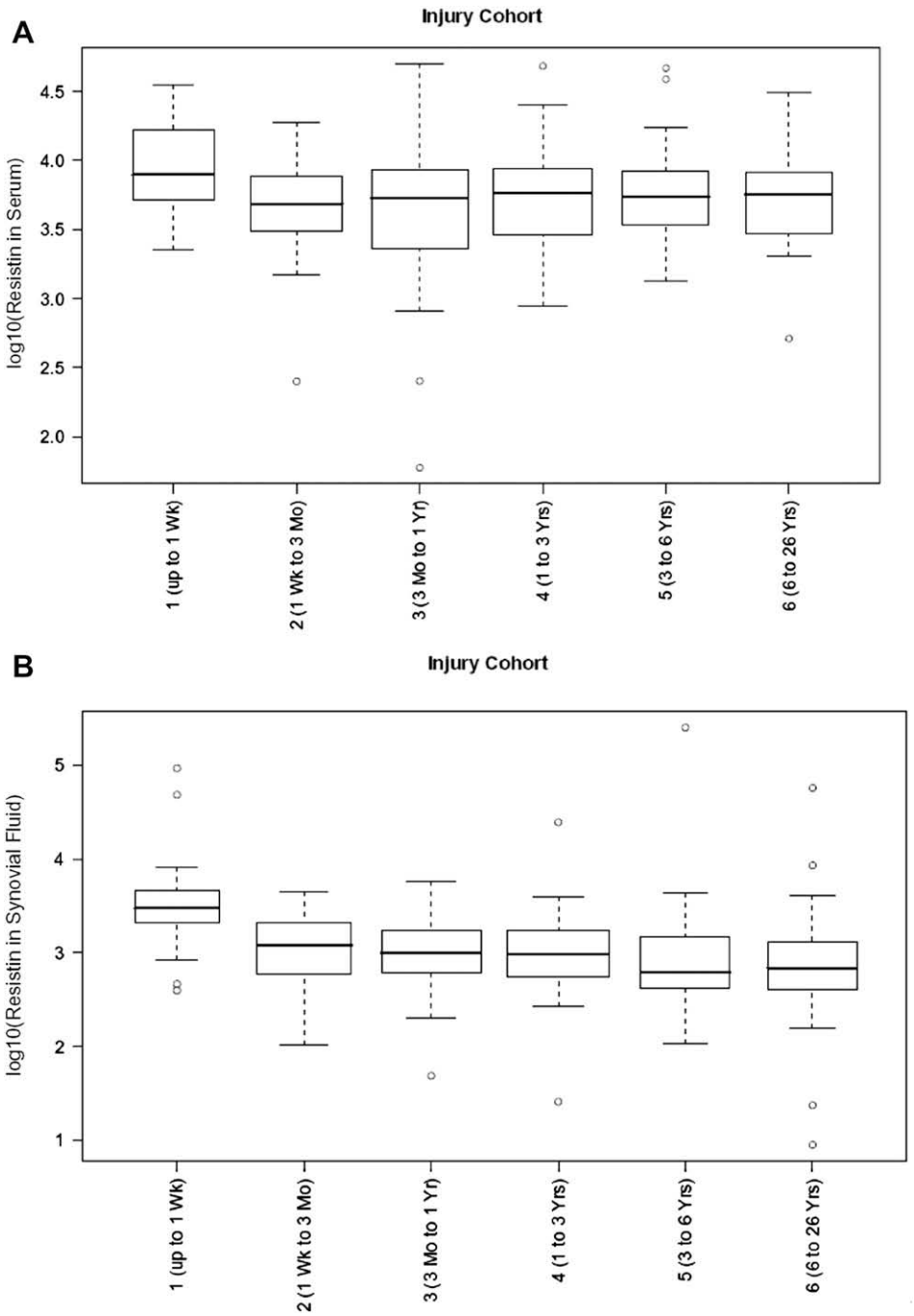

Fig. 1. Resistin levels in SF (A) and serum (B) samples from patients taken at different times since posttraumatic joint injury. Time intervals were chosen to produce an approximately equal number of samples in each group ($n = 29$–43 in each interval). The data presented in box and whiskers plots where the line across the middle of the box is the median, the limits of the box are the 25th and 75th percentiles The ''whiskers'' extending above and below the box end at the most extreme data point that is no more than 1.5 times the interquartile range away from the ends of the box. Any data points beyond this are displayed as circles. The highest resistin values in both SF and serum were observed at shorter time points – up to 5 weeks after injury.

