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Regulation of IL-10 and IL-17 mediated experimental autoimmune encephalomyelitis by S-nitrosoglutathione

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ABSTRACT

In this study, we investigated IL-10 and IL-17 specific immunomodulatory potential of S-nitrosoglutathione (GSNO), a physiological nitric oxide carrier molecule, in experimental autoimmune encephalomyelitis (EAE). In active EAE model, GSNO treatment attenuated EAE severity and splenic $CD4^+$ T cells isolated from these mice exhibited decreased IL-17 expression without affecting the IFN-γ expression compared to the cells from untreated EAE mice. Similarly, adoptive transfer of these cells to nave mice resulted in reduction in IL-17 expression in the spinal cords of recipient mice with milder EAE severity. $CD4^+$ T cells isolated from GSNO treated EAE mice, as compared to untreated EAE mice, still expressed lower levels of IL-17 under T_H17 skewing conditions, but expressed similar levels of IFN-γ under T_H1 skewing condition. Interestingly, under both T_H17 and T_H1 skewing condition, $CD4^+$ T cells isolated from GSNO treated EAE mice, as compared to untreated EAE mice, expressed higher levels of IL-10 and adoptive transfer of these T_H17 and T_H1 skewed cells seemingly exhibited milder EAE disease. In addition, adoptive transfer of $CD4^+$ T cells from GSNO treated EAE mice to active EAE mice also ameliorated EAE disease with induction of spinal cord expression of IL-10 and reduction in of IL-17, thus suggesting the participation of IL-10 mechanism in GSNO mediated immunomodulation. GSNO treatment of mice passively immunized with $CD4^+$ T cells either from GSNO treated EAE mice or untreated mice further ameliorated EAE disease, supporting efficacy of GSNO for prophylaxis and therapy in EAE. Overall, these data document a modulatory role of GSNO in IL-17/IL-10 axis of EAE and other autoimmune diseases.

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is a $CD4^+$ T helper (T_H) cell mediated autoimmune disease of central nervous system (CNS) that serves as an animal model of multiple sclerosis (MS) (Lublin, 1985). The disease is characterized by differentiation and expansion of myelin specific immune cells, their infiltration into the CNS, and chronic encephalitogenic inflammation leading to damage to myelin, oligodendrocytes, and axons (Compston and Coles, 2002). Earlier, IFN-γ producing T_H1 cells were believed to be solely responsible for the initiation and progression of MS and EAE (Lovett-Racke et al., 2004; Racke et al., 1994). However, there is now a general consensus that IL-17 producing T cells (T_H17) are also involved in the onset and progression of EAE (Cua et al., 2003; Komiyama et al., 2006; Langrish et al., 2005). Moreover, recent studies have also described an important role for IL-10 producing regulatory T cells (Tregs) in regulation T_H1 and T_H17 mediated immune responses in EAE disease (Fiorentino et al., 1991; Huber et al., 2011).

There is a growing body of evidence that nitric oxide (NO) plays an important role in regulation of cellular processes involved in immune and inflammatory responses (Bogdan, 2001). Cellular NO is synthesized by three distinct forms of NO synthases (NOS): neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) (Griffith and Stuehr, 1995). NO is reported to play an inhibitory role in differentiation of T_H17 cells and their release of IL-17 (Jianjun et al., 2013; Nath et al., 2010). Accordingly, genetic ablation of iNOS (but not nNOS and eNOS) increased the severity of EAE disease via inducing T_H17 cells (Jianjun et al., 2013). NO was also reported to induce a specific subset of Treg cells, called NO-Treg, that reduce EAE disease via inducing the release of IL-10 (Niedbala et al., 2007).

