Osteoarthritis and rheumatoid arthritis are characterized by focal loss of cartilage due to an up-regulation of catabolic pathways, induced mainly by pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumour necrosis factor α (TNFα). Since reactive oxygen species are also involved in this extracellular-matrix-degrading activity, we aimed to compare the chondrocyte oxidative status responsible for cartilage damage occurring in primarily degenerative (osteoarthritis) and inflammatory (rheumatoid arthritis) joint diseases. Human articular chondrocytes were isolated from patients with osteoarthritis or rheumatoid arthritis, or from multi-organ donors, and stimulated with IL-1β and/or TNFα. We evaluated the oxidative stress related to reactive nitrogen and oxygen intermediates, measuring NO₂⁻ as a stable end-product of nitric oxide generation and superoxide dismutase as an antioxidant enzyme induced by radical oxygen species. We found that cells from patients with osteoarthritis produced higher levels of NO₂⁻ than those from patients with rheumatoid arthritis. In addition, IL-1β was more potent than TNFα in inducing nitric oxide in both arthritides, and TNFα alone was almost ineffective in cells from rheumatoid arthritis patients. We also observed that the intracellular content of copper/zinc superoxide dismutase (Cu/ZnSOD) was always lower in rheumatoid arthritis chondrocytes than in those from multi-organ donors, whereas no differences were found in intracellular manganese SOD (MnSOD) or in supernatant Cu/ZnSOD and MnSOD levels. Moreover, intracellular MnSOD was up-regulated by cytokines in osteoarthritis chondrocytes. In conclusion, our results suggest that nitric oxide may play a major role in altering chondrocyte functions in osteoarthritis, whereas the harmful effects of radical oxygen species are more evident in chondrocytes from patients with rheumatoid arthritis, due to an oxidant/antioxidant imbalance.

**INTRODUCTION**

The progressive deterioration and loss of articular cartilage leading to an irreversible impairment of joint motion are the final pathogenic events common to osteoarthritis (OA) and rheumatoid arthritis (RA). In both arthritides the loss of integrity of the cartilage extracellular matrix depends on an imbalance between anabolic and catabolic pathways [1,2]. It is clearly established that pro-inflammatory cytokines, mainly

**Key words:** chondrocytes, nitric oxide, osteoarthritis, oxygen radicals, rheumatoid arthritis, superoxide dismutases.

**Abbreviations:** IL-1, interleukin-1; MD cells, cells from multi-organ donors; OA, osteoarthritis; RA, rheumatoid arthritis; RNI, reactive nitrogen intermediates; ROI, reactive oxygen intermediates; SOD, superoxide dismutase; TNFα, tumour necrosis factor α.

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interleukin-1 (IL-1) and tumour necrosis factor α (TNFα), can activate chondrocytes to enter a catabolic condition and prime a matrix-degrading activity [3], including protease secretion [4], radical species production [5,6], down-regulation of matrix and protease inhibitor synthesis [7,8], inhibition of chondrocyte proliferation [9] and cell death [10].

Radical species with oxidative activity, which include reactive nitrogen intermediates (RNI) and reactive oxygen intermediates (ROI), play an important role in this chondrocyte catabolic activity, since they represent the mediators and effectors of cartilage damage [11,12]. RNI are derived from the oxidation of the guanido nitrogen of L-arginine, with production of a nitrogen-centred radical, nitric oxide (NO). NO production depends on a large family of NO synthases, which includes constitutively expressed isoforms (neuronal and endothelial NO synthases) and an inducible isoform [13]. Evidence is accumulating that there is an overproduction of inducible NO synthase and NO in OA and RA cartilage compared with normal tissue [13,14]. It has been demonstrated that high local concentrations of NO may exert detrimental effects on chondrocyte functions, including inhibition of collagen and proteoglycan synthesis [7], activation of metalloproteinases [15], decreased expression of IL-1 receptor antagonist [16], inhibition of chondrocyte proliferation [9] and induction of apoptotic death [10]. On the other hand, it has been suggested that the ROI produced inside the joints may also contribute significantly to the pathogenesis of arthritis, since these inorganic oxidants are able to degrade matrix components by direct action or by indirect activation of latent collagenases [17]. Moreover, it has been suggested that ROI act as intracellular second messengers mediating IL-1-induced collagenase gene expression [18]. To prevent ROI being toxic, cells possess a co-ordinated antioxidant enzyme system. Superoxide dismutase (SOD), being the first enzyme in this radical-scavenging system and catalysing the dismutation of superoxide anion into hydrogen peroxide ($H_2O_2$), plays a critical and limiting role. Indeed, if the levels of some antioxidant enzymes are lower or higher than others, $H_2O_2$ can be stored as an intermediate of scavenging reactions and may exert detrimental effects [19]. It has been demonstrated that cytokines, besides inducing ROI production [6], may in parallel modulate the expression and activity of oxygen radical scavengers [20,21]; in particular, IL-1 and TNFα are able to induce SOD expression [22,23].

