View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

OsteoArthritis and Cartilage (2003) **11**, 747–755
© 2003 OsteoArthritis Research Society International. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/S1063-4584(03)00150-X

Osteoarthritis and Cartilage

The role of reactive oxygen species in homeostasis and degradation of cartilage

Y. E. Henrotin Ph.D. Professor†*, P. Bruckner Ph.D. Professor‡ and J.-P. L. Pujol Ph.D. Professor§
† *Bone and Cartilage Research Unit, Institute of Pathology, level 5, CHU Sart-Tilman, Liège, Belgium*
‡ *Institute of Physiological Chemistry and Pathobiochemistry, Muenster University Hospital, 48149 Muenster, Germany*
§ *Laboratory of Connective Tissue Biochemistry, University of Caen, France*

Summary

Objectives: The metabolism of cells in articular joint tissues in normal and pathological conditions is subject to a complex environmental control. In addition to soluble mediators such as cytokines and growth factors, as well as mechanical stimuli, reactive oxygen species (ROS) emerge as major factors in this regulation. ROS production has been found to increase in joint diseases, such as osteoarthritis and rheumatoid arthritis, but their role in joint diseases initiation and progression remains questionable.

Method: This review is focused on the role of ROS, mainly nitric oxide, peroxynitrite and superoxide anion radicals, in the signaling mechanisms implied in the main cellular functions, including synthesis and degradation of matrix components. The direct effects of ROS on cartilage matrix components as well as their inflammatory and immunomodulatory effects are also considered.

Results: Some intracellular signaling pathways are redox sensitive and ROS are involved in the regulation of the production of some biochemical factors involved in cartilage degradation and joint inflammation. Further, ROS may cause damage to all matrix components, either by a direct attack or indirectly by reducing matrix components synthesis, by inducing apoptosis or by activating latent metalloproteinases. Finally, we have highlighted the uncoupling effect of ROS on tissue remodeling and synovium inflammation, suggesting that antioxidant therapy could be helpful to treat structural changes but not to relieve symptoms.

Conclusions: This review of the literature supports the concept that ROS are not only deleterious agents involved in cartilage degradation, but that they also act as integral factors of intracellular signaling mechanisms. Further investigation is required to support the concept of antioxidant therapy in the management of joint diseases.
© 2003 OsteoArthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

Key words: Cytokines, Oxygen, Free radicals, Osteoarthritis, Inflammation.

Introduction

In normal conditions, chondrocytes are living in an avascular environment, with low oxygen supply as a consequence. Nevertheless, some of their metabolic functions are dependent on oxygen, which is mainly supplied by the synovial fluid. Chondrocytes display a metabolism adapted to anaerobic conditions. In pathological conditions, oxygen tension in synovial fluid is subject to fluctuation as a consequence of ischemia–reperfusion phenomenon, pathological acceleration of tissue metabolism and sustained abnormal strains on the joint[1]. In response to partial oxygen pressure (pO_2) variations, mechanical stress, immunomodulatory and inflammatory mediators, chondrocytes produced abnormal levels of reactive oxygen species (ROS) that are generally produced by immune cells to assume host defense[2–5]. The main ROS produced by chondrocytes are nitric oxide ($^{\cdot}NO$) and superoxide anion ($O_2^{\cdot -}$) that generate derivative radicals, including peroxynitrite ($ONOO^-$) and hydrogen peroxide (H_2O_2)[6,7]. $NO^{\cdot}$ is synthesized by $^{\cdot}NO$ synthase (NOS) enzymes. Of the three NOS isoforms, two are constitutively expressed, endothelial NOS (eNOS, NOS3) and neuronal NOS (nNOS, NOS1), and one is inducible (iNOS, NOS2). Chondrocytes express both eNOS and iNOS. The inducible form is regulated at the gene level by a variety of growth factors, cytokines and endotoxins. $^{\cdot}NO$ production is stimulated by interleukin (IL)-β, tumor necrosis factor (TNF)-α, interferon (IFN)-γ and lipopolysaccharides (LPS), and inhibited by transforming growth factors (TGF)-β, IL-4, IL-10 and IL-13[8–11]. Superoxide anion radicals are produced by the enzyme complex NADPH, which catalyzes the reduction of molecular oxygen to superoxide anion radicals. NADPHoxidase complex consists of essentially two membrane-bound peptides. A flavocytochrome consists of two peptides of 22 and 91 kDa ($p22^{phox}$ and $gp91^{phox}$, respectively) and a regulatory peptide named Rap1A. Activation of the oxidase requires the translocation to the membrane of at least three further cytosolic components of 40, 47 and 67 kDa ($p40^{phox}$, $p47^{phox}$ and $p67^{phox}$, respectively). Articular chondrocytes express cell-specific components of NADPHoxidase complex such as $p22^{phox}$, $p40^{phox}$, $p47^{phox}$, $p67^{phox}$ and $gp91^{phox}$. NADPHoxidase is activated by

This paper has been supported by an unrestricted educational grant from UCB Pharma.
*Address correspondence and reprint requests to: Dr Yves E. Henrotin, Bone and Cartilage Research Unit, Institute of Pathology, level 5, CHU Sart-Tilman, 4000 Liège, Belgium. Tel: 32-4-366-24-67; Fax: 32-4-366-47-34; E-mail: yhenrotin@ulg.ac.be
Received 13 January 2003; revision accepted 25 June 2003.

