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Review Article

Roles of Reactive Oxygen Species in Rheumatoid Arthritis Pathogenesis

Su-Jin Yoo[2,*], Eunbyeol Go[1,*], Ye-Eun Kim[1], Sunyoung Lee[2], Jaeyul Kwon[1]
Departments of [1]Medical Education and [2]Internal Medicine, Chungnam National University School of Medicine, Daejeon, Korea

Rheumatoid arthritis (RA) is an autoimmune disease that starts with decreased tolerance to modified self-antigens and eventually leads to synovitis and destruction of bone and cartilage. Age is a risk factor for developing RA. Major changes in the immune system come with age due to chronic oxidative stress on the deoxyribonucleic acid (DNA) damage pathway, somatic mutation, modifications of auto-antigens, T cell tolerance and activation of fibroblast-like synoviocytes (FLS). Reactive oxygen species (ROS) generated by nicotinamide adenine dinucleotide phosphate oxidase 2 (NADPH oxidase 2) suppress T cell receptor signaling. Sirtuin 1 (SIRT1) is a critical immune suppressor of T cell activation and a key regulator of oxidative stress. When oxidative stress reduces activity of SIRT1, the breakdown of tolerance to modified self-antigens is expected. Generation of ROS can be perpetuated by enhanced DNA damage and dysfunctional mitochondria in a feedback loop during the development of RA. Through major T cell loss and selective proliferation of peripheral T cells, pro-inflammatory T cell pools with abnormal features are established in the T cell compartment. Hypoxic and inflammatory condition in synovium perpetuates ROS generation, which leads to the activation of FLS. In both T cell and synovium compartment, oxidative stress reshapes the immune system into the development of pre-clinical RA. **(J Rheum Dis 2016;23:340-347)**

Key Words. Rheumatoid arthritis, Reactive oxygen species, NADPH oxidase, Sirtuin 1, Oxidative stress

INTRODUCTION

Reactive oxygen species (ROS) include superoxide, hydrogen peroxide and hydroxyl radicals produced by the sequential reduction of oxygen. It is commonly thought that ROS are pro-inflammatory agents because inflammatory diseases have been linked to chronically elevated ROS production ("oxidative stress"). However, it is not clear what pathological processes are initiated or regulated by ROS in the immune system. Recently ROS have been studied as specific and critical regulators of immune system signaling [1,2]. ROS are generated from various sources including mitochondria and nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase, Nox). The discovery of a family of NADPH oxidases related to the phagocyte oxidase (Nox/Duox family) provides new opportunities to investigate the distinct roles of ROS generation by genetically manipulating these sources [3].

Rheumatoid arthritis (RA) is a long-lasting autoimmune disease that primarily affects joints due to the inflammation of the synovium and consequently causes damage to the cartilage and bones [4]. The etiology of RA is unclear. However, inflammatory cytokines are known to play important roles in the pathogenesis of RA to promote autoimmunity, chronic inflammation and tissue destruction. Oxidative stress has also been shown to be closely correlated with the pathogenesis of RA [5]. This review aims to provide an exploration of the possible roles of ROS generation in the immune systems involved in the initiation and development of RA.

The effects of ROS at different stages of RA develop-

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Corresponding to : Jaeyul Kwon, Department of Medical Education, Chungnam National University School of Medicine, 266 Munhwa-ro, Jung-gu, Daejeon 35015, Korea. E-mail : kwonja@cnu.ac.kr
*The first two authors contributed equally to this work.