At the molecular level, NO exerts its physiological activities by interacting with and modulating the activities of enzymes containing

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iron-sulfur centers (e.g. guanylyl cyclase) (Cooper, 1999) and/or by secondary modification of protein thiols directly or by low-molecular-weight-nitrosothiol mediated trans-S-nitrosylation (Singh et al., 1996). S-nitrosoglutathione (GSNO), the most abundant cellular low-molecular-weight-nitrosothiol, is formed by a redox based reaction between NO and glutathione (GSH) (Singh et al., 1996). GSNO has been identified as a potential carrier and reservoir of NO (Singh et al., 1996) and is known to inhibit platelet activation (de Belder et al., 1994) and inflammatory processes (Peng et al., 1995a, b), and is also reported to induce cardiovascular protection (Konorev et al., 1995).

We previously evaluated the immuno-modulatory efficacy of GSNO in different EAE models and reported prophylactic and therapeutic efficacy of GSNO against the clinical disease of EAE (Nath et al., 2010; Prasad et al., 2007). GSNO inhibited the IL-6-induced STAT3 activation (Tyr^{705} phosphorylation) by S-nitrosylation of the STAT3 protein on Cys^{259} (Kim et al., 2014) and also downregulated the IL-6 and TGF-β induced expression of RORγt, a T_H17 cell specific transcription factor (Nath et al., 2010). Additionally, GSNO treatment also inhibited the T_H17 cell polarization induced by IL-6 and TGF-β and their effector function induced by IL-23 in in vitro cell culture conditions, but without significant changes in T_H1 (IFN-γ) and T_H2 (IL-4) immune responses, suggesting GSNO as a potential T_H17 specific immuno-modulatory agent under EAE conditions (Nath et al., 2010). Previous studies from our laboratory and others have described that GSNO directly modulates the activities of proinflammatory transcription factors, such as NF-κB, AP-1, and STAT3 (Corti et al., 2014; Prasad et al., 2007; Won et al., 2013) and thus modulates gene expression for various proinflammatory effectors, such as iNOS, ICAM-1, and VCAM-1 (Corti et al., 2014; Khan et al., 2005; Kim et al., 2014; Prasad et al., 2007; Won et al., 2013). It is also of interest to note that some of these transcription factors (e.g. NF-κB and AP-1) play a role in IL-17 mediated proinflammatory processes (Song and Qian, 2013). These studies, therefore, indicate that GSNO-mediated mechanisms effectively modulate both differentiation and effector function of T_H17 cells and thus suggest its potential for prophylactic and therapeutic intervention of EAE and other autoimmune diseases.

In this study, we report that GSNO modulates autoimmune responses in EAE by specifically targeting the T_H17 cells and by inducing IL-10 expression. This conclusion is supported by (1) splenic $CD4^+$ T cells isolated from GSNO treated EAE animals expressed lower levels of IL-17, while did not exhibited any difference in IFN-γ expression compared to the cells from untreated EAE animals; accordingly, adoptive transfer of these cells to naïve mice exhibits milder disease with lesser expression of IL-17 in the spinal cord; (2) $CD4^+$ T cells from GSNO treated EAE animals express higher levels of IL-10 under both T_H1 and T_H17 skewing conditions, than the cells from untreated EAE animals, and adoptive transfer of both T_H1 and T_H17 skewed cells exhibited milder EAE disease; (3) Adoptive transfer of $CD4^+$ T cells from GSNO treated EAE mice to active EAE mice ameliorated active EAE disease with induction of spinal cord expression of IL-10 and reduction in of IL-17; (4) GSNO treatment of mice passively immunized with $CD4^+$ T cells either from GSNO treated EAE mice or untreated mice further ameliorated EAE disease.

2. Materials and methods

2.1. Mice

Female SJL and C57BL/6 mice, purchased from the Jackson Laboratory (Bar Harbor, ME), were housed in the animal care facility of Medical University of South Carolina and received standard laboratory food and water ad libitum. Paralyzed mice were provided with Transgel (Charles River Laboratories, Wilmington, MA) as an alternate food/water source. All animal protocols were in accordance with the animal experiment guidelines of the Medical University of South Carolina and National Institute of Health and accepted by Institutional Animal Care and Use Committee in Medical University of South Carolina (Approved number: AR#1644).