In the present study, we wanted to compare the chondrocyte oxidative status responsible for cartilage damage occurring in primarily degenerative or inflammatory joint diseases, in order to clarify its role in the pathogenic mechanisms of the two different arthritic disorders, OA and RA. It is generally accepted [13,19] that a reliable estimation of RNI and ROI production can be obtained by evaluating the level either of radical byproducts or of the enzymes induced by these species, whereas the precise measurement of free radical levels is difficult because of their extremely short half-lives. Therefore we evaluated the RNI- and ROI-related oxidative stress indirectly, by measuring the production of NO$_2$ and SOD respectively by human OA- and RA-affected chondrocytes, stimulated in vitro with the two pro-inflammatory cytokines IL-1β and/or TNFα.

**METHODS**

**Specimens**

Articular cartilage fragments were obtained from the tissues of 14 patients with OA (mean age 59 years, range 28–77 years; mean disease duration 4 years, range 3–6 years) and 14 patients with RA (mean age 62 years, range 43–75 years; mean disease duration 17.5 years, range 1–40 years) undergoing hip and knee replacement. The diagnosis of OA was based on clinical, laboratory and radiological evaluations [24,25]. The diagnosis of RA was made according to American College of Rheumatology classification criteria [26]. Cartilage samples were also obtained from three multi-organ donors with no history of joint diseases at the time of explantation (MD cells) (mean age 60 years, range 56–64 years). Informed consent was obtained from all patients, and the Institutional Review Board approved the study. All OA patients were receiving non-steroidal anti-inflammatory drugs and all RA patients were on immunosuppressive treatment.

**Chondrocyte isolation**

Chondrocytes were isolated from articular cartilage by sequential enzymic digestion: 30 min with 0.1% hyaluronidase (Sigma, St. Louis, MO, U.S.A.), 1 h with 0.5% Pronase (Sigma), and 1 h with 0.2% collagenase (Sigma) at 37 °C in Dulbecco’s modified Eagle’s medium (GIBCO BRL, Grand Island, NY, U.S.A.) containing 25 mM Hepes (Sigma), 100 units/ml penicillin (Biological Industries, Kibbutz Beilt Haemek, Israel), 100 μg/ml streptomycin (Biological Industries), 50 μg/ml gentamicin (Flow Laboratories, Biaggio, Switzerland) and 2.5 μg/ml amphotericin (Biological Industries). The isolated chondrocytes were filtered using 100 μm nylon meshes, washed and centrifuged. The pellet was seeded at high density ($2 \times 10^6$ cells/cm$^2$) and cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal calf serum at 37 °C in a humidified atmosphere of 5% CO$_2$. For all experiments, only primary cultures were used, to ensure the stability of the chondrocyte phenotype.

**Chondrocyte stimulation**

At 24 h after seeding, the cell monolayers were incubated for 72 h with or without recombinant human IL-1β
(100 units/ml; specific activity 5 × 10^7 units/mg; Boehringer Mannheim, Mannheim, Germany) and/or recombinant human TNFα (100 units/ml; specific activity 1.0 × 10^8 units/mg; Boehringer Mannheim). The cytokine concentrations and incubation time were standardized based on results obtained in previous dose-dependence and kinetic experiments. After 72 h the supernatants were collected, divided into aliquots, and stored frozen at −80 °C until analysis. Cell monolayers were lysed directly in the culture plate by adding 0.25 ml of 0.5% Triton in PBS per cm², incubating for 30 min on ice and sonicating in iced water (5 × 20 s) with an Ultrasonic 300 sonicator equipped with a sonotrode (Branson Scientific Instruments, NJ, U.S.A.). The suspensions were then harvested and stored frozen at −80 °C until analysis.