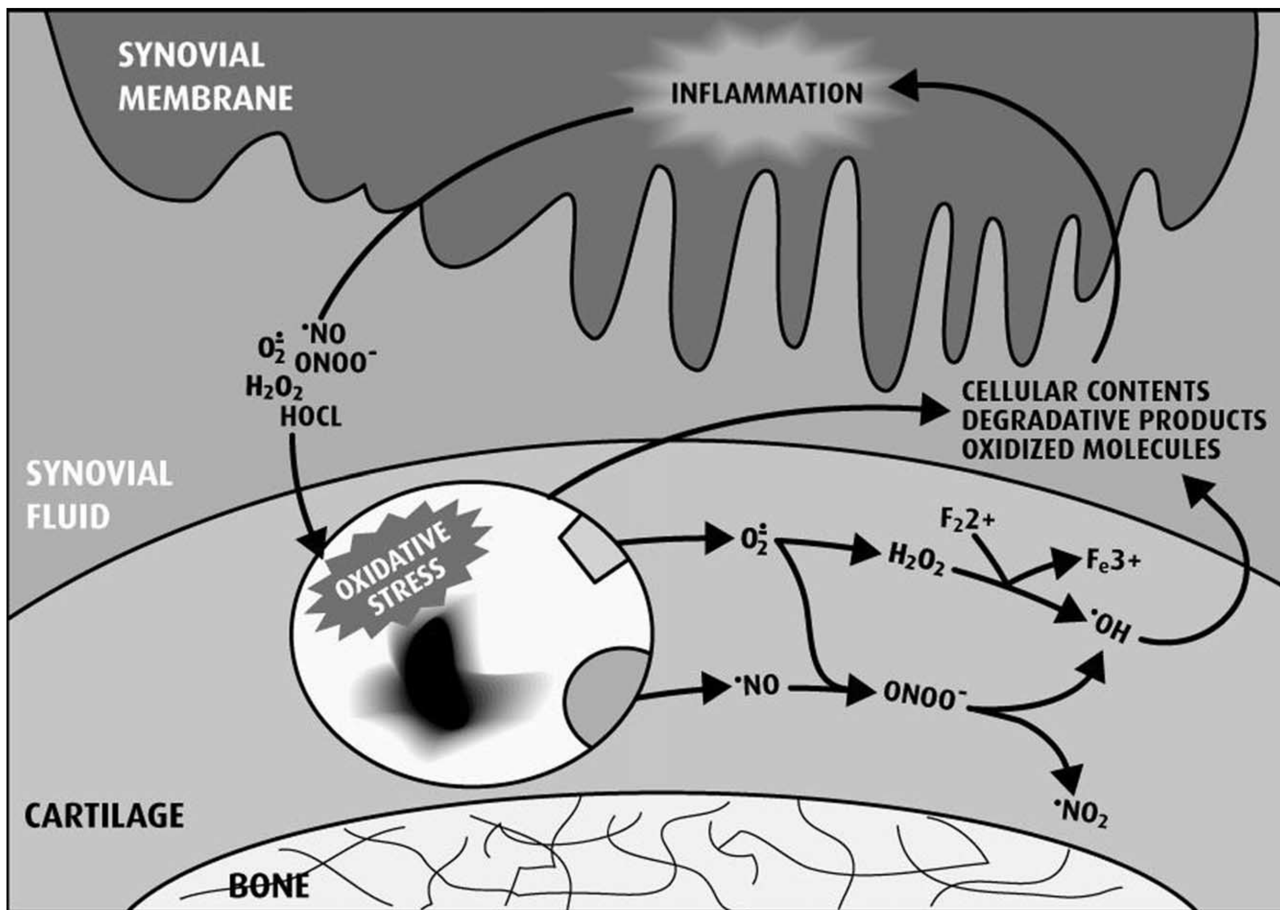

Fig. 1. Implication of ROS in cartilage degradation and related synovium inflammatory reaction. H_2O_2=hydrogen peroxide; $ONOO^-$=peroxynitrite; $^{\cdot}OH$=hydroxyl radical; $O_2^{\cdot-}$=superoxide anion.

calcium ionophore inomycin, phorbol myristate acetate but inhibited by $^{\cdot}NO$[12–14]. Recently, it was reported that chondrocytes synthesize myeloperoxidase and that myeloperoxidase mRNA level is increased in osteoarthritis (OA), suggesting that chondrocytes produce hypochlorous acid[15]. In the presence of iron Fe^{2+} and H_2O_2, chondrocyte releases hydroxyl radicals ($OH^{\cdot}$) that react with unsaturated fatty acids of membrane lipids to initiate chain reactions, resulting in the formation of other, more long-lived lipidic radicals ($RO^{\cdot}$, $ROO^{\cdot}$)[16].

Indirect evidence for ROS implication in cartilage degradation comes from the presence of lipid peroxidation products[17], nitrite[18], nitrotyrosine[19], a nitrated type II collagen peptide[20], modified low-density lipoprotein (LDL) and oxidized IgG[21] in the biological fluids of patients with arthritis[13–15]. Furthermore, nitrotyrosine, nitrated proteins and oxidized LDL (ox-LDL) have been found to be accumulated in cartilage of arthritic patients demonstrating the direct implication of ROS in some joint diseases[22–24]. Recently, it was demonstrated that intraarticular injection of *N*-iminoethyl-L-lysine (L-NIL), a selective inhibitor of iNOS, reduced the progression of cartilage erosion in an experimental osteoarthritic dog model[25] incriminating $^{\cdot}NO$ and/or derived ROS as potent mediators of cartilage degradation.

Cellular responses to ROS generation are dependent on the cellular redox status. When the oxidant level does not exceed the reducing capacities of cells, ROS are strongly involved in the control of cellular functions including signal transduction. In contrast, in some pathological situations, when the cellular antioxidant capacity is insufficient to detoxify ROS, oxidative stress may occur that degrades not only cellular membranes and nucleic acids but also extracellular components including proteoglycans and collagens. Furthermore, ROS can modify proteins by oxidation, nitrosylation, nitration or chlorination of specific amino acids, leading to impaired biological activity, changes in protein structure and accumulation of damaged proteins in the tissue. Oxidative stress may also cause cell death and release of cellular content into extracellular environment. Altogether, degradation products and cellular content containing oxidized molecules may contribute to the exacerbation of synovial inflammation and form a vicious circle, constituted by newly formed ROS and further degradation products (Fig. 1).

Effects of ROS on intracellular signaling

During the last 10 years, increasing evidence has been provided that a large number of intracellular signaling pathways are regulated by intracellular ROS. Several growth factors and cytokines binding to different types of cell membrane receptors can elicit a rise in intracellular ROS. These include cytokine receptors, receptor tyrosine

