Oxidative Stress and Immune Thrombocytopenia

Bing Zhang and James L. Zehnder

Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by increased platelet destruction or decreased platelet production. The mechanism of the disease has been extensively studied so that we now have a much improved understanding of the pathophysiology; however, the trigger of the autoimmunity remains unclear. More recently, oxidative stress was identified to be involved in the pathogenesis of ITP and provides a new hypothesis for the initiation of autoimmunity in patients with ITP. In this review, oxidative stress and its impact on autoimmunity, particularly ITP, will be covered. Semin Hematol 50:e1–e4. © 2013 Elsevier Inc. Open access under CC BY-NC-ND license.

PUBLISHER'S NOTE

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REACTIVE OXYGEN SPECIES AND REACTIVE NITROGEN SPECIES

Free radicals, with high reactivity, are molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals.[1] The production of reactive oxygen species (ROS) can result from exposure to extracellular stressors such as irradiation and inflammatory cytokines, as well as byproducts of normal cellular metabolism of oxygen by enzymatic reactions, such as the mitochondrial respiratory chain, xanthine oxidase, NADPH oxidases, or NO synthases.[2] Superoxide anion radical ($O_2^{\bullet -}$), the primary form of ROS, is mostly produced within the mitochondria of a cell and can further interact with other molecules to generate secondary ROS (eg, hydrogen peroxide, hydroxyl radical).[1] $NO^{\bullet}$ is generated in biological tissues by nitric oxide synthases and present as an abundant reactive radical. During the respiratory burst in response to inflammatory processes, the superoxide anion and nitric oxide produced by immune cells may react together to produce significant amount of peroxynitrite anion ($ONOO^-$), a much more oxidatively active molecule.[1]

OXIDATIVE STRESS AND DAMAGE

At low to moderate concentrations, ROS function as important molecules involved in many physiological processes.[1] However, an over-production of ROS/reactive nitrogen species (RNS) and a deficiency in enzymatic (eg, superoxide dismutase, glutathione peroxidase, catalase, thioredoxin reductase) and non-enzymatic antioxidant (eg, vitamin E, vitamin C, glutathione) defense mechanisms will result in the disturbance of the equilibrium status of pro-oxidant and antioxidant reactions. Oxidative stress and nitrosative stress refer to the overproduction of free radicals in excess of the system's ability to scavenge them, consequently leading to potential biological damage.[1]

The targets of oxidative damage include lipids, proteins, carbohydrates, and DNA. ROS can stimulate the peroxidation of polyunsaturated fatty acids (PUFA) to generate lipid hydroperoxides (PUFA-OOH). This is an oxidative event reversible through reduction by peroxiredoxin (PRX) and glutathione peroxidase (GPX). Lipid hydroperoxides are unstable and form a variety of reactive aldehydes, including the α,β-unsaturated aldehydes 4-hydroxynonenal (4-HNE), trans-4-oxo-2-nonenal (4-ONE), 4-hydroxy-(2E)-hexanal (4-HHE), (2E)-hexenal, crotonaldehyde, and acrolein, as well as the dialdehydes glyoxal and malondialdehyde

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(MDA). The highly reactive α,β-unsaturated aldehydes are conjugated to glutathione catalyzed by GSTA4, leading to their efflux from the cell by the glutathione conjugate transporter RLIP76. In addition, free aldehydes can be converted to less toxic molecules through oxidation by aldehyde dehydrogenase or reduction by alcohol dehydrogenase, aldehyde reductase, or aldose reductase. Those aldehydes that escape cellular metabolism act as electrophiles in the covalent modification of proteins via non-enzymatic Michael addition.[3] Compared with free radicals, the aldehydes can covalently modify proteins throughout a cell and diffuse across cell membranes to attack remote targets. The modification of amino acids by aldehydes happens mainly on the nucleophilic residues Cys, and to a lesser extent, His and Lys. ROS/RNS modification of proteins may alter every level of protein structure from primary to quaternary, causing major and mostly irreparable physical changes.[4]