**NO production**

NO production was measured by estimating the stable NO metabolite, nitrite, in conditioned medium using a microplate adaptation of the Griess assay. Briefly, 100 μl of each supernatant was incubated with 100 μl of Griess reagent (Molecular Probes, Eugene, OR, U.S.A.) for 10 min at room temperature and the absorbance at 540 nm was measured with an ELISA plate reader (Multiskan Ex; Labsystems, Helsinki, Finland). Concentrations of nitrite were calculated using sodium nitrite in fresh culture medium as a standard. The results were expressed as nmol/10^6 cells.

**Cu/ZnSOD and MnSOD determination**

The cell lysates were thawed and centrifuged for 15 min at 4 °C at 13,000 g, giving clear supernatants. The cell lysates and cell culture supernatants were then examined for concentrations of copper/zinc-dependent (Cu/Zn) and manganese-dependent (Mn) SOD by means of two specific enzyme immunoassays (Cu/ZnSOD ELISA: Bender MedSystems, Vienna, Austria; MnSOD ELISA: Scoti, Tokyo, Japan). The results were expressed as ng/10^6 cells.

**Statistical analysis**

Non-parametric ANOVA tests for multiple comparisons of unpaired and paired data were used (distribution-free Kruskall–Wallis test and Friedman ANOVA), followed by Dunn’s test or the Wilcoxon test respectively for unpaired and paired data. Comparisons between only two independent groups were performed using the Mann–Whitney U-test. Statistical computations were performed using CSS Statistica statistical software (Statsoft Inc., Tulsa, OK, U.S.A.).

**RESULTS**

**NO production**

Unstimulated chondrocytes released NO₃⁻ spontaneously, and NO₃⁻ levels from OA-derived cells were higher than those from RA-derived cells (P < 0.05) (Figure 1). OA chondrocytes also generated more NO₃⁻ than RA cells in response to IL-1β or TNFα (P < 0.05) (Figure 1). However, NO₃⁻ release induced by the combination of the two cytokines was not significantly different between the two diseases.

We also evaluated the efficacy of the different stimuli in NO₃⁻ induction. In OA chondrocytes both IL-1β and TNFα increased NO₃⁻ synthesis significantly compared with the basal condition (P < 0.005); IL-1β was more potent than TNFα (P = 0.002). In RA cells, by contrast, NO₃⁻ production was increased only slightly in response to TNFα, but IL-1β had a significant effect (P < 0.01). Finally, the combined use of IL-1β and TNFα induced a remarkable release of NO₃⁻ in both arthritides (P < 0.05); this effect was probably due primarily to the activity of IL-1β, since a statistically significant difference was only found between the combined treatment with the two pro-inflammatory cytokines and the treatment with TNFα alone (P < 0.05).

**Cu/ZnSOD and MnSOD content and modulation**

Under basal conditions, different amounts of intracellular Cu/ZnSOD were found in chondrocytes from the various groups of subjects (Table 1). RA chondrocytes had the lowest levels of the enzyme, whereas MD cells had the highest levels (P < 0.05). Treatment with cytokines failed to cause a statistically significant increase in the amount of intracellular Cu/ZnSOD, and the difference among groups paralleled the results obtained in absence of stimuli (RA compared with MD: P < 0.05)
Cu/ZnSOD release into the supernatant by OA (n = 14), RA (n = 14) and MD (n = 3) chondrocytes

Results are expressed as absolute amounts of enzyme released into the supernatant (ng/10^6 cells) after 72 h of culture in the basal condition or after stimulation with IL-1β and/or TNFα. Values are means ± S.E.M. Friedman ANOVA (comparison between cytokine-treated cells and unstimulated cells) and ANOVA Kruskal–Wallis (comparison among different disease-affected cells and normal cells) showed no significant differences.