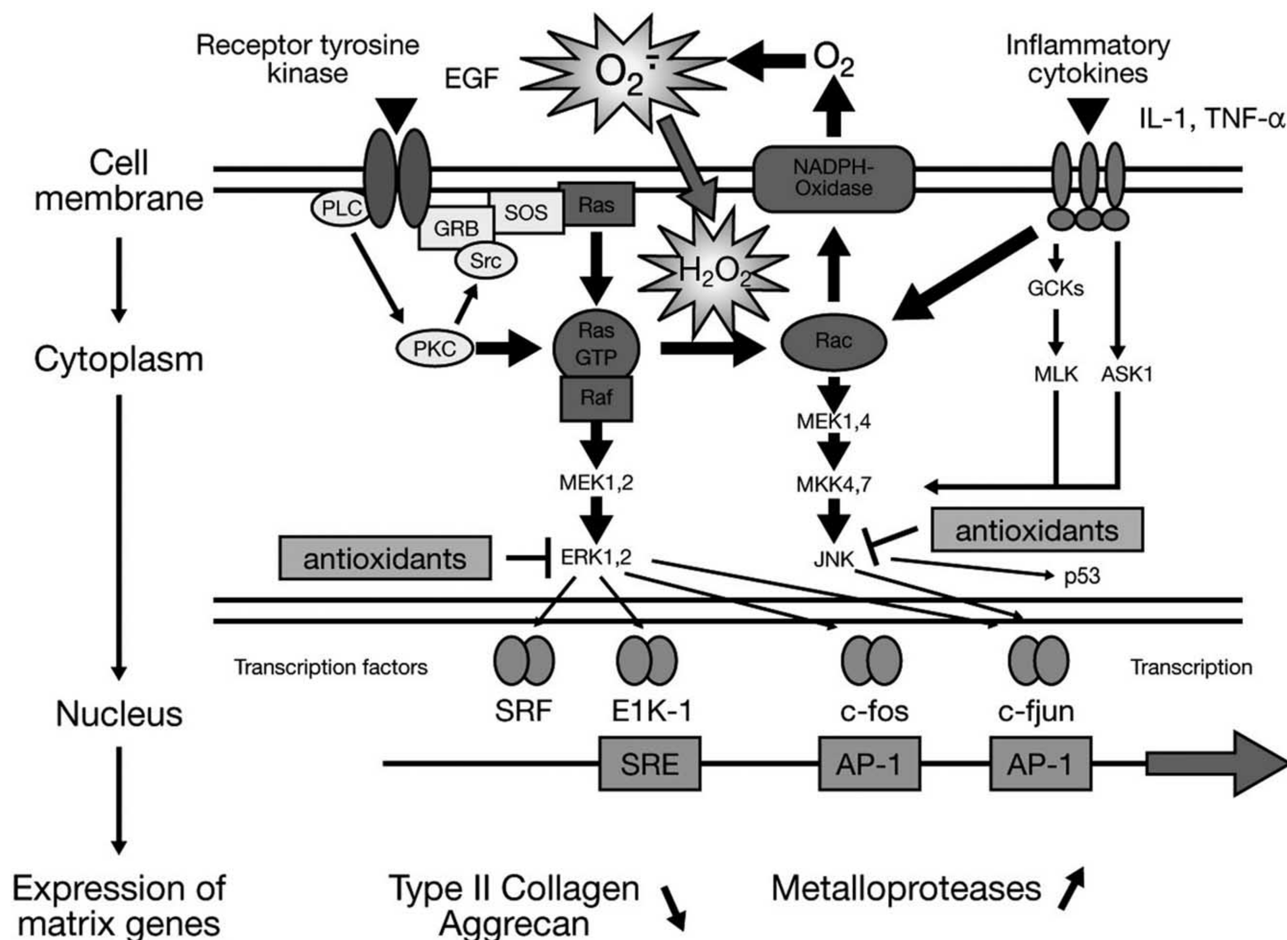

Fig. 2. ROS as signaling molecules in chondrocyte transduction pathways and gene expression. Several growth factors, in particular those acting through tyrosine kinase receptors, and inflammatory cytokines, such as IL-1 and TNF-α, activate signaling cascades in the cytoplasm. Directly or via induction of the Ras pathway, the small GTPase Rac is activated and associated with membrane-bound NADPHoxidase, which generates $O_2^{\cdot-}$. The generated superoxide or the dimutation product H_2O_2 then regulates the ERK1/2 and JNK pathways. The signaling events can be inhibited with antioxidants. Finally, the activity of transcription factors and subsequent gene expression are modulated. Furthermore, ROS can directly interfere with the activity of transcription factors through reactive cysteines that constitute redox-sulfuhydryl switches (modified from reference79). ASK-1=apoptosis signal regulating kinase; ERK=extracellular signal related kinase; GCK=glucokinase; MAPK=mitogen-regulated kinase; MKK=MAP kinase kinase; MLK=mixed lineage kinase; PLC=phospholipase C; PKC=protein kinase C; Sos=son of sevenless; SRF=serum response factor; SFR: S gene family receptor kinase.

kinases, receptor serine/threonine kinases and G protein-coupled receptors. Further, it has been reported that ROS activate mitogen-regulated kinase (MAPK) pathways in several systems, including extracellular signal-regulated kinase (ERK)1/2, Jun-NH2-terminal kinase and P38 MAPK cascades (Fig. 2).

In addition to the activation of different members of signaling cascades involved in cell growth and differentiation, ROS may directly regulate the activity of transcription factors through oxidative modifications of conserved cysteines for example. Several transcription factors have been shown to be redox-sensitive, including nuclear factor (NF)-κB, activating protein (AP)-1, specificity protein (Sp)-1, C-Myb, p53, early growth response (egr)-1 and hypoxia inducible factor (HIF)-1α. The reactive cysteines may constitute redox-sulfhydryl switches, which directly regulate gene expression[26]. Redox regulation can be exerted also at the level of protein degradation. HIF-1α is degraded by the ubiquitin pathway which is regulated by the intracellular redox state and is activated by elevated levels of ROS. Generally, degradation of HIF-1α prevails under normoxia, whereas hypoxia inhibits the ubiquitin pathway, resulting in upregulation of HIF-1α expression[27].

In articular chondrocytes evidence has been provided some years ago that ROS were involved as signaling intermediates for cytokines and growth factors. TNF-α and basic fibroblast growth factor (bFGF) were found to induce ROS production in bovine articular chondrocytes through a NADPHoxidase enzyme complex, resulting in upregulation of c-fos expression[28]. Similarly, IL-1β induction of c-fos and collagenase expression in articular chondrocytes were found to be ROS-dependent[29]. Although ·NO does not upregulate c-fos expression in chondrocytes[28], the inhibition of its production by a specific iNOS inhibitor partially reduced the IL-1 induction of collagenase expression. Also, treatment of chondrocytes with a ·NO donor is able to stimulate collagenase gene expression[29]. In that case, it is likely that AP-1 activity is directly modulated by post-translational modification of its components, involving oxidation–reduction of a key cysteine residue which affects

deoxyribonucleic acid (DNA) binding activity[30]. Further evidence suggests that the redox regulation of AP-1 DNA binding is facilitated by the reducing activity of redox factor-1 protein that may act directly on this critical cysteine residue[31]. Similar redox regulation has also been demonstrated with the small GTP-binding protein Ras. In this case, ·NO modulates the activity of Ras through nitrosylation of a critical cysteine residue[32]. Finally, IL-1-induced collagenase may be mediated by an autocrine loop involving Rac 1, ROS and NF-κB[33].

Altogether, these studies show that ROS may induce transcription factors binding activity and then act as signaling intermediates of cytokines and growth factors. These findings suggest that responses of cells to cytokines and growth factors are dependent on the cell redox status. The redox status results from a subtle equilibrium between ROS production and the intracellular antioxidants level. This balance is subtly modulated by exogenous factors, such as oxygen tension or cytokines. In pathological circumstances, the redox status can be altered and the responses of cells to biochemical factors fully modified. This point should be considered before concluding on the role of ROS in the homeostasis of tissue and physiopathology of arthritis.

Effects of ROS on chondrocyte apoptosis

Chondrocyte death is now considered as an important factor contributing to the breakdown of extracellular matrix in joint diseases. The loss of cells is likely to be of multifactorial origin, with both necrosis and apoptosis being responsible. Damaged chondrocyte viability impairs self-repair in cartilage and may in fact accelerate the progression of the lesion[34–36]. Apoptosis is a complex intracellular pathway resulting from the imbalance between apoptotic and non-apoptotic factors and implicating complex processes (recently in reference37). ·NO has long been considered as the primary inducer of chondrocyte apoptosis[38] mediated by caspase-3 and tyrosine kinase activation. However, it has become clear that ·NO by itself cannot initiate apoptosis and that the concomitant production of $O_2^{\cdot-}$ is required[39], suggesting the role played by $ONOO^-$ in this process.

