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The Emerging Potentials of Human Regulatory B Cells Mediated Therapies in Myasthenia Gravis.

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ABSTRACT: Regulatory B cells (Bregs) with immunosuppressive function are critical in maintaining immune tolerance. In recent years, Bregs is an essential part of the study due to its therapeutic relevance and function in immune tolerance. The positive and negative regulatory role of human Bregs in immune tolerance is being discussed in several pathologies, including in autoimmune diseases, cancers, chronic infections, strokes in multiple reports. The negative regulatory roles of human Bregs are associated with lesser numbers and functional abnormalities in most of these studies, including myasthenia gravis (MG). In this review, the potential findings regarding human Bregs in MG, and Bregs mediated potential therapeutic strategies with its pros and cons have been discussed based on previous and current reports.

Keywords: Human Bregs; IL-10; IL-35; TGF- ß; myasthenia gravis; Bregs expansion

1 INTRODUCTION

Regulatory B cells, a subset of B cells that predominantly responsible for regulating the immune responses [1]. In addition to their crucial function antigen-presenting and antibody production, B cells can regulate immune responses through the secretion of cytokines. In autoimmune diseases, severe infections, tumors, and transplantation immune conditions activate the body's own immune system, thereby enabling the humoral immune regulatory system resulting in the activation of regulatory B cells, secreting anti-inflammatory cytokines, e.g., interleukin (IL)-10, transforming growth factor (TGF)-β. In healthy individuals, they maintain dynamic homeostasis at a certain level. In the disease state, the body's immune system is destroyed, causing their dynamic homeostasis to be broken, thus playing a role. As of the current state of knowledge, three cytokines have identified that are produced by Bregs, among which IL-10 considered the hallmark cytokine of Breg cells [2-5]. However, TGF-ß and IL-35 have also associated with B cell-mediated immunosuppression.

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TGF-ß is a pleiotropic cytokine that plays a role in a wide range of processes as well as immune regulation [6], whereas IL-35 plays a role in immunosuppression through induction of Treg cell proliferation while suppressing T_H17 responses. IL-35 primarily produced by Treg cells [7]. The importance of human Bregs in the maintenance of immune homeostasis comes from various immune-related pathologies such as cancers, and chronic infections, autoimmune diseases, including myasthenia gravis, which are often associated with abnormalities in Breg numbers or function [8]. Through the understanding of the regulatory role of human Bregs in myasthenia gravis, being able to identify the defect in numbers and function, and initiation of in vitro and in vivo therapeutic interventions targeting Bregs can anticipate a useful therapeutic method for MG. In this review, an overall discussion has been made based on previous and current reports on human Bregs in MG and their potential therapeutic strategies with all its positive and negative impacts.

2 OVERVIEWS OF MG AND BREGS

2.1 An overview of myasthenia gravis

Myasthenia gravis (MG) was first discovered as a distinct clinical entity by T. Willis in his account in Latin in 1672 [9]. However, MG was not recognized until S. Wilks gave the first modern description in 1877 [10], where he described bulbar and peripheral muscular weakness and was struck by the absence of pathology in the central nervous system (CNS). The first full descriptions of MG were described by W. Erb in 1879 and afterward described by W. Goldflam in 1893 [11-12]. The first description of a myasthenic patient with a thymic mass was made by C. Weigert in 1901 [13]. The remarkable discovery of symptomatic treatment of MG was made by M. B. Walker in 1934 [14]. J. A. Simpson made the first suggestion that MG is an autoimmune disease in 1960 [15], and the role of an autoimmune genesis of MG was confirmed as well as established a classification by K. E. Osserman and G. Genkins in 1971 [16] (see Figure 1). Further investigations of MG were done and are being conducted by multiple investigators.

2.2 An overview of regulatory B cells

The idea of regulatory B cells (Bregs) first came into attention during delayed-type hypersensitivity (DTH) responses in guinea pigs in 1974 [17-18]. It took more than 20 years until it came into the researcher's re-attention. In 1996, in a murine autoimmune model of experimental autoimmune encephalitis (EAE), it was noticed that the absence of B cells aggravated the inflammation [19]. In 1997, in chronic colitis, it was observed that the disease worsened in the B-cells deficient group [20]. In 2000, its suppressive ability in inflammatory

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bowel disease was observed and described using the term "regulatory B cell" [21]. In 2002, this subset of B cells was described again as IL-10-producing B cell [2] due to its IL-10 expressibility (see Figure 2). Afterward, many studies have described the role of Bregs in various disease or conditions in both mice [22-28] and humans [29-34], more studies are required for phenotypic identification and characterization in human Bregs. Phenotypic identification of mice Bregs has been well established [3, 30], and human Bregs phenotypic identifications are now defined in studies as well [2, 30, 32-33, 35].

