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The Role of Reactive Oxygen Species in Immunopathogenesis of Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis is a disease associated with painful joints that affects approximately 1% of the population worldwide, and for which no effective cure is available. It is characterized by chronic joint inflammation and variable degrees of bone and cartilage erosion. Oxygen metabolism has an important role in the pathogenesis of rheumatoid arthritis. Reactive oxygen species (ROS) are produced in many normal and abnormal processes in humans, including atheroma, asthma, joint diseases, aging, and cancer. TNF-α overproduction is thought to be the main contributor to increased ROS release in patients with RA. Increased ROS production leads to tissue damage associated with inflammation. The prevailing hypothesis that ROS promote inflammation was recently challenged when polymorphisms in Neutrophil cytosolic factor 1(Ncf1), that decrease oxidative burst, were shown to increase disease severity in mouse and rat arthritis models. It has been shown that oxygen radicals might also be important in controlling disease severity and reducing joint inflammation and connective tissue damage. In this review article, our aim is to clarify the role of ROS in immunopathogenesis of Rheumatoid arthritis.

Key words: CIA; Immunopathogenesis; Rheumatoid arthritis; ROS;

INTRODUCTION

Rheumatoid arthritis (RA) is a common autoimmune disease and a systemic inflammatory disease that involves hyperplasia of synovial tissues and structural damage to cartilage, bone, and ligaments.[1,2] A combination of genetic susceptibility and environmental factors are also necessary for mounting this self-reacting response.[3] Like many other autoimmune diseases, a significant part of the genetic contribution is associated with the MHC region.[1,4] In humans, susceptibility to RA is highly associated with the HLA-DR1 and HLA-DR4 MHC class II molecules. Similarly, the mouse strains of DBA/1 and B10.Q have the I-Aq and I-Ar haplotypes and are highly susceptible to collagen-induced arthritis (CIA), as experimental model of RA. It has been also demonstrated that the expression of a restricted T-cell receptor (TCR) repertoire is connected with the development and susceptibility to CIA in mice.[5-8] One of the most frequent causes for local bone erosion is arthritis which involves destruction of juxta-articular bone. This process is a hallmark of rheumatoid arthritis but it also occurs within the context of other chronic forms of ar-

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thritis, particularly in psoriatic arthritis.[9] Immunostaining in arthritic synovial tissue of mice with collagen arthritis clearly identifies RANKL production by numerous cell types, including macrophages and fibroblasts. In patients with RA bone erosion can be prevented with anti-TNF therapy, identifying TNF as a crucial bone-erosive cytokine in this disease.[10]

In rheumatoid arthritis, oxidative stress has been described as an important mechanism that underlies destructive proliferative synovitis.[11,12] Moreover, oxidative stress was also found to influence functional characteristics of synovial T lymphocytes with critical implications for TCR signaling events.[13,14]

Several lines of evidence suggest a role for oxidative stress in the pathogenesis of RA. Numerous studies indicate that NADPH oxidase-derived oxygen radicals may have a harmful effect in arthritis: the increased NADPH oxidase activity of circulating neutrophils and monocytes in RA patients was reported earlier.[15,16]

Contrary to the conventional view that high reactive oxygen species (ROS) levels mediate inflammation, it was shown that reduced capacity to produce ROS , due to allelic polymorphism in respiratory burst oxidase component neutrophil cytosolic factor 1 (Ncf1 or p47phox) gene promoted activation of arthritogenic T cell, leading to severe arthritis in rats.[17] Interestingly collagen-induced arthritis in mice and rats and pristane-induced arthritis in rats were shown to be more severe and of higher incidence in animals with *Ncf1* variants associated with lower burst capacity.[18,19] ROS has been recently thought not only to act on invading pathogens but also to play an important regulatory role in the immune system. Although granulocytes are the main ROS-producing cell type, a preventive effect on arthritogenic T cells could clearly be seen after treatment.[20,21]

REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and hydroxyl radicals, and their reactive products were classically described as harmful products of aerobic metabolism which could be capable of causing DNA mutations, lipid peroxidation, and protein oxidation.[22] Oxidative stress can damage to cell membranes, lipids, nucleic acids, proteins, and constituents of the extracellular matrix (ECM) such as proteoglycans and collagens.[23]