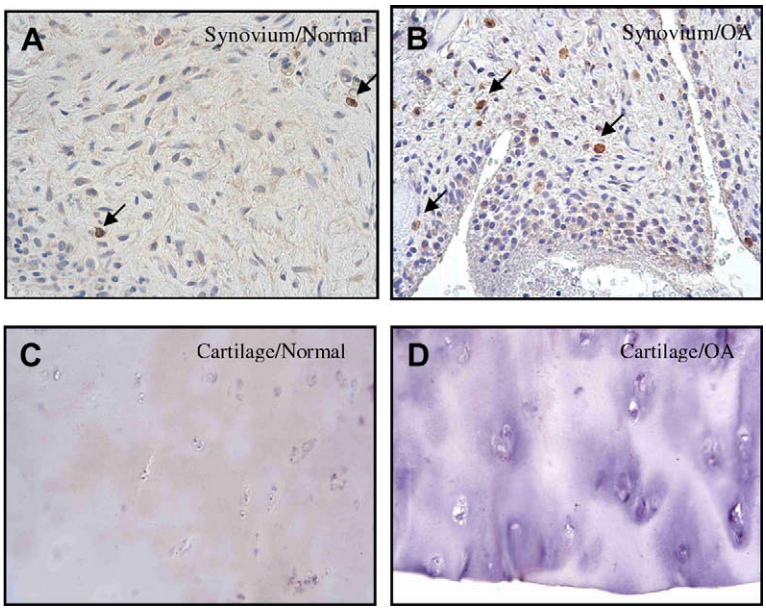

Fig. 2. IHC staining of synovium (A, B) and cartilage (C, D) tissues from normal (A, C) and OA (B, D) patients with an anti-resistin antibody. The arrows indicate examples of the cells positively stained with anti-resistin antibody. Magnification is 40×. The images are representative of sections taken from three patients.

Table I

Human primary monocytes isolated from two healthy donors were treated for 48 h with and without recombinant human resistin (10 μg/ml). Cytokines/chemokines released from monocytes were assayed with a human 30-plex cytokine kit. Results are presented as mean (pg/ml) ± SD

Cytokine	Donor 1		Donor 2	
	Untreated	Resistin	Untreated	Resistin
IL-1α	N/D	41±5	20±10	601±10
IL-1β	28±10	120±12	493±10	3116±17
IL-1Rα	277±6	5804±12	783±15	16,318±20
IL-6	1738±20	11,895±10	2255±5	14,479±20
IL-7	4.6±2	25±3	12±3	50±9
CCL4/MIP-1α	110±10	360±28	331±42	3118±15
CCL3/MIP-1β	226±6	1123±10	793±15	6185±20
CXCL8/IL-8	5021±14	29,470±50	8096±2	47,421±60
IL-10	210±7	2046±15	414±13	4491±10
CCL2/MCP-1	2237±10	13,943±20	607±6	10,630±30
G-SCF	176±3	1025±8	359±28	3413±11
TNF-α	42±9	273±10	68±20	576±7
EGF	N/D	17±2	N/D	26±4
GMSCF	N/D	N/D	N/D	22±5
CCL5/Rantes	N/D	N/D	N/D	52±5
CCL11/Eotaxin	N/D	N/D	N/D	N/D
CXCL10/IP-10	N/D	N/D	N/D	N/D
IL-2	N/D	N/D	N/D	N/D
IL-5	N/D	N/D	N/D	N/D
IL-4	N/D	N/D	N/D	N/D
TGFα	N/D	N/D	N/D	N/D
CX3CL1/fractalkine	N/D	N/D	N/D	N/D
IL-12p70	N/D	N/D	N/D	N/D
IL-12p40	N/D	N/D	N/D	N/D
IL-13	N/D	N/D	N/D	N/D
IL-15	N/D	N/D	N/D	N/D
IL-17	N/D	N/D	N/D	N/D
sCD40L	N/D	N/D	N/D	N/D
VEGF	N/D	N/D	N/D	N/D
IFNγ	N/D	N/D	N/D	N/D

N/D – below the limit of detection.