OXIDATIVE STRESS AND AUTOIMMUNITY

Proteins modified by aldehydes are highly immunogenic. Autoantibodies directed against epitopes in MDA- and HNE-modified low-density lipoproteins (LDLs) develop in rabbits and mice immunized with oxidized LDL particles.[5] The adaptive immune response may be enhanced by oxidative modification of protein antigens.[5] Oxidative modification of proteins has been shown to induce pathogenic antibodies in a variety of autoimmune diseases, such as, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), type 1 diabetes mellitus (T1DM), scleroderma, and Behcet's disease.[5,6]

In patients with SLE, there is an increase in total serum protein carbonyl levels, which is largely due to an increase in oxidized albumin. The SLE autoantibodies were revealed to have higher reactivity towards hydroxyl radical damaged human serum albumin (HSA) over native albumin. The ROS modification leads to conformational alterations of HSA, which can present unique antigenic determinants for the production of autoantibodies.[7] Similarly, an elevation in the levels of MDA-modified proteins was observed among SLE patients; in addition, IgG autoantibodies in the sera of SLE patients exhibited a significant enhanced reactivity against MDA-modified bovine serum albumin (BSA).[8] Furthermore, the serum levels of anti-MDA- or anti-HNE-protein antibodies in SLE patients were reported to be correlated with disease activity.[9] Findings from some animal studies are also in agreement with the role of ROS in the pathogenesis of autoimmune diseases. In the lupus-prone $MRL^{+/+}$ (Murphy Roths Large) mice treated with dichloroacetyl chloride (DCAC), which is a metabolite of trichloroethene (TCE) and known to induce autoimmune response, induction of serum anti-MDA antibodies was shown, suggesting the presence of lipid peroxidation and a putative role of oxidative stress in inflammatory autoimmune disease.[10] $MRL^{+/+}$ mice exposed to TCE were also reported to show increased nitration and carbonylation of proteins, as well as significant increase in Th1 specific cytokine (interleukin [IL]-2, interferon-γ), indicating an association between protein oxidation and induction/exacerbation of TCE- induced autoimmune response.[11] In rabbits immunized with the HNE-modified lupus-associated 60-kd Ro protein, intromolecular and intermolecular epitope spreading occurred preferentially, compared to rabbits immunized with the unmodified Ro. This suggests that oxidative stress facilitates epitope spreading in SLE.[12]

Evidence has been accumulating supporting the involvement of oxidative stress in the development of autoimmune rheumatoid arthritis. Griffiths[13] pointed out in her review that two conditions are considered necessary for neoantigenic determinants generated by free radicals to lead to autoimmunity: failure to remove or repair ROS/RNS damaged biomolecules, and an associated defect, probably in T-cell signal processing and/or antigen presentation.

Type 1 diabetes mellitus (T1DM) is another autoimmune disease with a well-documented role of oxidative stress in the disease onset and complications. The total protein carbonyl content was found to be significantly increased in T1DM patients compared with normal controls, and this increase was largely due to elevated oxidized albumin. The serum albumin of T1DM patients was conformationally altered with more exposure of its hydrophobic regions, thus presenting unique antigenic determinants for the production of T1DM autoantibodies.[14] Hydroxyl radical modified glutamic acid decarboxylase-65 (GAD_{65}), an immunological marker of T1DM, was also reported to be a potential autoantibody in T1DM. The in vitro modification of GAD_{65} causes conformational perturbation and generates highly immunogenic unique neoepitopes. The circulating autoantibodies from T1DM patients exhibited high recognition with ROS modified GAD_{65} over unmodified GAD_{65}.[15] Oxidative stress is usually mediated by genetic lack of antioxidant enzymes and environmental triggers like viral infection. Following viral infection, ROS is generated from activated phagocytes. The highly permeable H_2O_2 can act on the T cells and stimulate T-cell–specific nuclear factor-κB activity and subsequently proinflammatory cytokine production, such as tumor necrosis factor-α, IL-6, and IL-1β. In the case of T1DM, β cells are more prone to oxidative damage due to their exceptionally low level of antioxidant enzymes, and thus become easy targets for cytokine-mediated autoimmune attack. ROS are implicated in all stages of autoimmune T1DM, from the primary trigger to the acquisition of T-cell–mediated autoreactivity.[16]