Among ROS produced by living cells, O_2^- is a pro-inflammatory compound that damages cells and the ECM. For instance, O_2^- damages endothelial cells, increasing the permeability of the microvasculature and promoting the migration of neutrophils to foci of inflammation.[24,25] Superoxide anion radicals are produced by the enzyme complex NADPH, which catalyzes the reduction of molecular oxygen to superoxide anion radicals. NADPH oxidase complex consisting 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.[26-28]

Several defense mechanisms have evolved to protect cellular structure from oxidative damage. These include intracellular enzymes such as superoxide dismutase, glutathione peroxidase, catalase and other peroxidases, thioredoxin reductase, the sequestration of metal ion cofactors such as Fe and Cu by binding to proteins, and endogenous antioxidants.[29] The antioxidant enzymes that scavenge ROS are ubiquitously distributed in the body. In addition, hydrosoluble antioxidants (vitamin C, and uric acid) and liposoluble antioxidants (vitamin E, carotenoids, and bilirubin) are particularly important for protecting cell membranes and plasma lipoproteins.[24] An epidemiological study suggested that low selenium (chemical antioxidant) status may be a risk factor for rheumatoid factor-negative RA and low alpha-tocopherol status may be a risk factor for RA independently of rheumatoid factor status.[30]

The main role of ROS function is to form an integral part of the organism's defense against invading microbial agents. The formed ROS are mainly used to kill invading pathogens[31] and therefore, lack of a functional NADPH oxidase complex results in low resistance to bacterial and fungal infections in humans and chronic granulomatous disease.[32]

In addition to the role in phagocyte function and host defense, a large amount of evidence points to important roles for ROS in cell proliferation, apoptosis, angiogenesis, endocrine-related functions, and oxidative modification of the extracellular matrix. Indeed, increased ROS have been documented at sites of inflammation, such as synovial joints of patients with inflammatory arthritis. It has been reported that circulating neutrophils and monocytes from patients with RA increase NADPH oxidase activity.[33,34] The redox potential of the neutrophil is regulated by scavengers of ROS. Moreover, the redox poten-

tial of a cell can be modulated by cytokines, such as tumor necrosis factor (TNF). The TNF-induced adjustment of the redox potential is thought to be important for the cell to generate one of two opposing signals, survival or apoptosis. TNF responses involved in the induction of apoptosis is mediated by mitochondrial ROS generation, activation of caspases, and DNA fragmentation. In contrast, TNF signals for survival activate release of ROS, induce ROS activation of NFκB and increase expression of genes such as Bcl-2 and superoxide dismutase. NFκB blocks caspase activation and Bcl-2 blocks mitochondrial leakage/oxidant release.[35] ROS may directly regulate the activity of transcription factors through oxidative modifications of conserved cysteines. 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.[26]

ROS are produced as signaling intermediates for cytokines and growth factors. TNF-α and basic fibroblast growth factor (bFGF) were shown to induce ROS production in bovine articular chondrocytes through a NADPHoxidase enzyme complex, resulting in upregulation of c-fos expression.[36] Induction of c-fos and collagenase expression by IL-1β in articular chondrocytes were found to be ROS-dependent. These findings suggest that responses of cells to cytokines and growth factors are dependent on the cell redox status.[37]

The oxidation status of T cells exposed to extracellular ROS might also influence the function of phosphatases that are known to be exquisitely sensitive to the oxidation of cysteine in the catalytic site.[38] Oxidation of proteins on cell surfaces has been shown to be crucial for function. The protein LAT (linker for activation of T cells) is highly sensitive to redox alterations, resulting in lower T cell receptor signalling after oxidation.[39]

ROS signals are important during lymphocyte transendothelial migration. It was shown that ROS production is stimulated by lymphocyte binding to the adhesion molecule, vascular cell adhesion molecule-1 (VCAM-1).[40] VCAM-1 stimulates endothelial cell NADPH oxidase for the production of low levels of ROS (1 μM H_2O_2) and this is required for VCAM-1-dependent lymphocyte migration.[41]

ROS are also involved in antigen processing and oxidation of target structures. Ag presentation could be affected by altered ROS production from the NADPH oxidase complex. Phagosomes are relying on ROS production to regulate protein degradation into peptides that can be presented. Recent data suggest that mice with a deficiency in ROS production related to NADPH oxidase complex have an accelerated Ag degradation due to a more acidic environment in dendritic cell phagosomes which leads to defective cross-presentation to T cells.[42] Another intriguing newly reported finding is that redox status regulates editing of the MHC class repertoire. Presumably, oxidation of the peptide-reactive site is required for optimal peptide loading.[43]