**Table 3 CuZnSOD release into the supernatant by OA (n = 14), RA (n = 14) and MD (n = 3) chondrocytes**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OA</th>
<th>RA</th>
<th>MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated</td>
<td>135.1 ± 21.8</td>
<td>128.8 ± 25.0</td>
<td>200.2 ± 43.7</td>
</tr>
<tr>
<td>IL-1β</td>
<td>143.7 ± 25.0</td>
<td>129.2 ± 27.6</td>
<td>171.7 ± 46.1</td>
</tr>
<tr>
<td>TNFα</td>
<td>132.9 ± 18.2</td>
<td>120.8 ± 23.9</td>
<td>178.1 ± 45.2</td>
</tr>
<tr>
<td>IL-1β + TNFα</td>
<td>117.3 ± 21.7</td>
<td>120.0 ± 33.8</td>
<td>184.3 ± 60.1</td>
</tr>
</tbody>
</table>

Cu/ZnSOD release into the supernatant by OA (n = 14), RA (n = 14) and MD (n = 3) chondrocytes was statistically significant only in OA disease condition, the up-regulation of MnSOD by cytokine-treated cells with unstimulated cells within each group showed no significant differences between cytokine-stimulated and untreated cells. Statistical analysis showed no significant differences between intracellular Cu/ZnSOD levels in cytokine-stimulated and untreated cells.

**Table 2 Intracellular MnSOD levels in chondrocytes from OA (n = 5) and RA (n = 5) patients and MD (n = 3) subjects**

Results are expressed as absolute amounts of enzyme released into the supernatant (ng/10^6 cells) after 72 h of culture in the basal condition or after stimulation with IL-1β and/or TNFα. Values are means ± S.E.M. Significance of differences (Friedman ANOVA and Dunn’s test): *P < 0.05 for OA compared with RA; †P < 0.05 for RA compared with MD. Statistical analysis showed no significant differences between intracellular MnSOD levels in cytokine-stimulated and untreated cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OA</th>
<th>RA</th>
<th>MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated</td>
<td>191.2 ± 45.7</td>
<td>107.8 ± 23.9</td>
<td>376.3 ± 35.4</td>
</tr>
<tr>
<td>IL-1β</td>
<td>249.1 ± 22.2</td>
<td>130.7 ± 25.8</td>
<td>419.5 ± 62.9</td>
</tr>
<tr>
<td>TNFα</td>
<td>285.4 ± 46.2 †</td>
<td>103.2 ± 26.3 †</td>
<td>357.3 ± 56.7</td>
</tr>
<tr>
<td>IL-1β + TNFα</td>
<td>253.0 ± 30.0 †</td>
<td>84.6 ± 12.2 †</td>
<td>380.2 ± 81.9</td>
</tr>
</tbody>
</table>

Table 1 Intracellular Cu/ZnSOD levels in chondrocytes from OA (n = 5) and RA (n = 5) patients and MD (n = 3) subjects

Results are expressed as absolute amounts of intracellular enzyme (ng/10^6 cells) after 72 h of culture in the basal condition or after stimulation with IL-1β and/or TNFα. Values are means ± S.E.M. Significance of differences (ANOVA Kruskal–Wallis and Dunn’s test): *P < 0.05 for OA compared with RA; †P < 0.05 for RA compared with MD. Statistical analysis showed no significant differences between intracellular Cu/ZnSOD levels in cytokine-stimulated and untreated cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OA</th>
<th>RA</th>
<th>MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated</td>
<td>190.6 ± 131.1</td>
<td>786.3 ± 459.1</td>
<td>901.9 ± 137.1</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1906.2 ± 422.4 *</td>
<td>1293.0 ± 502.9</td>
<td>1859.1 ± 635.3</td>
</tr>
<tr>
<td>TNFα</td>
<td>2533.3 ± 867.3 *</td>
<td>1136.9 ± 442.1</td>
<td>2158.5 ± 938.0</td>
</tr>
<tr>
<td>IL-1β + TNFα</td>
<td>2166.3 ± 840.5 *</td>
<td>1082.1 ± 330.7</td>
<td>1610.1 ± 463.2</td>
</tr>
</tbody>
</table>

Cu/ZnSOD release into the supernatant was abundant under basal conditions for all samples studied (Table 3). MnSOD was also released into the culture medium of unstimulated chondrocytes, but was less abundant (Table 4). Neither Cu/ZnSOD nor MnSOD levels in the supernatant were influenced significantly by stimulation with pro-inflammatory cytokines, and there were no differences between chondrocytes from OA, RA and MD subjects (Tables 3 and 4).