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ment vary with the level and location of ROS production and the cell type or tissues involved [4]. ROS generally function as damaging or modifying agents of cellular components or as signaling molecules in an immune response. ROS are generated from several sources by stimulation of inflammatory cytokines such as tumor necrosis factor (TNF) or angiogenic factor such as vascular endothelial growth factor (VEGF) [6,7]. Mitochondria and NADPH oxidase are well studied sources of ROS [8]. Nox2 and Duox1 have been suggested to be critical [9,10] in T cell receptor (TCR) signaling of T cells and Nox2-dependent ROS generation was identified even as suppressor of arthritis [11]. Hypoxia-driven ROS generation is also very important in terms of RA pathogenesis because hypoxia develops even in the pre-clinical stage of synovitis and worsens the inflammation which in turn further promotes hypoxic conditions and creates a vicious cycle that may contribute to the establishment and progression of RA [12].

As aging progresses, biochemical imbalance between the formation and clearance of ROS generates a state referred to as "oxidative stress", leading to the damage of various cell components including proteins, lipids and deoxyribonucleic acid (DNA) [12]. Based on the concept that autoimmune diseases are a consequence of immune aging, age-related changes such as chronic oxidative and inflammatory stress are relevant to the initiation of RA [13-17]. Oxidative stress has already been shown to be involved in autoimmune responses. Surprisingly the p47phox subunit of Nox2 was first discovered as a protective factor in arthritis models, which suggested that Nox2-originated oxidative bursts suppressed autoimmune T cells [11,18,19]. ROS generation was proposed to regulate the expression of inflammatory cytokines and chemokines and to affect tissue damage in RA [20]. Excessive production of ROS may be critical for joint destruction and osteoclast activation [21,22]. ROS generation derived from the hypoxia-activated Nox2 is an initiating factor in angiogenesis for joint inflammation [23].

MAIN SUBJECTS

Effects of ROS in the pathogenesis of RA

1) Somatic mutation

Elevated ROS generation at the site of chronic inflammation causes somatic mutations [24]. Somatic mutations in the p53 gene have been observed in the RA synovium and cultured fibroblast-like synoviocytes (FLS) [25]. Many mutations produced by oxidative stress are present in the mitochondrial genome. A high frequency of mitochondrial somatic mutations was reported in synovial tissue of patients with RA and was strongly associated with low level of oxygen in the synovium as well as with high synovial lipid peroxidation [26].

2) Defect in DNA damage repair pathways

Impairments of DNA damage repair pathways increase the risk of RA in older people [27]. Naïve T cells in old people have more chance to accumulate genomic DNA damage than those in young people because these cells in older people have a relatively long life span in the periphery and are exposed to oxidative stress [28]. DNA damage such as DNA double-strand breaks needs to be detected and repaired by DNA damage repair pathways in order to maintain genomic stability. Increases in DNA-dependent protein kinases and deficiencies in ataxia telangiectasia mutated (ATM) and p53 in RA T cells have been shown to impair these repair pathways and lead to markedly increased DNA damage and apoptosis in naïve $CD4^+$ T cells [27,29]. The significant loss of naïve T cells imposes lymphopenia-induced proliferation, leading to premature immunosenescence and possibly an autoimmune-biased T cell repertoire [17,27]. Dysfunctional T cells in patients with RA display the characteristics of inflammation-activated cells and sustain chronic inflammatory immune responses in the synovium [30].

3) Oxidative modification of auto-antigens

Oxidative stress-induced modifications in protein, lipid and DNA may have important roles in the pathogenesis of RA [31]. A strong correlation between levels of ROS and disease activity score with markers of oxidative damage was observed in patients with RA. Measurement of oxidatively modified proteins, lipids or DNA could serve as a biomarker for monitoring disease activity of RA [32]. Type II collagen oxidized by ROS (ROS-CII) were strongly detected in the serum and synovial fluid of patients with RA. 92.9% of sera from disease-modifying antirheumatic drug (DMARD)-naïve patients with early RA showed autoreactivity to ROS-CII [33]. Neo-epitopes can be generated by oxidative modification of proteins and be involved in autoimmune responses [34]. The immune system via pattern recognition receptors (PRRs) such as scavenger receptors, receptor of advanced glycation end products (RAGE) and toll-like receptor 4 (TLR4) can

sense neo-epitopes as pathogen- or danger-associated molecular patterns (PAMPs/DAMPs) [35].