2.3 Regulatory role of human Bregs in MG

Myasthenia gravis is an autoimmune disease caused by a defect of nerve impulses transmission to muscles at the postsynaptic neuromuscular junction (NMJ). Around 85% of patients with MG possess anti-AChR antibodies that block acetylcholine receptors (AChRs) at NMJ, and 38-47% of patients with MG possess muscle-specific kinase (MuSK) autoantibodies [36-39]. B cells produce anti-AChR or MuSK autoantibodies with the aid of T cells and other lymphocytes, thus playing a negative role in MG pathophysiology [39]. B-cell subsets, called regulatory B cells, possess immunosuppressive functions that are linked to anti-inflammatory cytokines production and disease progression in MG patients. Studies have found the inverse correlations between abnormalities in human Bregs number and function with disease progression in patients with MG in vitro [33, 40-43].

3 THERAPEUTIC POTENTIAL OF BREGS IN MG

Most of the available treatments for various immune-related pathologies, including myasthenia gravis are symptomatic. Long-term therapies using these available medications may have a significant level of side effect, maybe ineffective or even may increase life-threatening infections. Thus, cell medicated therapy targeting specific immune cells that drive disease progression is becoming increasingly popular and proven effective in the treatment of cancers [8, 44]. According to the current state of knowledge of the phenotypes and markers of human regulatory B cells, they produce IL-10, IL-35, and TGF- ß [33-34, 45]. However, IL-10 is the only consensus marker for the majority of functional regulatory B cell subsets; therefore, regulatory B cells are well recognized as IL-10 producing B cells [2, 46]. Multiple studies have shown a significant decrease in B10 cells in neuroimmunological disorders, including myasthenia gravis [33, 41, 47-49]. A study found that the treatment of experimental autoimmune myasthenia gravis (EAMG) mice with low-dose granulocyte-macrophage colony-stimulating factor (GM-CSF) increased the proportion of $CD1d^{hi}CD5^{+}$ B cells and B10 cells in vitro.

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In contrast, $CD1d^{hi}CD5^{+}$ B cells inhibited B cell proliferation and its autoantibody production in an IL-10–dependent manner. The study suggested that in vivo or in vitro expansion of $CD1d^{hi}CD5^{+}$ B cells or B10 cells may represent an effective strategy in the treatment of human myasthenia gravis [24]. Another study found that patients with MG had relatively lowered percentages of $CD19^{+}CD5^{+}CD1d^{+}$ Bregs as compared to healthy controls (HCs) and, the production of IL-10 and TGF-β_1 was relatively lesser in patients with MG than HCs, which were linked with more severe of MG disease status. The study suggested that MG Bregs could be cocultured in vitro with particular drug medium and in vitro research to develop B cell-mediated therapies of MG [33]. Thus, in vitro expansion of Bregs; ex vivo expansion or, in vivo manipulation of Bregs; and depletion therapy of Bregs strategies would provide a new window of opportunity to treat MG (see Figure 3).

3.1 In vitro expansion of Bregs

Different combinations of stimuli have been used to quantify Bregs in various diseases [8]. IL-10-producing B cells (Bregs) from patients with myasthenia gravis could be treated with certain medications such as steroids e.g., dexamethasone in vitro culture model. Bregs isolated from myasthenia gravis patient-derived peripheral drug mononuclear cells (PBMCs) using B cell isolation techniques and magnetic separation [34]. As the IL-10-producing B cells, $CD19^{+}CD5^{+}CD1d^{+}$ Bregs had been described to reduce in vitro in patients with myasthenia gravis, after in vitro treatment for a specific duration, in vitro expansion of $CD19^{+}CD5^{+}CD1d^{+}$ Bregs to be observed using flow cytometry, and the induced IL-10 expression, which is the hallmark of regulatory B cells, can be observed using enzyme-linked immunosorbent assay (ELISA) or, cytometric bead array (CBA). The in vitro expansion of MG patient-derived $CD19^{+}CD5^{+}CD1d^{+}$ Bregs that are treated in the drug culture medium can be compared with MG patient-derived $CD19^{+}CD5^{+}CD1d^{+}$ Bregs that are drug-free medium.