**Table 4 MnSOD release into the supernatant by OA (n = 4), RA (n = 4) and MD (n = 3) chondrocytes**

Results are expressed as absolute amounts of enzyme released into the supernatant (ng/10^6 cells) after 72 h of culture in the basal condition or after stimulation with IL-1β and/or TNFα. Values are means ± S.E.M. Friedman ANOVA (comparison between cytokine-treated cells and unstimulated cells) and ANOVA Kruskal–Wallis (comparison among different disease-affected cells and normal cells) showed no significant differences.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OA</th>
<th>RA</th>
<th>MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated</td>
<td>65.6 ± 11.6</td>
<td>56.9 ± 32.4</td>
<td>170.5 ± 73.9</td>
</tr>
<tr>
<td>IL-1β</td>
<td>83.9 ± 16.5</td>
<td>33.8 ± 13.5</td>
<td>195.8 ± 95.7</td>
</tr>
<tr>
<td>TNFα</td>
<td>77.8 ± 18.2</td>
<td>34.0 ± 15.8</td>
<td>207.7 ± 103.1</td>
</tr>
<tr>
<td>IL-1β + TNFα</td>
<td>90.7 ± 22.0</td>
<td>25.0 ± 19.8</td>
<td>197.0 ± 94.4</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The breakdown of articular cartilage in RA and OA is likely to be related to the synthesis and release of catabolic factors in the microenvironment of the joint. We demonstrated previously [27] that pro-inflammatory cytokines and inducible NO synthase were highly expressed in synovial membranes from patients with inflammatory arthritides, but rarely expressed by synovial cells in OA conditions (Table 1). Finally, OA chondrocytes possessed always more Cu/ZnSOD than RA cells, but this difference became significant only after addition of TNFα (P < 0.05) (Table 1).

With regard to intracellular MnSOD, we found that the content of MnSOD was not significantly different among OA, RA and MD cells, both in the basal condition and upon activation with cytokines (Table 2). On comparing cytokine-treated cells with unstimulated cells within each disease condition, the up-regulation of MnSOD by cytokines was statistically significant only in OA chondrocytes (P < 0.05) (Table 2). Finally, when we grouped all the chondrocyte samples, a higher degree of statistical difference compared with the basal condition was obtained with treatment either IL-1β or TNFα alone (P < 0.005 for IL-1β or TNFα alone; P < 0.05 for the combined treatment).
and traumatic arthritis. In contrast, these mediators were frequently expressed by OA-affected chondrocytes, and were almost absent from cartilage obtained from patients with inflammatory joint disease. In the present study, we showed that the production of NO by isolated chondrocytes cultured in vitro was significantly higher in OA than in RA chondrocytes both under basal conditions and after stimulation with IL-1β or TNFα. These findings further support the hypothesis that OA chondrocytes are a more important source of NO than RA cartilage. The spontaneous overproduction of NO in OA compared with RA may indicate the presence of inflammatory factors such as autocrine cytokines or growth factors in osteoarthritic cartilage [13], or it may be due to a constitutively up-regulated NO synthase in OA [28]. OA chondrocytes also released higher levels of NO2- after cytokine treatment. It has been shown that the number of type I IL-1 receptors is significantly increased in OA chondrocytes [29], and it is well known that the increased release of NO by IL-1β-stimulated OA chondrocytes inhibits synthesis of the IL-1 receptor antagonist. Furthermore, TNFα receptor expression is significantly increased on OA chondrocytes [30]. On the other hand, pro-inflammatory cytokines and their specific receptors seem to be more highly expressed in synovial tissue and at the cartilage/pannus junction in RA than in OA [31,32]. On comparing the efficacy of the different stimuli in the induction of NO release in the two diseases, we confirmed an enhanced susceptibility of OA cells to cytokine-induced NO production compared with RA chondrocytes. It is noteworthy that IL-1β induced the most prominent effects and was more potent than TNFα, which was ineffective when added alone to RA-affected chondrocytes, further underlining the lower responsiveness of these cells. It has been reported previously that normal cartilage releases lower levels of NO than arthritic cartilage [13,16], but no information was available concerning the comparison between OA and RA, which was the aim of the first part of our study.

