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Activation of the receptor for advanced glycation end products (RAGE) exacerbates experimental autoimmune myasthenia gravis symptoms

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Abstract RAGE belongs to immunoglobulin superfamily and serves as a ligand for various immunoregulatory molecules including S100B that has been demonstrated important to T cell mediated autoimmune diseases. In this context, we hypothesized that RAGE could also impact B cell mediated, T cell-dependent autoimmune diseases. This was tested using myasthenia gravis (MG) animal model, EAMG. We show that expression of both RAGE and S100B are increased during EAMG and the interaction between RAGE and S100B affected the Th1/Th2/Th17/Treg cell equilibrium, up-regulate AChR-specific T cell proliferation. Furthermore, addition of S100B in vitro stimulated splenocyte activity linked to COX-2 up-regulation. NS-398, a selective COX-2 inhibitor, effectively diminished S100B mediated activity of AChR-specific antibody secreting splenocytes. These findings suggested that a reciprocal relationship between RAGE and S100B promoted the development of EAMG, highlighting the importance of understanding the mechanisms of EAMG disease as a means of developing new therapies for the treatment of MG.

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1. Introduction

Myasthenia gravis (MG) is a B cell-mediated, T cell-dependent autoimmune disease of neuromuscular junctions [1]. Muscle weakness and fatigue (hallmarks of MG) are a function of improper signaling between T- and B-cells which results in the elicitation of an antibody-mediated autoimmune responses against the acetylcholine receptor (AChR) located at neuromuscular junctions.

Experimental autoimmune myasthenia gravis (EAMG) is a widely studied animal model of MG that has helped shape our current understanding of the pathophysiology of MG [2,3]. Like MG, some inflammatory cytokines (IL-17) [4,5] that affect T cell and/or B cell function are associated with the onset of EAMG.

The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily and has been shown to be expressed by diverse cell types, including activated T cells, B cells, macrophages and neuronal cells [6]. RAGE signaling has also been found to be stimulated by members of the S100 family of proteins, including S100B and AGE (advanced glycation end products) [7,8]. It has previously been demonstrated that RAGE played a critical role (in the context of its various ligand interactions) in mediating immune and inflammatory responses. For example, blocking RAGE activation with soluble RAGE was shown to prevent delayed type sensitivity reactions [8]. Expression of RAGE and S100B in patients with MS was observed in neurons, the vasculature and inflammatory cells and RAGE and S100B expression was also shown to be elevated in experimental autoimmune encephalomyelitis (EAE). Interactions between RAGE and S100B were shown to modulate encephalitogenic $CD4^+$ T cells and inhibition of RAGE signaling inhibited the elicitation of the EAE inflammatory phase following T cell activation with myelin basic protein (MBP) [9]. In patients with type I diabetes, RAGE/ligand interactions were shown to be involved in late-stage disease in NOD (non-obese diabetic) mice and blocking this interaction inhibited the autoimmune response that lead to type I diabetes [10]. Although RAGE/S100B interactions have been implicated in T cell-mediated autoimmune diseases, few studies have examined the effects of B cell-mediated autoimmune responses.

Cyclooxygenase-2 (COX-2) is one cyclooxygenase isoform whose production is significantly up-regulated by cytokines and growth factors and can lead to the formation of potent inflammatory prostaglandin-mediated responses. Several studies have demonstrated that the RAGE ligands, including AGE and S100B can increase COX-2 levels and up-regulate inflammatory genes in vascular cells and monocytes. Shanmugam et al. indicated that S100B-treated THP-1 monocytes significantly up-regulated COX-2 mRNA and protein expression levels and that these processes could be blocked using anti-RAGE antibodies, suggesting that COX-2 up-regulation via RAGE ligation led to monocyte activation [11]. Bianchi and colleagues found that S100B/RAGE up-regulated COX-2, IL-1β and TNF-α expression in microglia that was associated with NF-κB and AP-1 activation [12] and Daniel and Biao also demonstrated that COX-2 played a role in B cell activation.

In the context of these observations, we hypothesized that RAGE and S100B were involved in the development of EAMG via a yet to be defined mechanism. In the present study, we demonstrate for the first time that RAGE expression and its ligand S100B were up-regulated in EAMG and that this up-regulation led to an activation of T cells and a significant increase in splenocyte COX-2 expression which in turn stimulated antibody production associated with EAMG elicitation. Inhibition of COX-2 by NS-398 diminished the S100B-mediated AChR-specific splenocyte-secreted antibodies. These experiments characterized the role of RAGE and S100B in EAMG that may further illuminate our understanding of the immune mechanisms associated with MG presentation.

2. Methods

2.1. Animals

Female Lewis rats 6–8 weeks (160–180 g) were purchased from the Peking Vital River Laboratory Animal Ltd. (Beijing, China). All rats were bred and maintained in accordance with the Care and Use of Laboratory Animal guidelines published by the China National Institute of Health.

2.2. Reagents

The peptide corresponding to the 97–116 region of the rat AChR subunit, R97–116 (DGDFAIVKFTKVLLDYTGHI), was synthesized by AC Scientific, Inc. (Xi'an, China). The rat myelin basic protein (MBP) 68–86 peptide (YGSLPQKSQRSQDENPV) was synthesized by Sangon Ltd. (Shanghai, China). *Mycobacterium tuberculosis* (strain H37RA) was purchased from Difco (Detroit, MI, USA) and Incomplete Freud's Adjuvant (IFA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The following antibodies were purchased from commercially available sources: anti-rat RAGE (175410 R&D, Minneapolis, MN), mouse anti-rat RAGE (RD9C 2, Santa Cruz Biotechnology, Santa Cruz, CA, USA), FITC-conjugated anti-rat CD4 (W3/25, Caltag Laboratories, Burlingame, CA) and PE-conjugated anti-rat Foxp3 (FJK-16 s, eBioscience, San Diego, CA). PE-conjugated anti-rat IFN-γ (DB-1), PE-conjugated anti-rat IL-4 (OX-1) and mouse anti-rat CD3 (G4.18, BD Biosciences, San Jose, CA), rabbit anti-rat IL-17 (H-132, Santa Cruz Biotechnology, Santa Cruz, CA), Cy3-conjugated goat anti-rabbit IgG and PE-Cy3-conjugated goat anti-mouse IgG (Caltag Laboratories, Burlingame, CA), rabbit anti-rat COX-2 (Abcam, USA) and the fixation and permeabilization kit used for flow cytometry was from eBioscience, RAGE-Fc (R&D Systems Inc., Minneapolis, MN), NS-398 (Cayman Chemical, USA).

2.3. Induction and clinical scoring of EAMG

Female Lewis rats (160–180 g) were randomly divided into 2 groups: CFA or EAMG. The EAMG rats were anesthetized and immunized subcutaneously at the base of the tail with 200 μl inoculums containing 50 μg of R97-116 peptides in 100 μl IFA supplemented with 2 mg *M. tuberculosis* and 100 μl phosphate-buffered saline (PBS) on day 0 followed by a boost on day 30 with the same antigens in incomplete Freund's adjuvant (IFA). The CFA control rats received the same emulsion except PBS was used instead of the peptide.

After the primary immunization, animals were weighed every other day until they were sacrificed (6–8 weeks). The severity of EAMG was scored by measuring muscular weakness