In contrast, other recent reports have proposed that ·NO could be anti-apoptotic, primarily when intracellular antioxidant level is very low[39]. The mechanism proposed involves scavenging action against other ROS and the inhibition of Fas-induced caspase-3 activation[40].

Effects of ROS on matrix synthesis

Exposure of the chondrocytes to H_2O_2 inhibits proteoglycan and DNA synthesis and depletes intracellular adenosine triphosphate (ATP) as a result of a simultaneous inactivation of glyceraldehyde-3-phosphate dehydrogenase[41,42]. ·NO is implicated in the IL-1 inhibition of aggrecan synthesis by rabbit articular chondrocyte in explant culture. Treatment of cartilage fragments with the ·NOS inhibitor *N*-monomethyl-L-arginine (L-NMMA) reduces the response to IL-1 and restores proteoglycan synthesis. Exogenous ·NO has similar suppressive effects on the proteoglycan production[43,44]. However, an ·NO donor does not inhibit proteoglycan biosynthesis as extensively as IL-1, suggesting that ·NO is only one of the effectors by which IL-1 exerts its inhibition on cartilage matrix synthesis[45]. It has also been reported that IL-1 causes an inhibition of proteoglycan sulfation in human articular chondrocytes, which preferentially affects the 6-sulfated isomer of chondroitin sulfate. This effect is reversed by an inhibitor of ·NO production, *N*-iminoethyl-L-ornithine (L-NIO). Thus, IL-1-induced ·NO mediates the inhibition of sulfation and alters the sulfation pattern of newly synthesized glycosaminoglycan chains[46]. Similar observations were made with chondrocytes transduced with the iNOS (NOS-2 gene) and they confirmed the capability of sublethal endogenously produced ·NO to inhibit matrix synthesis. Further, *S*-nitroso-*N*-acetyl-L,D-penicillamine (SNAP; a donor of ·NO) and SIN-1 (SIN-1, 3-morpholinosydnimine; a compound generating both ·NO and $O_2^{\cdot-}$) reversibly mimic the IL-β inhibitory effect on glycosaminoglycan synthesis[43]. Superoxide dismutase reverses SIN-1 inhibited GAG synthesis by primary bovine chondrocytes in monolayer, indicating that the simultaneous generation of superoxide is essential to inhibit proteoglycan synthesis. The concurrent generation of $O_2^{\cdot-}$ and ·NO is required for the action of IL-1 to inhibit proteoglycan synthesis[47]. We have demonstrated that pretreatment of chondrocyte with SIN-1 or $ONOO^-$ (but not SNAP), downregulates aggrecan gene expression, suggesting the involvement of $ONOO^-$ in the inhibition of aggrecan synthesis[48].

IL-1 can also inhibit the production of type II collagen in cultured rabbit articular chondrocytes and this effect is partially prevented by L-NMMA[49]. The inhibition of prolyl hydroxylase by ·NO could be responsible for the reduction of collagen production by IL-1.

Another possible explanation of the ROS effect on synthesis of matrix components is their contribution to the loss of chondrocyte sensitivity to growth factors. A study with iNOS knock-out mice suggested that ·NO is responsible for part of the cartilage insensitivity to insulin-like growth factor (IGF)-1 by inhibiting IGF-1 receptor autophosphorylation[50]. This mechanism could explain why chondrocytes in arthritic cartilage respond poorly to IGF-1 and may then contribute to abrogate cartilage repair. In this context, ROS may also participate in the failure of repair by reducing the capacity of chondrogenic precursor cells to migrate and proliferate within an injured area. ·NO was demonstrated to inhibit chondrocyte migration and attachment to fibronectin via modification of the actin cytoskeleton[51].

Effects of ROS on cartilage matrix breakdown

In vitro studies have largely suggested a role of ROS in cartilage degradation, as reflected by their effects on matrix components and chondrocyte behavior. However, only limited information is available so far on the potential role of ROS in the onset and progression of cartilage remodeling. High level of nitrite/nitrate has been found in synovial fluid, serum and urine of patients with rheumatoid arthritis (RA) and OA, suggesting the involvement of ·NO in the pathophysiology of these diseases (Table 1). Some potential structural effects of antioxidants have been suggested by animal studies. Pelletier and collaborators have demonstrated that L-NIL, a specific inhibitor of inducible ·NOS, prevents cartilage degradation in a dog model of OA initiated by section of the anterior cruciate ligament[52]. A diet supplemented with vitamins E, C, A, B6, B2 and selenium diminished the development of mechanically induced OA in male STR/1N mice, indicating that ROS might be involved in the mechanical induction of OA[53]. In the transgenic

Table I
Effects of ˙NO and derived peroxynitrite in arthritis

Cartilage matrix synthesis
↘ Aggrecan synthesis
↘ Type II collagen synthesis
↗ IL-β inhibitory effect on matrix components synthesis
↘ Chondrocyte responses to growth factors (IGF-1)
↘ Chondrocyte migration and attachment to fibronectin
Cartilage matrix breakdown
↗ Chondrocyte apoptosis
↗ MMP-3 mRNA level in chondrocytes
↗ MMP-13 mRNA level in chondrocytes
↗ IL-β-stimulated collagenolytic activity released by chondrocytes
↘ Synthesis or activity of TIMPs by fibroblasts
Inflammatory mediators production
↘ NADPHoxidase activity
↘ Adhesion molecules expression by endothelial cells and leukocytes
↘ IL-β or LPS-stimulated IL-6 production by macrophages and chondrocytes
↘ IL-β or LPS-stimulated IL-8 production by chondrocytes
↘ or ↗ IL-β or LPS-stimulated PGE2 production by macrophages and chondrocytes

KRN/NOD mouse model of RA, α-tocopherol administration prevented joint destruction (bone and cartilage loss) without modifying the clinical and histological inflammatory aspects (articular index, pannus proliferation and invasion) of the disease[54].

ROS may cause damage to all matrix components. Several *in vitro* studies have reported the degradation of cartilaginous tissue slices by ROS-generating systems. Damage was supposed to be due to direct attack of proteoglycan and collagen molecules by free radicals. Indeed, the incubation of acid soluble type I collagen with superoxide anion radicals generated by the xanthine oxidase–hypoxanthine system degrades collagen and prevents the formation of fibrils by this collagen[55,56]. In the presence of oxygen, OH˙ degrades collagen and modifies the amino acid composition. The amino acid composition of the peptides obtained by the action of OH˙ showed a significant decrease of 4-hydroxyproline and proline residues and an increase of aspartic acid and glutamic acid[57]. Type I collagen exposure to HOCl fails to degrade collagen but induces the formation of cross-links of unknown nature. HOCl induces also hyaluronic acid cleavage and reduces synovial fluid viscosity, probably in a hydroxyl radical-dependent manner[58,59]. Further, the reaction of HOCl with hyaluronic acid and chondroitin sulfate A gives a novel carbon-centered radical involved in polymer fragmentation[60].