IHC WITH ANTI-RESISTIN ANTIBODY IN JOINT TISSUES

Positive staining with an anti-resistin antibody was detected in 3/3 normal and OA synovial tissue samples tested. The cell types stained with an anti-resistin antibody were predominantly macrophages and mast cells evenly distributed within the synovium [Fig. 2(A, B)]. Immunoreactive macrophages and mast cells appeared to be more numerous in OA synovium compared to synovium tissue samples from normal donors that might be due to an increase in joint inflammation often observed in OA patients[15–17]. Cartilage was negative for resistin staining in 3/3 tested samples from healthy and OA donors [Fig. 2(C, D)]. No staining with the isotype control antibody was detected (data not shown).

RESISTIN TREATMENT OF MONOCYTES

Recombinant human resistin induced the release of multiple cytokines and chemokines from primary monocytes isolated from the blood of healthy donors. The highest concentration (10 μg/ml) of resistin stimulated the secretion of 13 cytokines out of 30 tested (Table I). IL-6, interleukin 8 (CXCL8/IL-8) and CCL2/MCP-1 were among the cytokines whose secretion was highly increased in a dose dependent manner upon resistin treatment [Fig. 3(A–C)].

CARTILAGE CULTURES IN SUPERNATANTS FROM RESISTIN-TREATED MONOCYTES

To assess if cytokines released by resistin from immunocompetent cells affect cartilage degradation, mouse cartilage explants were incubated with the conditioned media from monocytes treated with recombinant human resistin. The conditioned media from resistin-treated monocytes caused the release of proteoglycans from the tissue into the culture media (Fig. 4). This effect was dose dependent; increasing resistin concentrations on immunocompetent cells resulted in increased levels of proteoglycan release from cartilage.

RESISTIN-TREATED CARTILAGE

We next sought to determine the direct effects of resistin on cartilage explant cultures. Resistin treatment led to release of s-GAG from the cartilage extracellular matrix to the medium [Fig. 5(A)] and decreased proteoglycan staining intensity with Toluidine Blue from the periphery of the mouse cartilage explants [Fig. 5(B–E)]. In addition, mouse cartilage explants treated with resistin released increased levels of IL-6, CXCL8/KC (mouse orthologue of human interleukin 8) and CCL2/JE (mouse orthologue of human chemokine (C-C motif) ligand 2) compared to untreated control cultures [Fig. 6(A)]. Other cytokines and chemokines were below the level of detection in conditioned media from resistin-treated mouse cartilage. In addition, resistin caused a dose dependent increase in the release of a proinflammatory molecule, PGE_2, from mouse cartilage explants [Fig. 6(B)].

To determine the effect of resistin on mature articular cartilage, human cartilage explants were isolated from two non-OA donors and treated with recombinant resistin. In contrast to mouse femoral head cultures, resistin did not significantly affect proteoglycan release in human explants (data not shown) within the culture period studied. This might be explained by higher resistance of adult cartilage to proteoglycan degradation compared to relatively young cartilage isolated from 3-week old mice. However, both concentrations of resistin (1 and 10 μg/ml) significantly inhibited proteoglycan synthesis as assessed by [^{35}S]-sulfate incorporation (Fig. 7).