Advanced glycation end products (AGEs) are accumulated by increased oxidative stress in aging and RA [36]. Advanced oxidation protein products (AOPPs) are accumulated in RA patients and are involved in various chronic inflammatory conditions through Nox-dependent ROS production [37]. As one of the receptors for AGEs and AOPPs, RAGE has been suggested as a risk factor for cardiovascular disease in RA patients [38]. Bone-targeting endogenous secretory RAGEs were shown to rescue RA in the murine collagen-induced arthritis (CIA) model [39].

4) Signaling role in T cell tolerance

Several types of ROS have been reported to be involved in T cell activation and differentiation in autoimmune responses. ROS generated from Nox2, Duox1 and mitochondria in T cells were reported to be relevant to these functions [9,10,40]. ROS from macrophages and other immune cells are also involved [41]. ROS have been shown to regulate autoimmune responses. Impairment of Nox2-dependent ROS generation in neutrophil cytosolic factor 1 (Ncf1)-mutated mice results in enhanced disease severity in several different animal models of arthritis [11,18,19]. Macrophage-restricted expression of functional Ncf1 restored arthritis resistance in a CIA model but not in a T cell-independent anti-collagen antibody-induced arthritis model. Restoration of Ncf1 in Ncf1-deficient mice suppressed T cell activation [41].

5) Regulation of T cell differentiation

Several types of ROS generation were reported to modulate differentiation of naïve $CD4^+$ T cells. In both mouse and human models oxidative stress led the differentiation of the naïve $CD4^+$ T cells towards Th2 phenotype [42,43]. In the absence of ROS T cells differentiated to Th1 type [9,41]. In addition, activation of naïve T cells from Nox2-deficient mice exhibited a skewed Th17 phenotype [19].

The immediate-early response gene X-1 (IEX-1, also known as IER3) is involved in preventing the production of ROS in mitochondria. Consequently the elevated generation of mitochondrial ROS from null mutation of IER3 facilitates the differentiation of Th17 cells and immunization with collagen lead to more severe arthritis in IER3 null mice than in wild-type mice. This finding indicates that mitochondrial alterations provide substantial contributions to the dominant T cells [44].

Naïve $CD4^+$ T cells from patients with RA have excess NADPH production. This leads to excessively reduced glutathione and reduced ROS generation [45]. ROS loss and ATM insufficiency in naïve $CD4^+$ T cells from patients with RA skew T cell differentiation into interferon (IFN)-γ and interleukin (IL)-17 producing effector T cells. These biases are reversed by increasing intracellular ROS by treatment with menadione that generates intracellular ROS via redox cycle [45]. These observations indicate importance of ROS-based signal transduction in shaping T cell differentiation in RA.

Accelerated immune aging

Aging is characterized by increasing inflammatory and oxidative stress. The main feature of the aging process is a chronic progressive increase in the proinflammatory status described originally as inflamm-aging [13]. Based on the close relationship between oxidative stress, inflammation and aging, the oxidation-inflammatory theory of aging (oxi-inflamm-aging) was proposed [14]. RA, closely associated with aging, displays the characteristics described in the oxi-inflamm-aging [16,17]. During chronic oxidative and inflammatory stress oxidative modification of cellular components leads to the status described in the inflamm-aging and influences the homeostasis and health of the body. The relationship between the redox state and the function of immune cells influences the speed of aging and lifespan of the cells. At old age the body maintains a pro-inflammatory status and innate immune responses are actively induced more than adaptive immune response [15]. These pathways bring about a constant low level activation of granulocytes, macrophages and dendritic cells. The oxidative burst associated with an innate immune response upregulates ROS formation and reduced cellular antioxidant capacity. Overproduced oxidants react with membrane lipids and proteins and impair their function and create a circular loop of DAMP signaling activation. Moreover DAMPs can activate immune cells and their signaling pathway mediators such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and NADPH oxidase to further increase ROS production.