3.2 Ex vivo expansion or, in vivo manipulation of Bregs

Studies suggest that the environmental milieu in which B cells differentiate plays a pivotal role in the induction of Bregs, and inflammatory cytokines such as Il-10 play a critical role in the induction of immunosuppressive Bregs. While several studies support the in vivo expansion of Bregs, they also suggest a potential risk of triggering undesirable pro-inflammatory responses [50-53]. Stimulation of IL-10-producing B cells (Bregs) isolated from myasthenia gravis patient-derived PBMCs, followed by adoptive transfer of fluorescence-activated cell sorting (FACS)-sorted Bregs, could reestablish tolerance. The expansion of Bregs by proinflammatory

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cytokines depends on the signals that they receive; thus, the accuracy concentration of the proinflammatory cytokine is crucial for the effector regulatory B cell expansion. Additionally, the identification of Breg differentiation-inducing stimuli provides new opportunities to induce a shift in B cells toward a more regulatory phenotype for the in vivo manipulation of Bregs [8]. A further observation can be made to find out whether modulating signals in vivo can provide a long-term favorable environment for Breg differentiation and whether it improves the function of MG disease status.

3.3 Depletion therapy of Bregs

Several studies found that the use of rituximab has shown some success in the treatment of autoimmune diseases [54]. Depleting of Bregs or effector B cell subsets would provide more advantages over currently used total B cell depletion therapies in the treatment of cancers and other immune disorders. Although there is a lack of surface markers specific for Bregs, several markers have been shown to identify the majority of IL-10–producing Bregs [2, 29, 55]. However, these are not precisely Breg-specific to use in cellular therapy [8]. Thus, further identification of Breg-specific markers could result in the development of depletion therapy of Bregs in patients with myasthenia gravis.

4 POTENTIAL CHALLENGES

Therapies targeted to modify Bregs exhibit great potential in myasthenia gravis treatment, but the identification of effective Breg subsets for cell-mediated therapy might be challenging. The current therapeutic approaches are based on in vivo immunosuppressive Bregs expansion or generation. Most of the studies on Breg generation were so far conducted in mice, among which one study reported that Bregs differentiate into antibody-secreting cells after transient IL-10 production in vivo [8, 56]. Thus, without further detailed studies of human Bregs, it is rather difficult to carry out further cell-mediated therapies on human Bregs. On the other hand, an important consideration should be the quantity of Bregs, which are to be transferred for effective Bregs mediated therapy for myasthenia gravis. The main difficulties in the development of Bregs mediated therapy for myasthenia gravis is due to the unclear human Bregs definition and diversity of mechanism, as the current studies on human Bregs are a handful and less described [8, 57].

5 CONCLUSION

Available evidence indicates that therapeutic interventions targeting Bregs could provide improved approaches for the treatment of myasthenia gravis which can be in the following methods, such as method (1) Bregs phenotypic identification in isolated B cells from MG patient-derived PBMCs and in vitro expansion in drug medium and comparison with non-drug medium; method (2) Once ex vivo expansion of MG Bregs is completed then the adoptive transfer of these expanded cells into the patient could suppress autoimmunity in MG; in vivo modulation to expand Bregs in patients with MG to suppress autoimmunity or disease progress; method (3) in vivo depletion of Bregs as specific subsets of B cells would provide more advantages than current total B cell depletion therapies. Therefore, a constant attempt to understand Breg biology in healthy individuals versus MG can provide new possibilities to establish Breg immunotherapy in patients with MG.

Disclosure of Conflicts of Interests

None.

Author Contributions

MRK: concept development, review plan and performing review, manuscript writing and review of the manuscript.

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FIGURES:

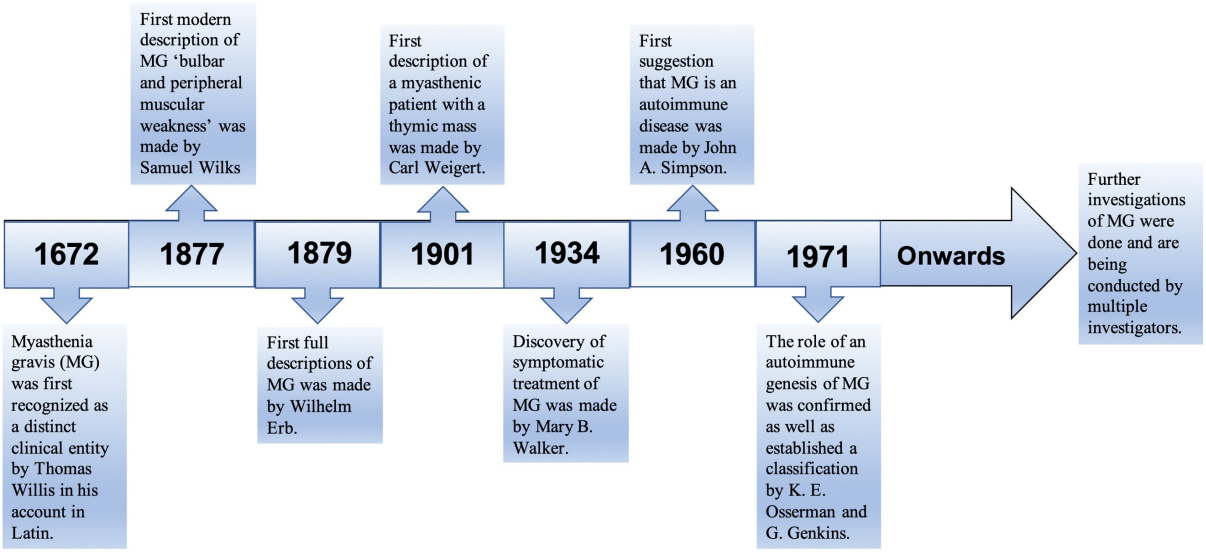

Figure 1: A timeline of the history of myasthenia gravis.