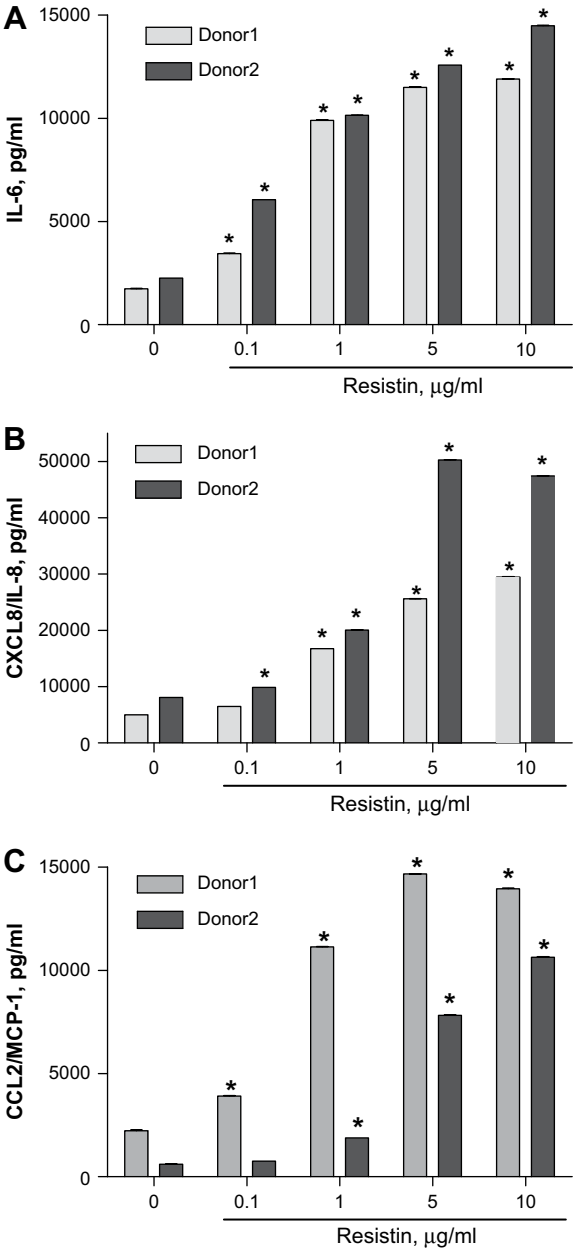

Fig. 3. Human monocytes from two donors were treated with recombinant human resistin for 48 h and resistin-stimulated (A) IL-6, (B) CXCL8/IL-8 and (C) CCL2/MCP-1 release were assayed by human multiplex cytokine kit. Each sample was run in duplicate. Results are presented as mean (pg/ml) ± SD, * indicates *P*-value < 0.05 by Dunnett's test.

Discussion

Over the past decade the role of inflammation in the pathogenesis of OA has received increased attention. As a result there is now increasing evidence that, at least in a subset of patients, the inflammatory cascade may be a prevalent

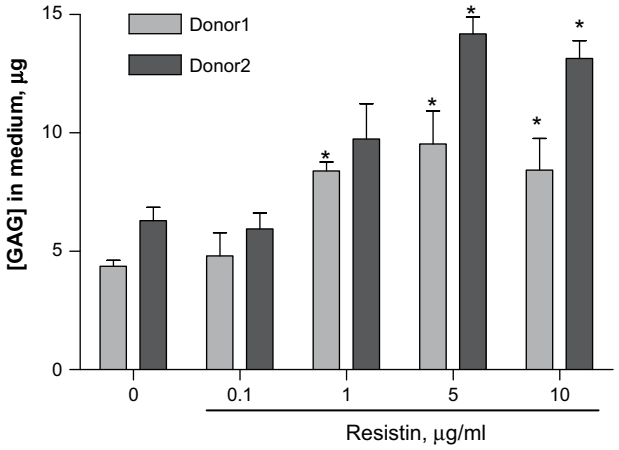

Fig. 4. Mouse articular cartilage was cultured in conditioned media from resistin-treated monocytes. An increase in the release of s-GAG was measured in the media following 3 days of culture using the DMMB assay. Results are presented as mean ± SE, $n = 4$; * indicates P-value < 0.05 by Dunnett's test.