THE ROLE OF OXIDATIVE STRESS IN THE PATHOGENESIS OF ITP

Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by low platelet count and increased

risk of mucocutaneous bleeding. Much progress has been made in understanding the pathophysiology of ITP, but the triggering event of autoantibody initiation remains unclear. By analyzing the blood expression profiles of patients with ITP and healthy controls, we identified the activation of ROS-related molecular signaling pathways in patients with ITP and proposed the involvement of oxidative stress in the pathogenesis of pediatric primary ITP. Often, the onset of ITP in children follows a viral infection, which triggers the ROS production from phagocytes. The overgeneration of ROS or inadequate antioxidant scavenging capacity leads to imbalanced redox state. The ratio of reduced to oxidized glutathione (GSH/GSSG) represents a good marker of the oxidative stress status, and a lower ratio indicates a higher level of oxidative stress. This marker is significantly higher in healthy controls than in patients with ITP; furthermore, the value in patients with chronic ITP is even lower than patients with acute ITP.[17] Persistent oxidative stress causes lipid peroxidation and reactive aldehydes are formed, evidenced by elevated MDA level in the plasma of ITP patients compared to controls.[18,19] Post-translational modification of proteins, as a result of either direct oxidation of amino acid residues or indirect oxidation by addition of aldehydes (more prevalent), ensues in the form of protein carbonylation. This process is confirmed by our unpublished experimental data on protein carbonyl content.

Wuttge et al[20] reported that a T-cell–dependent break of tolerance in mice immunized with homologous albumin covalently modified with aldehydes. The major histocompatibility complex-restricted and protein sequence-dependent responses were demonstrated by the T-cell hybridomas from immunized animals to modified albumin, but not to native albumin. Introduction of aldehyde adducts on self-proteins may also disturb tolerance to non-modified proteins by altered antigen processing to potentially induce autoimmune disease. In a later study performed by Rasheed et al,[21] HSA modified in vitro by hydroxyl radical was observed to be a potent antigenic stimulus inducing high-titer antibodies in rabbits as compared to native HAS, which induced low-titer antibodies. The substantially enhanced immunogenicity of ROS modified HSA could be due to the generation of neo-epitopes. Since the oxidatively modified proteins have been shown to be highly immunogenic, we speculate that a similar mechanism may at least in part account for the initiation of autoantigen in ITP.

The $CD4^+CD25hiFoxp3^+$ regulatory T cells (Tregs) have an important function in self-tolerance and defects in Tregs have been demonstrated to allow enhanced T-cell and B-cell autoreactivity in patients with ITP by a number of studies.[22–25] Interestingly, NO was shown to reduce Foxp3 expression and subsequently decrease Tregs in autoimmune disorders.[26] Therefore, ROS may contribute to the Treg deficiency in ITP and redox modulation may have an immune-regulatory role as well. Several small-scale studies were undertaken previously at different institutions to assess the usefulness of ascorbic acid in treating chronic ITP, and the results were quite controversial.[27–32] Based on these considerations, we believe that larger scale prospective clinical trials designed to evaluate the effectiveness of anti-oxidant (eg, N-acetylcysteine) in treating newly-diagnosed patients with ITP would be reasonable at this time.

CONCLUSIONS AND FUTURE DIRECTIONS

In summary, multiple lines of evidence are converging on oxidative stress as a potential initiating mechanism in autoimmune disease. In ITP patients, oxidative stress related gene expression pathways are activated and a higher level of oxidative stress is reflected by the low GSH/GSSG ratios compared to healthy controls.[17] As a consequence of oxidative damage, lipid peroxidation and oxidative protein modification are also present in patients with ITP. The ROS-modified proteins are known to be highly immunogenic and can induce autoantibody production in other autoimmune diseases. Important questions in the field include: (1) Are autoantibodies against oxidatively modified proteins present in patients with ITP? (2) Does the patients' redox status impact ITP disease susceptibility? (3) Does the level of oxidative damage correlate with ITP disease severity? (4) Is oxidative stress a cause, effect, or disease modifier of autoimmune disease in general and ITP specifically? (5) Will targeting antioxidant pathways alter the course of disease in patients with ITP?

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