THE ROLE OF ROS IN RA

The role of endogenously produced ROS in RA pathogenesis is probably a lot more complex and is yet to be clarified. While the pathogenesis of RA remains unclear, the full spectrum of cellular and humoral elements of the immune system are activated and coordinarily contribute to disease pathology.[44-46]

In many joint diseases, pro-inflammatory factors such as cytokines and prostaglandins, together with ROS and nitric oxide (NO) are released at sites of inflammation.[47] These factors are associated with very low SOD concentrations in joint fluid. SOD activity is a key component of the cellular antioxidant armamentarium that protects cells and the extracellular matrix (ECM) from the harmful effects of O_2^- and its derivatives.[24] Among ROS, the superoxide anion (O_2^-) plays a pivotal role in inflammation, particularly in patients with inflammatory joint disease. The main ROS produced by chondrocytes are nitric oxide ($^{\surd}$NO) and superoxide anion (O_2^-) that generate derivative radicals, including peroxynitrite ($ONOO^-$) and hydrogen peroxide (H_2O_2).[48,49] Oxygen and nitrogen radicals damage cellular element in cartilage and components of the extracellular matrix either by direct attack or indirectly by reducing matrix components synthesis (proteoglycans, Type II collagen) and reducing the sulfation of newly synthesized glycosaminoglycans, inducing apoptosis or by activation latent metalloproteinases.[50] It has been reported that the inhibitors of NOS activity reduce the development of arthritis, and these findings support the role for NO in the pathophysiology associated with inflammatory reactions.[51-53]

The therapy of RA is mainly based on methotrexate (MTX) and biological agents that directly target molecules involved in the pathogenesis of RA, such as etanercept, the human soluble tumor necrosis factor receptor.[20] Furthermore the treatment with glucuronoxylomannan (GXM) in collagen-induced arthritis

(CIA) has been shown that GXM increases secretion of IL-10 and reduces the production of TNF-α, IL-1β, GM-CSF and NO.[54-58]

In arthritis patients, increased reactive oxygen species are present at the sites of inflammation.[59] It has been demonstrated an increased oxidative enzyme activity along with decreased antioxidant levels in RA sera and synovial fluids.[60] NADPH oxidase-derived superoxide, a highly reactive molecule may have effector functions in RA which are detrimental for the joint.[61] SOD(scavenger of ROS) has a beneficial effect on arthritis that is induced by streptococcal cell walls, adjuvant, or via immunization with collagen type II.[62,63] Activation of the complement cascade plays a major role in type II collagen (CIA) pathogenesis. Activation of the complement cascade, especially C5a (a cleavage product of C5) recruits neutrophils and macrophages. The recruited neutrophils and macrophages are activated by FcγR ligation and secreting chemotoxic materials and pro-inflammatory cytokines, such as IL-1β, TNF-α, IL-8, IL-6, MIP-1α, nitric oxide (NO), and prostaglandins (PGE2).[2,64] A variety of cytokines have been implicated in the development of this disease. In particular, cytokines secreted from macrophages or synoviocytes, such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), as well as IL-6, IL-15, IL-17 and IL-18 are considered as major determinants in the perpetuation of arthritis.[65,66]

The recent evidence have been provided some data that in articular chondrocytes ROS are involved as signaling intermediates for cytokines and growth factors. TNF-α was found to induce ROS production in bovine articular chondrocytes through a NADPH oxidase enzyme complex, resulting in upregulation of c-fos expression.[36] Similarly, IL-1β induction of c-fos and collagenase expression in articular chondrocytes were found to be ROS-dependent.[37] On the other hand, it has been recently shown worsening of joint inflammation and granulomatous synovitis with extensive cartilage and bone erosion in the p47phox gene KO mice which is contrary to previous reports of the anti-rheumatic effects of SOD. The exacerbated joint inflammation in the NADPH oxidase-deficient mice could be caused by impaired phagocytosis of the immune complexes and zymosan used to elicited arthritis. Neutrophils from CGD patients are impaired in the phagocytosis of immune-complexes.[61]