2.2. Induction of EAE disease

EAE disease was induced in 8- to 12-week-old female SJL or C57BL/6 mice by immunization with proteolipid protein peptide ($PLP_{139–151}$; Peptide International, Louisville, KY) or MOG_{35-55} peptide (MOG_{35-55}; 200ug; Peptide International) emulsified (1:1) in 100ul complete Freund's adjuvant (CFA) on day 0 and day 7. Additionally, 300 ng of Pertussis toxin (Sigma-Aldrich, St Louis, MO) was given on day 0 and day 2 by i.p. injection. Pertussis toxin used as per the standardized protocol reported by us and other investigators for the induction of EAE (Nath et al., 2009). On the day of immunization, one group of mice received 100 µl phosphate buffered saline (PBS) and the second group of mice received daily GSNO (1.0 mg/kg, 100 µl/PBS) via oral route. GSNO was purchased from World Precision Instruments (Sarasota, FL) and its concentration was adjusted spectrophotometrically at 334 nm. Individual animals were observed daily for clinical disease severity by an investigator, blinded to experimental treatments, on a 0–5 scale as follows: 0 = no abnormality; 1 = piloerection, sluggish, 2 = limp tail; 2.5 = hind limb weakness (legs slip through cage top); 3 = hind limb paralysis; 4 = hind and forelimb paralysis; and 5 = moribund (Nath et al., 2010).

2.3. Ex vivo culture of T cells and characterization of $CD4^+$ T cell lineages

At the peak of EAE disease, the mice were sacrificed and $CD4^+$ T cells were purified from spleens by $CD4^+$ T cell isolation kit (Miltenyi, Auburn, CA). The purified T cells (2.5×10^6 cells/ml) were cultured in 96-well round-bottom microculture plates (Falcon Labware, Oxnard, CA) in RPMI-complete media containing RPMI 1640 (Life Technologies, Gaithersburg, MD), 10% FBS, and 100 µg/ml streptomycin and penicillin (Atlanta Biologicals Norcross, GA), 1 mM glutamine, 1 mM non-essential amino acids, and 50 µM 2-mercaptoethanol (Sigma-Aldrich).

For skewing of different $CD4^+$ T cell subsets and their expansion, the isolated $CD4^+$ T cells were stimulated with $PLP_{139–151}$ (5 µg/ml) with IL-2 (10 ng/ml) for T_H0, IL-2 (10 ng/ml), rhIL12p35 (10 ng/ml), and anti-IL-4 (1 µg/ml) for T_H1, or rmIL12/23p40 homodimer (10 ng/ml), anti-IFN-γ 1(µg/ml), anti-IL-4 (1 µg/ml) for T_H17. All cytokines and antibodies were purchased from BD Biosciences (San Diego, CA). Following stimulation, the cells were harvested for adoptive transfer of EAE disease and the culture supernatants were collected for analysis of IFN-γ, IL-17, and IL-10 expression by ELISA (BioLegend Cat# 430802, 432505, and 431411; San Diego, CA).

2.4. Adoptive transfer model of EAE

Cultured T cells ($20–30 \times 10^6$ T cells in 300 µl RPMI media per mouse) were injected to naïve female SJL or C57BL/6 mice (8–12 week old) via intraperitoneal route. The recipient mice were also given two doses of pertussis toxin (200 ng/300 µl of PBS/i.p.) on day 0 and 2 of post immunization. Clinical EAE disease was measured as described above.

2.5. Statistical analysis

Clinical disease scores are presented as average maximal scores over the treatment period (mean + SD) and analyzed using a nonparametric Kruskal-Wallis test. Statistical significance was set at 0.05. Statistics for proliferation and cytokine responses were analyzed with a one-way multiple-range analysis of variance (ANOVA). All analyses were conducted using Graph Pad Prism 3.0 software. Significances (p-value) between groups were determined using the Newman-Keul test. A value of $p < 0.05^*$ and above was considered significant.