However, ROS also contribute to cartilage degradation by mediating the activation of latent collagenase and by upregulating the expression of genes coding for matrix metalloproteinases (MMP). For example, N^G-monomethyl-L-arginine (L-NMMA) has been found to inhibit IL-β-induced MMP-9 gene expression[61]. L-NMMA depressed stromelysin and collagenase activity released by bovine and human explants and the SNAP induced MMP activity in a dose-dependent fashion. These data provide evidence that ˙NO plays a regulatory role in the activation of metal-dependent proteases in articular chondrocytes and cartilage[62]. HOCl could also be effectors of cartilage matrix destruction by directly activating proenzymes, including pro-MMP-8[63]. It is tempting to speculate that the oxidative potential of ROS may interfere with the propeptides by directly affecting the cysteine switch activation mechanism. Further, HOCl and oxygen singlet ($^1O_2$) may shift the balance of proteolytic potential by decreasing the production and/or the activity of tissue inhibitors of metalloproteinases (TIMPs) and other proteinase inhibitors such as α2-macroglobulin or α1-antiproteinases[64–67].

Recently, it was suggested that lipid peroxides could play a key role in the structural destabilization of cartilage matrix. Calcium ionophore treatment of primary rabbit chondrocytes significantly enhanced lipid peroxidation activity and the release of labeled matrix in a dose-dependent manner. This effect is blocked by vitamin E suggesting that the mechanism of matrix degradation and release is related to lipid peroxidation[68]. As a working hypothesis, it may be suggested that lipid peroxides affect cells matrix interactions by mediated by membrane-bound integrins, or by activating MT-MMPs or by generating other ROS and further metabolites in the pericellular environment. Another interpretation of these results emerges from the recent discovery of lectin-like ox-LDL receptor in cartilage of arthritic rat[24]. Interestingly, the induction of the expression of lectin-like ox-LDL receptor was accompanied by the accumulation of ox-LDL in chondrocytes, suggesting the possible interaction of ox-LDL with lectin-like ox-LDL receptor in cartilage. This observation is important since it was observed that lectin-like ox-LDL receptor blockade by anti-lectin-like ox-LDL receptor-1 antibody suppressed joint swelling, leukocyte infiltration and cartilage degradation in rat zymosan-induced arthritis[24] suggesting a new beneficial method for treating joint diseases such as RA or OA.

From these animal and *in vitro* studies, we can conclude that in pathological conditions, ROS, such as H_2O_2, ˙NO and/or ˙NO-derived nitrogen species contribute to cartilage degradation by inhibiting matrix synthesis, cell migration and growth factor bioactivity, by directly degrading matrix components, by activating MMPs and by inducing cell death. Altogether, these findings support the concept of antioxidant therapy to treat rheumatic disease.

ROS modulate the production of pro-inflammatory biochemical markers

In RA, inflammation of synovium largely contributes to the genesis of disease symptoms and tissue degradation. Although OA is considered as a degenerative disease of cartilage, some synovial inflammation is very often observed also in this pathology and may also contribute to the tissue degradation. The inflammatory reaction is controlled by several soluble biochemical factors, including prostanoids, cytokines and ROS produced by both synoviocytes and chondrocytes. Some exert proinflammatory effects whereas others may have anti-inflammatory properties, and it is likely that the balance between these two groups of factors determines the characteristics and the duration of the inflammatory disease. ROS, mostly ˙NO, play a pivotal role in the pathologic process mainly by contributing to the inflammatory-related tissue degradation. On the other hand, the potential role of ROS, particularly ˙NO, on the joint swelling, cellular infiltration and pain remains controversial. ROS have been found to have anti-inflammatory effects in some circumstances. Data from three animal studies tend to support this concept. First, clinical severity of inflammatory process is exacerbated in inducible ˙NOS deficient mice-type. This inflammatory flare was associated with enhancement of leukocyte infiltration

into perivascular tissues and overexpression of adhesion molecules (P-selectin and VCAM-1) in synovial tissue of those animals[69,70]. Second, in rat adjuvant arthritis, the delayed administration of L-NMMA during the development of arthritis fully blocks ·NO synthesis but fails to relieve, and even increases, clinical manifestations of arthritis[71]. Third, the administration of L-NIL, which preferentially inhibits iNOS and spares the constitutive isoforms (eNOS and cNOS), exacerbates clinical and histological manifestations of streptococcal cell wall-induced arthritis, suggesting that constitutive isoform of ·NOS also contributes to the development of acute and chronic inflammation[72]. Nevertheless, the effect of ·NO on inflammation seems to depend on the time of administration. When L-NMMA was administrated prophylactically, before the appearance of the symptoms, blockade of the ·NOS suppresses the development of adjuvant arthritis in rat, whereas ·NO inhibitors were only weakly efficient, or even detrimental, in established disease. This suggests that the major effect of ·NO may be upon immune recognition of the arthrogenic components, rather than upon delayed deleterious consequences of inflammation[71,72].

In vitro, the ROS effects appear varied and complex, depending on the cell type, the species and the agent used for their induction. Clearly, the synthesis of prostaglandin (PG) E_2 and proinflammatory cytokines are linked to ROS synthesis, but both inhibitory and stimulatory effects of ROS on these mediators have been reported. We and others have demonstrated in cultures of human primary chondrocytes that L-NMMA amplifies IL-β-stimulated PGE_2 production, but does not affect cyclooxygenase (COX)-2 mRNA levels[73,74]. Further, exposure of chondrocytes to either exogenously added $ONOO^-$, or its *in situ* generation by SIN-1 decomposition, decreases both IL-β-induced COX-2 gene and PGE_2 synthesis while SNAP, an ·NO donor, has no effect, suggesting that $ONOO^-$ is the species relevant to COX-2 inhibition[48]. In contrast, others have reported that $ONOO^-$, but not ·NO or $O_2^{\cdot-}$, induces COX in a macrophage-like cell line and chondrocytes[75,76].