Extracellular DAMPs such as S100 calcium-binding protein A8/A9 (S100A8/A9) are well known to act as critical alarmins. They modulate the inflammatory response and interact with the PRR, TLR4 and RAGE to promote cell activation and recruitment [46]. S100A8/A9 was identi-

fied as a potential biomarker for monitoring disease activity of RA and has been tested successfully in localizing sites of sterile injury in pre-clinical imaging studies. Surprisingly the S100A8/A9 protein works as a partner for the cytosolic factors of NADPH oxidase activation in neutrophils [47]. Neutrophils and monocytes are recruited to sites of inflammation during infection or sterile injury.

Mitochondrial defects

Mitochondrial defects are a crucial component of the aging process and several age-related diseases. Mitochondrial disturbances lead to the deterioration of protein quality control and can especially contribute to the decline in autophagic degradation with aging [48]. Elevated production of ROS due to mitochondrial disturbances increases with aging and enhances signaling of DAMPs [49,50]. Expressions of certain genes related to the function of mitochondria were altered in patients with RA. A functional annotation study of RA and osteoarthritis (OA) by integrative genome-wide gene expression profiling analysis indicates that both RA and OA can be classified as mitochondrial disorders [51]. A five-fold increase in mitochondrial ROS production in whole blood and monocytes of patients with RA relative to that of healthy subjects suggests that oxidative stress is a pathogenic hallmark in RA [32].

SIRT1

Sirtuin (SIRT), a class III protein deacetylase, has been considered to be a longevity factor for its ability to combat oxidative stress and promote cellular survival. NF-κB signaling is activated during aging [52] and is a potent inducer of the expression of several NADPH oxidase components including gp91phox and p22phox [53]. SIRT1 has also been suggested as a potent inhibitor of NF-κB signaling by suppressing oxidative stress and inflammatory responses [54]. In response to oxidative stress SIRT1 induced antioxidant expression via forkhead box O (FoxO) pathways. SIRT1 can deacetylate FoxO factors (FoxO1, FoxO3a, and FoxO4) to stimulate the expression of antioxidants such as catalase, manganese superoxide dismutase (MnSOD) and thioredoxin and also potentiate SIRT1 expression via an auto-feedback loop [55]. Phosphatase and tensin homolog (PTEN) activated by SIRT1-dependent deacetylation activates FoxO transcription factors, which stimulate the expression of several antioxidants and SIRT1 as well as many autophagy proteins. SIRT1 participates in the DNA damage repair process in an ATM-dependent way. The stress resistance was generally increased by these responses, which results in an extended life span [56].

Increased oxidative stress has been associated with the aging process and the expression and activity of SIRT1 was downregulated by chronic oxidative stress in inflammatory conditions [57]. For instance ROS can inhibit SIRT1 activity by evoking oxidative modifications on its cysteine residues. SIRT1 as a potent inducer of autophagy deacetylated Atg5, Atg7 and Atg8 proteins to stimulate autophagosome formation [58]. Furthermore FoxO1 and FoxO3 can act as downstream effectors of SIRT1 to promote autophagy [59], a process which declines with aging and is disturbed in several age-related diseases. Decreased activity of SIRT1 in aging leads to impairments in autophagy and subsequently enhances oxidative stress. A low-grade inflammatory phenotype was sustained in aging tissues because of impairments of autophagy. Consequently the deficiency in autophagy could enhance ROS and inflammatory responses in tissues and induce a state called inflamm-aging [60].