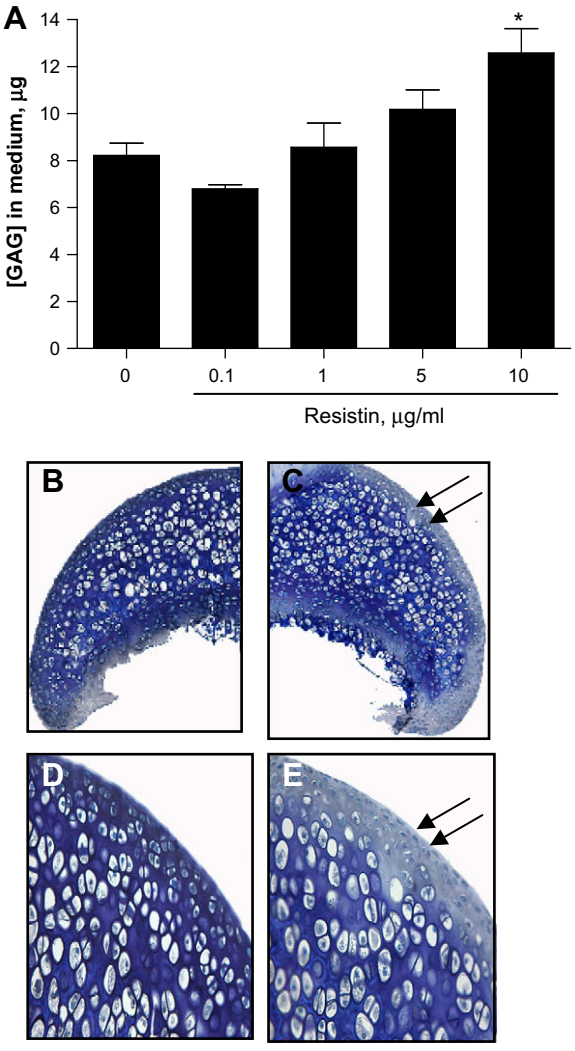

Fig. 5. Mouse articular cartilage was treated with human recombinant resistin for 3 days. (A) Culture supernatants were assayed for s-GAG content by the DMMB dye assay. Results presented are mean ± SE, $n = 4$; * indicates P-value < 0.05 in individual pairwise comparisons of each active group vs the control using Dunnett's test. Control (B, D) and 10 μg/ml resistin-treated (C, E) cartilage explants were fixed for histology and stained with Toluidine Blue to visualize loss of proteoglycans. Magnification is 10× (B, C) and 40× (D, E). The arrows indicate decreased staining intensity of the cartilage matrix in the periphery of the tissue explants.

cause of articular cartilage loss. The studies presented here implicate the involvement of a recently described pro-inflammatory cytokine, resitin, in the pathogenesis of OA.

Resistin was originally identified in mice as an adipokine that might provide the link between obesity and insulin resistance[33]. This idea primarily stems from studies demonstrating that serum resistin levels increase with obesity in mice, rats and humans[34–37]. Since these observations, the physiologic role of resistin and its involvement in insulin resistance and diabetes have been the subject of much controversy. It is worth noting that the resistin receptor has not been identified and that the signaling pathways are not well understood, adding further layers of complexity for understanding resistin biology. Recent research shows that human resistin is expressed in immune cells[1] and possesses many characteristics of a proinflammatory cytokine[4–7]. Senolt *et al.* implicated resistin in the development of RA based on the positive correlation of disease progression with resistin serum and SF levels in RA patients[12]. Injection of recombinant resistin into the healthy mouse knee results in inflammatory arthritis in the injected joint[4] indicating a potential role for resistin in driving joint inflammation. Resistin association with obesity together with its proinflammatory profile suggest that resistin might be one of the mediators that links OA with inflammation and obesity.

We establish for the first time a direct role for resistin in cartilage matrix turnover, and in the induction of inflammatory cytokines and PGE_2 secretion from cartilage cultures. Recombinant resistin stimulated proteoglycan degradation in mouse femoral head cultures and inhibited proteoglycan biosynthesis in human cartilage. Furthermore, we show that resistin is elevated in both serum and SF shortly after joint injury, a period that is often associated with enhanced joint inflammation[25–31]. Increased systemic and local resistin levels following injury, together with its effect on cartilage matrix turnover favoring breakdown over synthesis, suggest that resistin may be involved in the early stages of OA and could be a novel target for the treatment of injury-induced OA.

Resistin is expressed in monocytes/macrophages[2] and we further demonstrate that it can act in a paracrine manner to evoke inflammatory responses from primary monocytes. Cytokines secreted by monocytes in response to resistin drive cartilage matrix breakdown. We demonstrate that, in addition to its direct effect, resistin also indirectly affects cartilage matrix degradation through cytokines released from immune cells. Other inflammatory cytokines, such as TNF-α and IL-1, drive disease activity in RA and are considered to be promising targets in OA[38–40]. These cytokines have a similar ability to modulate inflammation and cartilage breakdown both directly and indirectly.

As was previously shown for primary OA[12,41], serum levels of resistin are higher than in paired SF samples following joint injury. Of interest here is that the median ratio of SF to serum resistin for individual patients was 0.31 during the first weeks following injury and gradually decreased