On the other hand, to our knowledge there has been no report dealing with the ability of chondrocytes to produce the superoxide-scavenging enzymes (Cu/ZnSOD and MnSOD) in response to cytokine-induced ROI generation. Therefore we performed this second part of the study on both cell lysates and supernatants from normal and arthritic chondrocytes. We found that the intracellular Cu/ZnSOD content was always significantly lower in RA chondrocytes than in MD cells. Although OA chondrocytes also possessed higher levels of Cu/ZnSOD than RA cells, we observed a significant difference only when the cells were treated with TNFα or with IL-1β plus TNFα. These results could lend themselves to different interpretations. We might hypothesize that RA cartilage is more susceptible to ROI attack because of a pre-existing disease-related deficit in antioxidant enzyme levels compared with normal chondrocytes. Indeed, since pro-inflammatory cytokines failed to modulate the amount of intracellular Cu/ZnSOD, the basal inadequacy of the antioxidant enzyme persists during stimulation with IL-1β and TNFα, while ROI production probably rises, thus worsening the oxidant/antioxidant imbalance. On the other hand, we did not observe any difference in intracellular MnSOD levels between arthritic cells and normal chondrocytes either in the basal condition or after stimulation with cytokines. Therefore, since cytoplasmic Cu/ZnSOD, in contrast with mitochondrial MnSOD, can be released upon cell activation and degranulation [33], we might alternatively postulate that RA chondrocytes have been depleted in vivo of Cu/ZnSOD by the persistent presence of pro-inflammatory cytokines inducing cell degranulation and release into the synovial fluid. Indeed, SOD activity in knee joint fluids from patients with RA has been found to be significantly higher than in those from control subjects [34,35], although conflicting data are presented in the literature [36]. Moreover, our group has reported elevated Cu/ZnSOD levels in RA sera [37], probably resulting from a high SOD concentration in synovial fluid. It has been hypothesized that a similar increase in Cu/ZnSOD could be inadequate to exert effective antioxidant protection, but could in turn increase the oxidative burden via overproduction of H2O2 and generation of the highly reactive OH· radical during the Fenton [38] or Haber–Weiss [39] reaction. Our findings, however, independent of their interpretation, provide evidence that the harmful effects of oxygen radicals seem to be involved primarily in articular destruction in RA compared with OA.

It is interesting to note that stimulation with IL-1β and TNFα increased the intracellular MnSOD content significantly in OA chondrocytes. The difference between cytokine-treated cells and unstimulated cells became more highly significant when grouping data from all chondrocyte samples, indicating that chondrocytes react to the increased oxidant stress by inducing compensatory changes in the levels of this antioxidant enzyme. This observation is fully in keeping with the concept that MnSOD is more inducible by cytokines than Cu/ZnSOD [20]. In contrast, investigation of the release of Cu/ZnSOD and MnSOD into the cell culture supernatant did not reveal any modulation by cytokines or any significant difference between arthritic and normal chondrocytes, suggesting that cells counteract the effect of ROI, which are indeed generated endogenously during intracellular aerobic reactions, mainly by increasing their intracellular antioxidant activities. These results underline the importance of our study performed directly on chondrocytes in order to assay SOD as a marker of local oxidative damage. Until now, the amount and activity of this antioxidant enzyme have been evaluated mainly in serum [35–37], synovial fluid [34–36] or erythrocytes [40] from patients with RA, with conflicting findings that
probably reflect the existence of complex systemic mechanisms or represent the contributions of different types of cells.

Taken together, our data may identify the types of molecules mainly responsible for local oxidant-induced joint injury in OA and RA. We can postulate that, in OA-affected chondrocytes, the harmful effects of free radicals are due mainly to more abundant NO production, whereas ROI attack is more prominent in RA chondrocytes due to an oxidant/antioxidant imbalance. Since it has been demonstrated that, in cytokine-activated chondrocytes, increased NO levels are associated with apoptosis only in the presence of oxygen radical scavengers, and increased levels of ROI cause necrosis only under conditions in which the simultaneous production of NO is lowered [10], we can also suggest that cell death in OA-affected chondrocytes is principally NO-mediated apoptosis [41,42], in contrast with minor apoptotic chondrocyte death [43], but more frequent ROI-mediated necrosis, in RA chondrocytes.

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