The Ncf1 protein (alias $p47^{phox}$) as one of six heterosubunits of the NADPH oxidase complex is responsible for the one electron reduction of oxygen-generating superoxide anions, which serve as precursors for ROS.[31,67] Oxidative stress has been shown to induce T cell hyporesponsiveness in RA through effects on proteins and proteosomal degradation.[68]

To date more focus has been paid on the immunoregulatory role of ROS, further highlighting the importance of *Ncf1* and ROS in autoimmune diseases.[67] The presence of the *Ncf1* mutation dramatically increased CIA.[69] CIA is an experimental rat model sharing a number of features with human RA. CIA develops in susceptible strains of rodents and is dependent on T-cell activation and local production of pro-inflammatory cytokines such as interferon-γ, IL-1 and TNF-α.[4,70,71] The exact mechanism behind immunoregulatory role of ROS is likely to be quite complex. It has been reported that altered oxidation status of T cell membrane proteins affect arthritogenic capacity. In the mouse model, a mutation in *Ncf1* results in an increased delayed-type hypersensitivity response and serum levels of anti-collagen type II (CII) IgG antibodies, indicating enhanced activation of autoreactive T cells.[19,72] On the other hand, increased oxidative burst in thymic epithelial cells or other APCs could affect the thymic selection, possibly by alteration of oxidation status of proteins on the cell surface.[73]

Exposure to ROS has been demonstrated to down-regulate T cell activity and a decrease in intracellular redox levels which impairs T cell function.[74-76] It was shown that an increase in cell surface thiols directly increases T cell activation and proliferation both *in vitro* and *in vivo* and thereby determines T cell arthritogenicity.[77] The first and more likely process is that T cell membranes normally become oxidized through interactions with Ncf1-expressing APCs.[78] There are the number of thiol groups on the cell surface of T cells which is regulated by means of ROS produced by the NADPH oxidase complex, and that the level of T cell surface −SH groups influences activation, proliferation, and arthritis development. It has been shown that oxidation by depletion of intracellular GSH led to a shift to a Th2 response, characterized by increased IL-4 production and inhibition of IFN-γ and IL-12 production.[79,80]

One of several proteins that are susceptible for redox regulation is CD4 molecule. It might be possible that the CD4–MHC class II interaction is altered upon redox changes in CD4.[80] However, ROS might affect signal transduction proteins such as LAT or ZAP70 that are located closely beneath the cell membrane.[82-84]

The Ncf1 is not only expressed in neutrophilic granulocytes but also in phagocytic APCs, with the highest expression in macrophages.[85] Ncf1- expressing macrophages mediate protection against arthritis by producing ROS that down-regulate T cell activation.[18] Macrophages have been suggested as being more activated in the absence of ROS, as shown in mice with a deficiency of the NADPH oxidase complex.[86,87] This was confirmed by a study showing that matrix metalloprotease activity of macrophages was lower in the presence of NADPH oxidase–derived ROS.[88] The ROS can operate as transmitters in the immune synapse, i.e., in the space formed between APCs and T cells where MHC class II, TCR, and costimulatory molecules interact since the Ncf1-containing NADPH oxidase complex is expressed in the phagosome and in the cell membrane lipid rafts.[89] It was observed that if a T cell had increased levels of cell surface −SH groups, it indicates that T cell surface redox levels are determined during T cell–APC interaction.[77] In addition to the extracellular redox level, intracellular redox levels also influence T cells; a decrease in the intracellular redox balance (oxidation) impairs T cell function.[90,91] The surprising finding with the *Ncf1* polymorphism in the susceptible animal models was that increased susceptibility to arthritis was associated with low oxidative burst capacity contradicting the hypothesis in current use that high levels of free radicals promote inflammation.[92] This finding led to propose that substances that increase the activation of the NADPH oxidase complex could ameliorate and possibly prevent severe arthritis.[20]

.However, the capacity of NADPH oxidase-derived ROS to mediate tissue damage in autoimmune inflammatory diseases has been controversial, as it is unclear whether activation of NADPH oxidase protects from or augments tissue damage.

CONCLUSION

In rheumatoid arthritis, oxidative stress has been described as an important mechanism that underlies destructive proliferative synovitis. TNF-α overproduction, as essential axis in RA pathogenesis is thought to be the main contributor in ROS release in patients with RA. The ROS damage endothelial cells, increase the permeability of the microvasculature and promote the migration of neutrophils to foci of inflammation. Therefore, antioxidant could be an appropriate option for research in the field of treatment for rheumatoid arthritis.

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