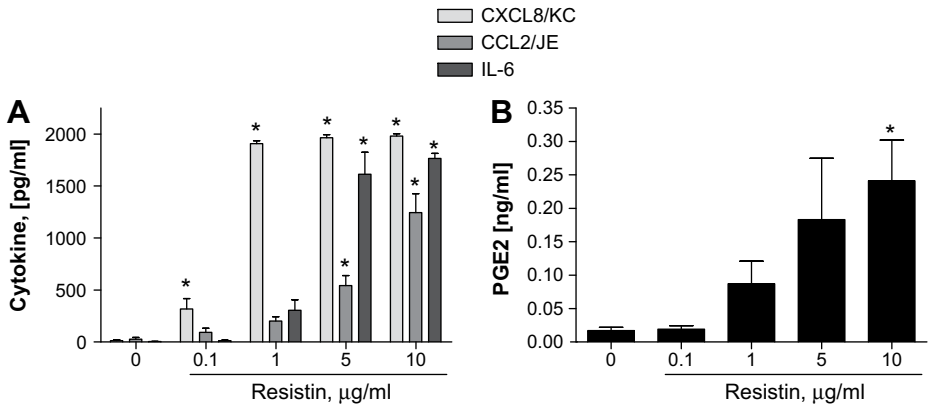

Fig. 6. Mouse cartilage explants were treated with different concentrations of recombinant human resistin for 3 days. Resistin-stimulated secretion of IL-6, CCL2/JE, CXCL8/KC (A) and (B) PGE_2 was measured by Luminex or ELISA kit, respectively. Results presented are mean ± SE, $n = 4$; * indicates P-value < 0.05 in individual pairwise comparisons of each active group vs the control using Dunnett's test.

to 0.15 in samples collected 6–26 years following injury. A post injury increase in the ratio of local to systemic levels suggests that resistin might accumulate in the joint from the circulation or alternatively local resistin production in the joint tissues might be up-regulated following an injury event. In agreement with a previous study[41], we detected resistin immunostaining in macrophage-like cells in normal and OA synovial tissues. We speculate that macrophage-like cells resident in the synovium might contribute to an increase in the local concentration of resistin in joints. Given their immune nature, these cells could be attracted to the joints and/or become activated in inflammatory conditions induced by injury. Although Presle *et al.* did not detect resistin in culture supernatants of joint tissues obtained at arthroplasty (cartilage, synovium, fat pad, meniscus, osteophyte, and bone), high levels of resistin were measured in homogenates of synovium and fat pad obtained from OA joints[41]. Further investigation is required to understand what stimulus might trigger resistin release from synovial tissue.

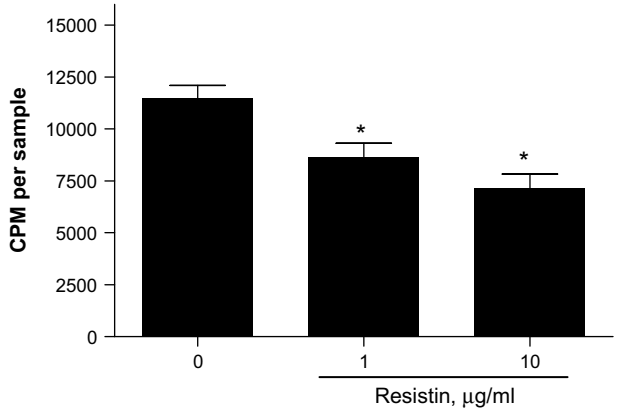

Fig. 7. Human articular cartilage explants isolated from a non-OA donor were treated with recombinant human resistin for 14 days. Proteoglycan synthesis was measured by [^{35}S]-sulfate incorporation during the last 16 h of treatment. The explants isolated from a second non-OA donor showed similar inhibition in proteoglycan synthesis in response to resistin treatment. Results presented are mean ± SE, $n = 6$; * indicates P-value < 0.05 in individual pairwise comparisons of each active group vs the control using Dunnett's test.

Our study is limited by the cross sectional nature of the clinical samples analyzed for resistin, and by lack of information on body mass index of the patients. Body fluid levels of many biomarkers, in particular those in serum, have been shown to change with age, body mass index and disease stage of OA. Resistin levels were not corrected for these confounders. To further understand how resistin levels change in injury patients over time, analysis of longitudinal samples is needed. To understand the role of resistin in human disease, studies with human cartilage at various disease stages and from different patient populations are required.

Intra-articular injection of resistin into healthy mouse joints caused inflammatory arthritis within 4 days characterized by synovitis, pannus formation, and cartilage destruction[4]. Our study further emphasizes the inflammatory nature of resistin as it triggers cytokine release not only from immune cells but also from cartilage explants. Inflammatory responses mediated by resistin directly from chondrocytes and/or from other cells present in femoral head explants, in turn, affect extracellular matrix metabolism and contribute to cartilage tissue destruction. The next step for *in vivo* analysis will be to determine if specific inhibitors of resistin are able to slow or prevent inflammatory or injury induced joint degradation.

Conflict of interest

Dr. Ort, Dr. Ma, Dr. Marsters, Dr. Picha, Dr. Carton, Dr. Baribaud and Dr. Blake are currently employed by Centocor. Dr. Lohmander received research grant support from Centocor through Lund University.

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