Experimental Autoimmune Uveitis

Reactive oxygen species (ROS) are produced by host phagocytes and play an important role in antimicrobial actions against various pathogens. Autoimmune uveitis causes blindness and severe visual impairment in humans at all ages worldwide. However, the role of ROS in autoimmune uveitis remains unclear. We used ROS-deficient (*Ncf1*$^{-/-}$) mice to investigate the role of ROS in experimental autoimmune uveitis (EAU). Besides, we also used the antioxidant N-acetylcysteine treatment to evaluate the effect of suppression of ROS on EAU in mice. The EAU disease scores of *Ncf1*$^{-/-}$ mice were significantly lower than those of wild-type mice. EAU induction increased the levels of cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-12, IL-17, and TNF-α) and chemokines (MCP-1) in the retinas of wild-type mice but not in those of *Ncf1*$^{-/-}$ mice. EAU induction enhanced the level of NF-κB activity in wild-type mice. However, the level of NF-κB activity in *Ncf1*$^{-/-}$ mice with EAU induction was low. Treatment with the antioxidant N-acetylcysteine also decreased the severity of EAU in mice with reduced levels of oxidative stress, inflammatory mediators, and NF-κB activation in the retina. We successfully revealed a novel role of ROS in the pathogenesis of EAU and suggest a potential antioxidant role for the treatment of autoimmune uveitis in the future.

Keywords: reactive oxygen species ; experimental autoimmune uveitis ; neutrophil cytosolic factor 1

1. Introduction

Uveitis is among the most important causes of blindness and severe visual impairment worldwide. Approximately 15% to 30% of uveitis occurs in the choroid and adjacent retina and is therefore classified as posterior uveitis or uveoretinitis [1]. Posterior uveitis tends to damage photoreceptor cells and leads to permanent blindness. According to epidemiological data from the United States of America, uveitis occurs in approximately 0.54% of the population, in which approximately 30% of cases of uveitis are idiopathic [2]. An autoimmune causality is supported by strong human leukocyte antigen (HLA) associations and by frequent responses to one or more unique retinal antigens. In addition, uveitis is often associated with autoimmune or inflammatory disorders, such as Behcet's disease, ankylosing spondylitis, sarcoidosis, psoriatic arthritis, Crohn's disease, and ulcerative colitis in patients [2]. Ocular trauma may precipitate uveitis, presumably through a breach of the blood–ocular barrier and the release of normally sequestered antigens [3][4]. In most uveitis cases, however, the etiologic triggers are unknown and have been postulated to include antigenic mimicry by microorganisms in conjunction with a concomitant adjuvant effect, leading to the priming of effector T lymphocytes capable of recognizing ocular antigens [5]. Autoimmune uveitis is a sight-threatening inflammatory disorder that affects humans at all ages [1]. Current therapies for uveitis are largely based on immunosuppressive treatment, including corticosteroids, antimetabolites, and alkylating agents. Due to the nonspecific nature and the dose-limiting side effects of these drugs, the results of current treatment for autoimmune-mediated uveitis remain unsatisfactory [6]. Each year, 17.6% of active uveitis patients experience a transient or permanent loss of vision, and 12.5% of uveitis patients will develop glaucoma [7]. An improved understanding of uveitis pathogenesis is needed to develop effective treatments.

A robust model for human uveitis is experimental autoimmune uveitis (EAU) in mice, which can be induced by immunizing susceptible mouse strains with a retinal antigen, such as interphotoreceptor retinoid binding protein (IRBP) and retinal arrestin (retinal soluble antigen or S-antigen) [8]. IRBP functions to transport retinoids, which are essential for the visual cycle, between the retinal pigment epithelium and the photoreceptors. S-antigen is the visual arrestin that quenches photoactivated rhodopsin in the process of visual signal transduction. Both proteins are highly evolutionarily conserved and are major components of the photoreceptor cell layer. The retinal antigens that are involved in the visual cycle and that can serve as targets in EAU are typically unique not only to the eye but also to the whole body. The only other site of expression (within the limits of detection of currently available methods) is the pineal gland ("third eye"), which controls the circadian rhythm and shares many vision-related proteins with the retina [9].

2. ROS

During EAU progression, the infiltration of inflammatory cells into the retina and/or uvea begins approximately seven days after induction. The stages before and after day seven postinduction are defined as the early and amplification phases, respectively. In the early phase, the upregulation of inducible nitric oxide synthase (iNOS), which catalyzes the production of nitric oxide (NO), is detected in the photoreceptor mitochondria of retina [10][11][12]. Inflammation is a natural defense mechanism against pathogens and it is associated with many pathogenic diseases such as autoimmune diseases. Oxidative stress refers to the excessive production of reactive oxygen species (ROS) in the cells and tissues and antioxidant system may not be able to neutralize them, which can lead to chronic inflammation [13]. ROS are strong stimulators of the transcription factor nuclear factor kappa B (NF-κB), which increases the transcription of inflammatory cytokines and chemokines [14]. An increase in the oxidative stress response with the generation of ROS, superoxide and hydrogen peroxide was also found. The major source of these oxidants is nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (NOX2) [15][16]. NO and superoxide rapidly react to form the highly toxic peroxynitrite $OONO^-$. NO and peroxynitrite are reactive nitrogen species (RNS). Oxidative stress induces the nitration of photoreceptor mitochondrial proteins and the peroxidation of membrane lipids. The ROS generated by oxidative stress and RNS are therefore proposed to be initial pathological events leading to the EAU-induced damage observed during the amplification phase. The role of ROS in EAU remains elusive.

The release of ROS and its downstream products from phagocytes, which is known as the respiratory burst, plays a significant role in fighting against invading pathogens. The importance of the innate immune defense with a functional phagocyte NOX2 is clearly exemplified in chronic granulomatous disease (CGD), a rare genetic disorder characterized by severe recurrent infections due to the inability of neutrophils and macrophages to mount a respiratory burst to kill invading pathogens [17]. In addition to recurrent and severe infections, inflammatory manifestations are also common in CGD patients, including the gastrointestinal tract (88.2%), lungs (26.4%), the urogenital tract (17.6%), and eyes (8.8%) [18][19]. NOX2 is composed of five subunits, including p47^{phox}, which is also called neutrophil cytosolic factor 1 (Ncf1) [20]. Ncf1 is an essential component of the NOX2 complex because the absence of Ncf1 leads to undetectable NOX2 activity as measured by the ROS response of neutrophils in mice [21]. The second most common genetic defect, responsible for approximately 30% of CGD cases, is an autosomal recessive mutation in *Ncf1* [17]. Mice without Ncf1 display augmented disease severity in two models of autoimmune disorders, experimental autoimmune encephalomyelitis (EAE) provoked by native myelin oligodendrocyte glycoprotein (MOG) and arthritis caused by collagen or serum [21][22]. To address the role of ROS in EAU in vivo, we compared wild-type mice and Ncf1-deficient mice [23] and assessed treatment with N-acetylcysteine (NAC), an ROS inhibitor used in the clinic. Surprisingly, we discovered that the suppression of ROS due to Ncf1 deficiency or NAC treatment decreases EAU severity in mice.

References

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