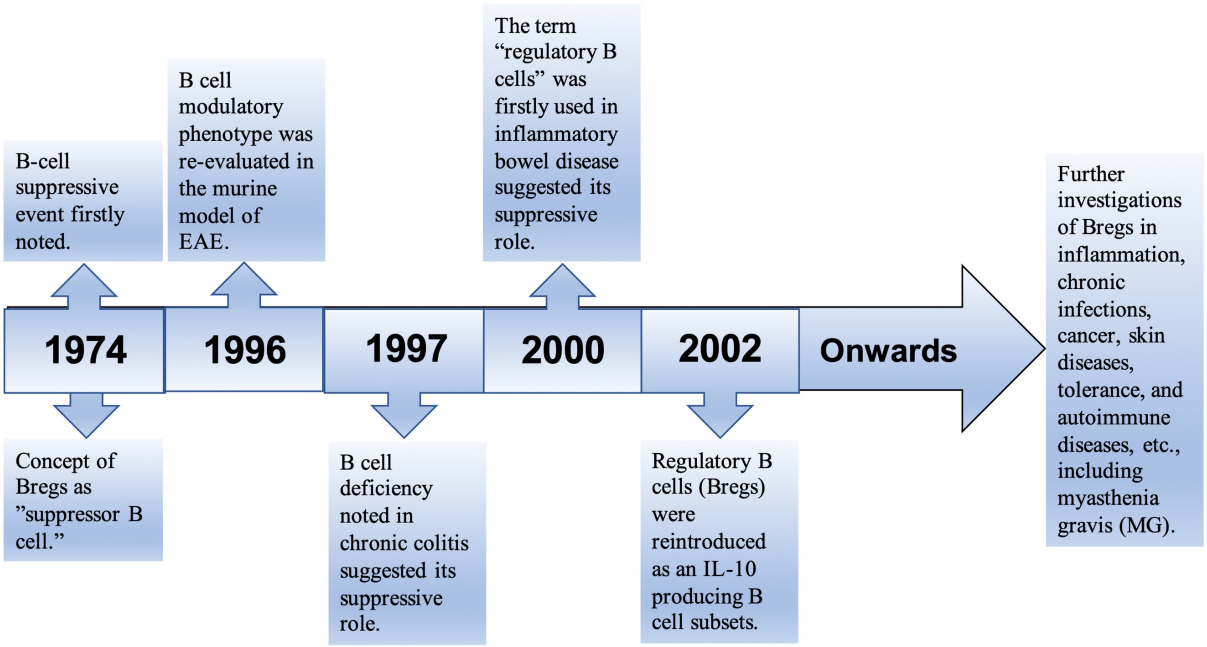

Figure 2: A timeline of the history of regulatory B cells.

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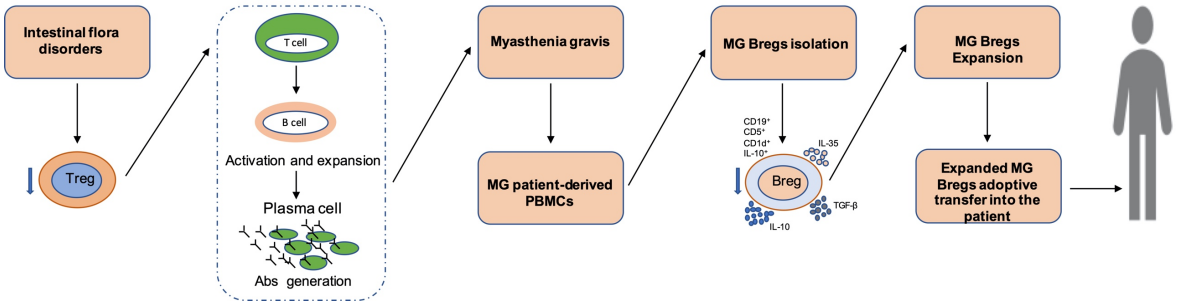

Figure 3: Expansion and adoptive transfer of regulatory B cells of myasthenia gravis.