SIRT1 expression and activity was found to be decreased in RA patients and anti-citrullinated protein antibody (ACPA)-positive patients with RA showed lower SIRT1 activity relative to ACPA-negative patients with RA. The rate of apoptosis of peripheral blood mononuclear cells (PBMCs) in patients with RA was increased and negatively correlated with SIRT1 expression levels. SIRT1 is required to maintain T-cell tolerance [61]. And the lack of SIRT1 resulted in hyperacetylation of c-Jun and the breakdown of T cell tolerance [62]. Therefore, the decreased activity of SIRT1 in aged people and RA patients may result in the activation of autoimmune T cells.

Treatment with resveratrol reduced synovial hyperplasia, cartilage destruction, leukocyte infiltration, macrophage and T cell activation, and collagen-specific immunoglobulin levels in both CIA and lipopolysaccharides-induced acute inflammatory arthritis models [63,64]. Resveratrol-induced SIRT1 activation leads to the inhibition of RelA acetylation and a reduction in NF-κB-induced expression of inflammatory factors such as TNF-α, IL-1β, IL-6, matrix metalloproteinases (MMPs) such as MMP1 and MMP3, and cyclooxygenase 2, all of which have been implicated in the pathogenesis of RA. Similarly resveratrol-treated bone-derived cells showed reduced receptor activator of nuclear factor kappa-B ligand (RANKL)-induced NF-κB acetylation and activa-

tion, as well as reduced osteoblastic activity associated with RA [65]. Resveratrol is also able to avoid excessive ROS induced lipid peroxidation and DNA damage.

Reshaping of peripheral naïve T cells

Although synovial inflammation-induced cartilage damage and destruction of bone is the dominant manifestation of clinical RA, systemic immune abnormalities that are not joint specific are already apparent many years before onset of the RA [4,66]. With advancing age, problems in the homeostasis of the T cell compartments and signaling thresholds for T cell activation lead to the loss of naïve T cells, the accumulation of inflammatory T cell populations and loss of tolerance to modified self-antigens [17,27].

The breakdown of tolerance to modified self-antigens-induced activation of self-reactive T cells could be driven by activation of DAMP signaling in addition to TCR signaling. DAMPs such as heat shock proteins and high mobility group box 1 (HMGB1) released from injured tissue can activate TLR4 and TLR2, respectively [46]. Similarly, increased production of ROS and post-translationally modified molecules such as oxidized lipoproteins activate the TLR8 and TLR2 pathway, respectively. TLR2, TLR4 and TLR8 activation will proceed to initiate an inflammatory response whose key mediators are IL-1, IL-6 and TNF-α. The increased expression of cytokines IL-1β, IL-6, and TNF-α play key roles in the initiation of arthritis and pathogenesis of destructive arthritis in experimental animal models [67].

As thymic activity decreases around the age of 40 to 50 years, prolonged residence of naïve T cells in the periphery progressively lead to the accumulation of oxidative DNA damage [28]. A defect in the maintenance of genomic integrity with age causes excessive loss of peripheral T cells that needs to be compensated by homeostatic proliferation to maintain compartment size and leads to the eventual emergence of senescence biomarkers. During this enforced T-cell proliferation in periphery infrequent self-reactive T cells could be clonally expanded and lead to overaged and autoreactive T cells [27].

Naïve $CD4^+$ T cells from patients with RA are metabolically reprogrammed, favoring NADPH production over adenosine triphosphate (ATP) generation [45]. Excessive NADPH supplies the cell with excessively reduced glutathione and depletes ROS. Such reductive stress fastens the cell cycle of T cells because they skip the G2/M cell cycle checkpoint due to insufficient ATM activation. ROS loss and ATM insufficiency promote T cell differentiation into Th1 and Th17 effector cells. p53 mRNA levels were significantly lower in PBMCs from patients with RA than from healthy controls. And PTEN expression down-regulated by p53 deficiency induced the activation of signal transducer and activator of transcription 3 (STAT3) [68].