Some cytokines are important contributors to cartilage degradation and synovium inflammation in arthritis. The association of IL-1β, IL-6 and oncostatin M seems to be particularly efficient for inducing cartilage degradation[77,78]. In previous studies, we have demonstrated that chondrocytes produce ·NO in response to IL-1β and LPS and that the inhibition of ·NO production by L-NMMA results in an increase of IL-6 and IL-8[73]. We have also reported that the antioxidant *N*-acetyl-cysteine (NAC) molecule enhances LPS-induced IL-β and iNOS gene expression in cultured human chondrocytes suggesting that ROS, other than ·NO, are also involved in the regulation of inflammatory gene expression[61]. Further, treatments of primary chondrocytes with sublethal concentrations of $ONOO^-$ and SIN-1, but not SNAP, inhibit IL-β-induced IL-β, IL-6, IL-8, COX-2 and iNOS gene expressions[48]. Inversely, SNAP inhibits LPS-induced gene expression, while H_2O_2 blocks both LPS and IL-β induction. These data support the hypothesis that ROS could have an anti-inflammatory effect by inhibiting the synthesis of pro-inflammatory mediators. Further, they suggest that this anti-inflammatory effect is dependent on the nature of the ROS tested and on the signaling pathway activated. ·NO is a regulator of the LPS activated signaling pathway whereas the IL-1β activated transduction factors are more sensitive to $ONOO^-$.

This paragraph points out the uncoupling effect of ROS on tissue remodeling and synovium inflammation, suggesting that antioxidant therapy could be helpful to treat structural changes but not to relieve symptoms. One explanation could be the overproduction by chondrocytes of pro-inflammatory mediators that contribute to the progression of synovium inflammation.

Conclusions

Today, ROS are no more considered as only detrimental agents capable of damaging the structure and function of several macromolecules, including the extracellular components. Evidence has been provided that they are produced at low level in articular chondrocytes and play great role as integral actors of intracellular signaling mechanisms. They modulate gene expression and, therefore, are likely to contribute to the maintenance of cartilage homeostasis. However, in joint diseases, ROS are produced in greater amounts and then become deleterious for joint tissue.

Further investigation is required to unravel the signaling mechanisms whereby ROS production may regulate the chondrocyte metabolism and its response to the cytokine/growth factor network that controls the physiopathology of cartilage. The knowledge of these mechanisms might offer new targets (i.e., intermediate radicals, redox sensitive transcription factors or kinases) to design future therapeutic approaches (i.e., sparing some ROS but specifically blocking the production of others) for the treatment of joint diseases and the prevention of cartilage ageing.

Acknowledgement

The authors thank Dr A. de Patoul for his editorial assistance.

References

1. Blake DR, Merry P, Unsworth J, Kidd BL, Outhwaite JM, Ballar R, *et al.* Hypoxic-reperfusion injury in the inflamed joint. Lancet 1989;1:289–93.
2. Tiku ML, Liesch JB, Roberston FM. Production of hydrogen peroxide by rabbit articular chondrocytes. J Immunol 1990;145:690–6.
3. Henrotin Y, Deby-Dupont G, Deby C, De Bruyn M, Lamy M, Franchimont P. Production of active oxygen species by isolated human chondrocytes. Br J Rheumatol 1993;32:562–7.
4. Hayashi T, Abe E, Yamate T, Taguchi Y, Jasin HE. Nitric oxide production by superficial and deep articular chondrocytes. Arthritis Rheum 1997;40:261–9.
5. Fermor B, Weinberg JB, Pisetsky DS, Misukonis MA, Banes AJ, Guilak F. The effects of static and intermittent compression on nitric oxide production in articular cartilage explants. J Orthop Res 2001;19: 729–37.
6. Hiran TS, Moulton PJ, Hancock JT. Detection of superoxide and NADPH oxidase in porcine articular chondrocytes. Free Radic Biol Med 1997;23:736–43.
7. Moulton PJ, Hiran TS, Goldring MB, Hancock JT. Detection of protein and mRNA of various components of the NADPH oxidase complex in an immortalized human chondrocyte line. Br J Rheumatol 1997;36:522–9.
8. Henrotin Y, Zheng SX, Labasse AH, Deby GP, Crielaard JM, Reginster JY. Modulation of human

chondrocyte metabolism by recombinant interferon. Osteoarthritis Cartilage 2000;8:474–82.
9. Borderie D, Hilliquin P, Hernvann A, Lamarechal H, Kahan A, Menkes CJ, *et al.* Inhibition of inducible NO synthase by TH2 cytokines and TGF beta in rheumatoid arthritic synoviocytes: effects on nitrosothiol production. Nitric Oxide 2002;6:271–82.
10. Stadler J, Stefanovic-Racic M, Billar TR. Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. J Immunol 1991; 147:3915–20.
11. Palmer RM, Hickerry MS, Charles IG, Moncada S, Bayliss MT. Induction of nitric oxide synthase in human chondrocytes. Biochem Biophys Res Commun 1993;193:398–405.
12. Hiran TS, Moulton PJ, Hancock JT. *In situ* detection of superoxide anions within porcine articular cartilage. Br J Biomed Sci 1998;55:199–203.
13. Moulton PJ, Goldring MB, Hancock JT. NADPHoxidase of chondrocytes contains an isoform of gp91phox subunit. Biochem J 1998;329:449–51.
14. Clancy RM, Leszczynska-Piziak J, Abramson S. Nitric oxide, an endothelial cell relaxation factor, inhibits neutrophil superoxide anion production via a direct action on the NADPHoxidase. J Clin Invest 1992; 90:1116–21.
15. Attur MG, Dave M, Akamatsu M, Katoh M, Amin AR. Osteoarthritis and osteoarthrosis: the definition of inflammation become a semantic issue in the genomic era of molecular medecine. Osteoarthritis Cartilage 2002;10:1–4.
16. Henrotin Y, Deby-Dupont G, Deby C, Franchimont P, Emerit I. Active oxygen species, articular inflammation and cartilage damage. In: Emerit I, Chance B, Eds. Free Radicals and Aging. Basel: Birkhaüser verlag 1992;308–22.
17. Situnayake RD, Thurnham DI, Kootathep S, Chirico S, Lunec J, Davis M, *et al.* Chain breaking antioxidant status rheumatoid arthritis: clinical and laboratory correlates. Ann Rheum Dis 1992;50:81–6.
18. Spreng D, Sigrist N, Schweighauser A, Busato A, Schawalder P. Endogenous nitric oxide in canine osteoarthritis: detection in urine, serum, and synovial fluid specimens. Vet Surg 2001;30:191–9.
19. Kaur H, Halliwell B. Aromatic hydroxylation of phenylalanine as an assay for hydroxyl radical. Measurement of hydroxyl radical formation from ozone and in blood from premature babies using improved HPLC methodology. Anal Biochem 1994;220:11–5.
20. Henrotin Y, Deberg M, Christgau S, Henriksen D, Seidel L, Reginster J-Y. Type II collagen derived fragment (Coll2-1) is a new marker predictive of osteoarthritic progression. Osteoporos Int 2002;13: S17 (O21).
21. Uesugi M, Yoshida K, Jasin HE. Inflammatory properties of IgG modified by oxygen radicals and peroxynitrite. J Immunol 2000;165:6532–7.
22. Fukuda K, Kumano F, Takayama M, Saito M, Ohtani K, Tanaka S. Zonal differences in nitric oxide synthesis by bovine chondrocytes exposed to interleukin-1. Inflamm Res 1995;44:430–7.
23. Loeser RF, Carlson CS, Del Carlo M, Cole A. Detection of nitrotyrosine in aging and osteoarthritic cartilage: correlation of oxidative damage with the presence of interleukin-1beta and with chondrocytes resistance to insulin-like growth factor-1. Arthritis Rheum 2002;46:2349–57.
24. Nakagawa T, Akagi M, Hoshikawa H, Chen M, Yasuda T, Mukai S, *et al.* Lectin-like oxidized low-density lipoprotein receptor 1 mediates leukocyte infiltration and articular cartilage destruction in rat zymosan-induced arthritis. Arthritis Rheum 2002;46:2486–94.
25. Pelletier JP, Lascau-Coman V, Jovanovic D, Fernandes JC, Manning P, Connor JR, *et al.* Selective inhibition of inducible nitric oxide synthase in experimental osteoarthritis is associated with reduction in tissue levels of catabolic factors. J Rheumatol 1999;26:2002–14.
26. Hentze MW, Rouault TA, Harford JB, Klauser RD. Oxidation–reduction and the molecular mechanism of a regulatory RNA–protein interaction. Science 1989; 244:357–9.
27. Groulx I, Lee S. Oxygen-dependent ubiquitination and degradation of hypoxia-inducible factor requires nuclear-cytoplasmic trafficking of the von Hippel–Lindau tumor suppressor protein. Mol Cell Biol 2002; 22:5319–36.
28. Lo YYC, Cruz TF. Involvement of reactive oxygen species in cytokine and growth factor induction of c-fos expression in chondrocytes. J Biol Chem 1995; 270:11727–30.
29. Lo YYC, Conquer JA, Grinstein S, Cruz TF. Interleukin-1β induction of c-fos and collagenase expression in articular chondrocytes: involvement of reactive oxygen species. J Cell Biochem 1998;69: 19–29.
30. Abate C, Petel L, Rauscher NIFJ, Curran T. Redox regulation of c Fos and Jun DNA-binding activity *in vitro*. Science 1990;249:1157–61.
31. Xanthoudakis S, Curran T. Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity. EMBO J 1992;11:653–65.
32. Lander HM, Milbank AJ, Tauras JM, Hajjar DP, Hempstead BL, Schwartz GD, *et al.* Redox regulation of cell signalling. Nature 1996;381:380–1.
33. Kheradmand F, Werner E, Tremble P, Symons M, Werb Z. Role of Rac 1 and oxygen radicals in collagenase-1 expression induced by cell shape change. Science 1998;280:898–902.
34. Blanco FJ, Guitan R, Vasquez-Martul E, de Torro FJ, Galdo F. Osteoarthritis chondrocyte die by apoptosis: a possible pathway for osteoarthritis pathology. Arthritis Rheum 1998;41:284–9.
35. Horton WE Jr, Feng L, Adam C. Chondrocyte apoptosis in development, ageing and disease. Matrix Biol 1998;17:107–15.
36. Hashimoto S, Takahashi K, Amiel D, Coutts RGD, Lotz M. Chondrocyte apoptosis and nitric oxide production during experimental induced osteoarthritis. Arthritis Rheum 1998;41:1266–74.
37. Aigner T, Kim HA. Apoptosis and cellular vitality. Issue in osteoarthritic cartilage degeneration. Arthritis Rheum 2002;46:1986–96.
38. Blanco FJ, Ochs RL, Schwarz RL, Lotz M. Chondrocyte apoptosis induced by nitric oxide. Am J Pathol 1995;146:75–85.
39. Del Carlo M, Loeser RF. Nitric oxide-mediated chondrocyte cell death requires the generation of additional reactive oxygen species. Arthritis Rheum 2002;46:394–403.
40. Migita K, Yamasaki S, Kita M, Ida H, Shibatomi K, Kawakami A, *et al.* Nitric oxide protects cultured rheumatoid cells from fas-induced apoptosis by inhibiting caspase-3. Immunology 2001;103:362–7.