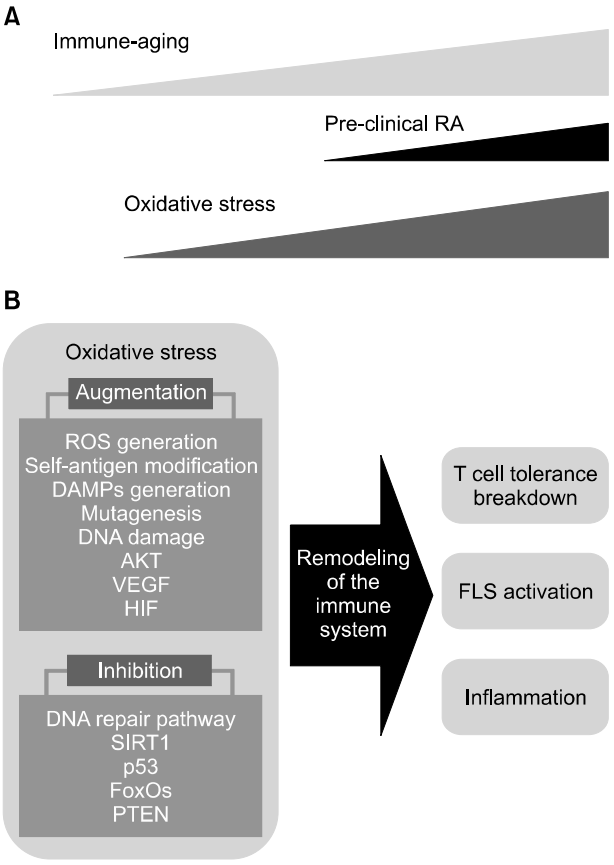

Figure 1. Role of oxidative stress in the pathogenesis of rheumatoid arthritis (RA). (A) With advancing age, oxidative stress plays critical roles in the generation of pre-clinical RA in the process of the immune-aging. (B) Important pathways regulated by the oxidative stress in the immune-aging process reshape the immune systems to lead breakdown of T cell tolerance, activation of fibroblast-like synoviocytes (FLS) and the generation of the inflammatory networks for pathogenesis of RA. Oxidative stress could contribute to development of RA in several ways. Enhanced generation of reactive oxygen species (ROS), oxidative modification of self-antigens, generation of danger-associated molecular patterns (DAMPs), mutagenesis of genomic deoxyribonucleic acid (DNA) and mitochondrial DNA, dysfunctional mitochondria, reduced autophagy, DNA damage, defects in DNA damage pathways, reduced activity of sirtuin 1 (SIRT1), p53, forkhead box O (FoxOs), and antioxidants, enhanced activity of AKT, activation of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF) pathway and inhibition of phosphatase and tensin homolog (PTEN).

Loss of p53 exacerbated autoimmune arthritis and dysregulated the population of Th17 and regulatory T (T_{reg}) cells. The oxidative stress-dependent inhibition of PTEN may have similar effects on the T cell differentiation [69].

CONCLUSION

There are various immune reaction steps and cell types in which different type of ROS may have specific roles in the pathogenesis of RA. Here we focus on the possible roles of ROS in the development of pre-clinical RA (Figure 1). Chronic oxidative stress in old age could generate mutations in both genomic and mitochondrial DNA, leading to enhanced ROS generation in a feedback loop and an eventually remodeling of immune systems. ROS production may contribute the breakdown of T cell tolerance through several pathways. Nox2-generated ROS was identified as a negative regulator for Th17 differentiation and T cell activation. ROS from mitochondria on the other hand works in an opposite way. SIRT1 also has been shown to be a critical immune suppressor of both T cell and macrophage activation. SIRT1 activity which is down-regulated by oxidative stress may augment ROS generation through several signaling pathways involving NF-κB, hypoxia-inducible factor (HIF), FoxOs or PTEN. With advancing age, FLS are activated in synovium and take tumor-like properties in which ROS generation is strongly involved. Several ROS-based signaling pathways appear to play critical roles in reshaping the compartment of T cells and synovium in the pre-clinical phase of RA.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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