41. Vincent F, Brun H, Clain E, Ronot X, Adolphe M. Effects of oxygen free radicals on proliferation kinetics of cultured rabbit articular chondrocytes. J Cell Physiol 1989;141:262–6.
42. Baker MS, Feigan J, Lowther DA. The mechanism of chondrocyte hydrogen peroxide damage. Depletion of intracellular ATP due to suppression of glycolysis caused by oxidation of glyceraldehyde-3-phosphate dehydrogenase. J Rheumatol 1989;16:7–14.
43. Taskiran D, Stefanovic-Racic M, Georgescu H, Evans C. Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1. Biochem Biophys Res Commun 1994;200:142–8.
44. Stefanovic-Racic M, Meyers K, Meschter C, Coffey JW, Hoffman RA, Evans C. Comparison of the nitric oxide synthase inhibitors methylarginine aminoguanidine as prophylactic and therapeutic agents in rat adjuvant arthritis. J Rheumatol 1995;22:1922–8.
45. Hauselmann HJ, Oppliger L, Michel BA, Stefanovic-Racic M, Evan CH. Nitric oxide and proteoglycan biosynthesis by human articular chondrocytes in alginate culture. FEBS Lett 1994;352:361–4.
46. Hickery MS, Bayliss MT. Interleukin-1 induced nitric oxide inhibits sulphation of glycosaminoglycan chains in human articular chondrocytes. Biochim Biophys Acta 1998;1425:282–90.
47. Oh M, Fukuda K, Asada S, Yasuda Y, Tamaka S. Concurrent generation of nitric oxide and superoxide inhibits proteoglycan synthesis in bovine articular chondrocytes: involvement of peroxynitrite. J Rheumatol 1998;25:2169–74.
48. Mathy-Hartert M, Martin G, Deby-Dupont G, Pujol JP, Reginster JY, Henrotin Y. Reactive oxygen species down-regulate pro-inflammatory gene expression by human chondrocytes. Inflamm Res 2003;52:111–8.
49. Cao M, Westerhausen-Larson A, Niyibizi C, Kavalkovich K, Georgescu HI, Rizzo CF, *et al.* Nitric oxide inhibits the synthesis of type II collagen without altering Col2A1 mRNA abundance: prolyl hydroxylase as a possible target. Biochem J 1997; 324:305–10.
50. Studer RK, Levicoff E, Georgescu H, Miller L, Jaffurs D, Evans CH. Nitric oxide inhibits chondrocyte response to IGF-I: inhibition of IGF-1Rbeta tyrosine phosphorylation. Am J Physiol Cell Physiol 2000; 279:C961–9.
51. Frenkel SR, Clancy RM, Ricci JL, Di Cesare PE, Rediske JJ, Abramson SB. Effects of nitric oxide on chondrocyte migration, adhesion, and cytoskeletal assembly. Arthritis Rheum 1996;39:1905–12.
52. Pelletier JP, Jovanovic D, Lascau-Coman V, Fernandes JC, Manning PT, Connor JR, *et al.* Selective inhibition of inducible nitric oxide synthase reduces progression of experimental osteoarthritis *in vivo*. Arthritis Rheum 2000;43:1290–9.
53. Kurz B, Jost B, Schunke M. Dietary vitamins and selenium diminish the development of mechanically induced osteoarthritis and increase the expression of antioxidative enzymes in the knee joint of STR/1N mice. Osteoarthritis Cartilage 2002;10:119–26.
54. De Bandt M, Grossin M, Weber AJ, Chopin M, Elbim C, Pla M, *et al.* Suppression of arthritis and protection from bone destruction by treatment with TNP-470/AGM-1470 in a transgenic mouse model of rheumatoid arthritis. Arthritis Rheum 2000;43:2056–63.
55. Greewald RA, Moy WW. Inhibition of collagen gelation by action of superoxide radicals. Arthritis Rheum 1979;22:251–9.
56. Monboisse JC, Braquet P, Borel JP. Non enzymatic degradation of acid soluble collagen by superoxide anion: protective effect of flavonoids. Biochem Pharmacol 1983;32:53–8.
57. Monboisse JC, Borel JP. Oxidative damage of collagen. In: Emerit I, Chance B, Eds. Free Radicals and Aging. Basel: Birkhaüser Verlag 1992;323–8.
58. Green SP, baker MS, Lowther DA. Depolymerization of synovial fluid hyaluronic acid (HA) by the complete myeloperoxidase (MPO) system may involve the formation a HA–MPO ionic complex. J Rheumatol 1990; 17:1670–5.
59. Saari H, Konttinen YT, Friman C, Sarsa T. Differential effects of reactive oxygen species on native synovial fluid and purified human umbilical cord hyaluronan. Inflammation 1993;17:403–15.
60. Hawkins CL, Davies MJ. Degradation of hyaluronic acid, poly-monosaccharides and model compounds by hypochlorite: evidence for radical intermediates and fragmentation. Free Radic Biol Med 1998; 24:1396–410.
61. Sasaki K, Hattori T, Fujisawa T, Takahashi K, Inoue H, Takigawa M. Nitric oxide mediates interleukin-1-induced gene expression of matrix metalloproteinases and basic fibroblast growth factor in cultured rabbit articular chondrocytes. J Biochem 1998; 123:431–9.
62. Murrell GA, Jang D, Williams RJ. Nitric oxide activates metalloprotease enzymes in articular cartilage. Biochem Biophys Res Commun 1995;206:15–21.
63. Burkhardt H, Swingel M, Menninger H, Macartney HW, Tschesche H. Oxygen radicals as effectors of cartilage destruction. Direct degradative effect on matrix components and indirect action via activation of latent collagenase from polymorphonuclear leukocytes. Arthritis Rheum 1986;29:379–87.
64. Michaelis J, Vissers MC, Winterbourn CC. Different effects of hypochlorous acid on human neutrophil metalloproteinases: activation of collagenase and inactivation of collagenase and gelatinase. Arch Biochem Biophys 1992;292:555–62.
65. Shabani F, McNeil J, Tippett L. The oxidative inactivation of tissue inhibitor of metalloproteinases-1 (TIMP-1) by hypochlorous acid (HOCl) is suppressed by anti-rheumatic drugs. Free Radic Res 1998; 28:115–23.
66. Stief TW, Kropf J, Kretschmer V, Doss MO, Fareed J. Singlet oxygen ((1)O_2) inactivates plasmatic free and complexed alpha2-macroglobulin. Thromb Res 2000; 98:541–7.
67. Siwik DA, Pagano PJ, Colucci WS. Oxidative stress regulates collagen synthesis and matrix metalloproteinases activity in cardiac fibroblasts. Am J Physiol Cell Physiol 2001;280:53–60.
68. Tiku ML, Shah R, Allison GT. Evidence linking chondrocyte lipid peroxidation to cartilage matrix protein degradation. Possible role in cartilage aging and the pathogenesis of osteoarthritis. J Biol Chem 2000; 275:20069–76.
69. Van de Loo FA, Arntz OJ, van Enckevort FH, van Lent PL, van den Berg WB. Reduced cartilage proteoglycan loss during zymosan-induced gonarthrosis in NOS2-deficient mice and anti-interleukin-1-treated

wild-type mice with unabated joint inflammation. Arthritis Rheum 1998;41:634–46.
70. Veihelmann A, Landes J, Hofbauer A, Dorger M, Refior HJ, Messmer K, *et al.* Exacerbation of antigen-induced arthritis in inducible nitric oxide synthase-deficient mice. Arthritis Rheum 2001;44:1420–7.
71. Stefanovic-Racic M, Meyers K, Meschter C, Coffey JW, Hoffman RA, Evans CH. *N*-monomethyl arginine, an inhibitor of nitric oxide synthase, suppresses the development of adjuvant arthritis in rats. Arthritis Rheum 1994;37:1062–9.
72. McCartney-francis NL, Song XY, Mizel DE, Wahl S. Selective inhibition of inducible nitric oxide synthase exacerbates erosive joint disease. J Immunol 2001; 166:2743–50.
73. Henrotin YE, Zheng SX, Deby GP, Labasse AH, Crielaard JM, Reginster JY. Nitric oxide down-regulates interleukin 1β (IL-β) stimulated IL-6, IL-8 and prostaglandin E2 production by human chondrocytes. J Rheumatol 1998;25:1595–601.
74. Mathy-Hartert M, Deby-Dupont G, Reginster J-Y, Pujol JP, Ayache N, Deby-Dupont G, *et al.* Regulation by reactive oxygen species of interleukin-β, nitric oxide and prostaglandin E2 production by human chondrocytes. Osteoarthritis Cartilage 2002;10: 547–55.
75. Tsai AL, Wei C, Kulmacz RJ. Interaction between nitric oxide and prostaglandin H synthase. Arch Biochem Biophys 1994;313:367–72.
76. Landino LM, Crews BC, Timmons MD, Morrow JD, Marnett LJ. Peroxynitrite, the coupling product of nitric oxide and superoxide, activates prostaglandin biosynthesis. Proc Natl Acad Sci U S A 1996;93: 15069–74.
77. Milner JM, Elliott SF, Cawston TE. Activation of procollagenases is a key control point in cartilage collagen degradation. Interaction of serine and metalloproteinases pathways. Arthritis Rheum 2001;44: 2084–96.
78. Rowan AD, Koshy PJT, Shingleton WD, Degnan BA, Heath JK, Vernallis AB, *et al.* Synergic effects of glycoprotein 130 binding cytokines in combination with interleukin-1 on cartilage collagen breakdown. Arthritis Rheum 2001;44:1620–32.
79. Sauer H, Wartenberg M, Hescheler J. Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cell Physiol Biochem 2001;11:173–86.