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Functional defect in regulatory T cells in myasthenia gravis

Muthusamy Thiruppathi[1,2], **Julie Rowin**[1], **Qin Li Jiang**[1], **Jian Rong Sheng**[1,2], **Bellur S. Prabhakar**[2], and **Matthew N. Meriggioli**[1]

[1]Department of Neurology and Rehabilitation, College of Medicine, University of Illinois Hospital and Health Sciences System, Chicago, Illinois

[2]Department of Microbiology and Immunology, College of Medicine, University of Illinois Hospital and Health Sciences System, Chicago, Illinois

Abstract

Forkhead box P3 $(FOXP3)^+$ is a transcription factor necessary for the function of regulatory T cells (T_{reg} cells). T_{reg} cells maintain immune homeostasis and self-tolerance, and play an important role in the prevention of autoimmune disease. Here, we discuss the role of T_{reg} cells in the pathogenesis of myasthenia gravis (MG) and review evidence indicating that a significant defect in T_{reg} cell *in vitro* suppressive function exists in MG patients, without an alteration in circulating frequency. This functional defect is associated with a reduced expression of key functional molecules such as FOXP3 on isolated T_{reg} cells and appears to be more pronounced in immunosuppression-naive MG patients. *In vitro* administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) enhanced the suppressive function of T_{reg} cells and up-regulated FOXP3 expression. These findings indicate a clinically relevant T_{reg} cell–intrinsic defect in immune regulation in MG that may reveal a novel therapeutic target.

Keywords

myasthenia gravis; regulatory T cells; FOXP3; GM-CSF

Introduction

Acquired myasthenia gravis (MG) is an autoimmune disorder of the neuromuscular junction in which patients experience fluctuating skeletal muscle weakness that often affects selected muscle groups preferentially. The target of the autoimmune attack in most cases is the skeletal muscle acetylcholine receptor (AChR), but in others, non-AChR components of the neuromuscular junction, such as the muscle-specific receptor tyrosine kinase, are targeted (reviewed in Ref. 1). The precise origin of the autoimmune response in MG is unknown, but abnormalities of the thymus gland (hyperplasia and neoplasia) almost certainly play a role in patients with anti-AChR antibodies[2,3] and genetic predisposition is also likely to influence which patients develop the disorder.[4–6] High-affinity, anti-AChR antibodies bind to the neuromuscular junction, activate complement and accelerate AChR destruction, while causing failure of neuromuscular transmission and the resulting myasthenic symptoms (reviewed in Ref. 1). However, auto reactive AChR-specific $CD4^+$ T cells, which can be detected in most MG patients,[7–10] likely play an important role in MG, through cognate interactions with B cells leading to and the synthesis of anti-AChR antibodies.[11]

Corresponding author: Matthew N. Meriggioli, MD, Department of Neurology and Rehabilitation, University of Illinois College of Medicine, 912 S. Wood Street, M/C 796, Chicago, IL60612, mmeriggioli@gmail.com.

Conflict of Interest
The authors declare no competing financial interests.

Regulatory T cells and immune tolerance

Immune tolerance to self antigens is initially achieved during thymic development by the clonal deletion of potentially autoreactive T cells. However, some of these pathogenic cells, including some with reactivity to skeletal muscle AChR, survive clonal deletion in normal individuals, and are kept in check by peripheral tolerance mechanisms, most notably by a specialized subset of $CD4^+$ T cells called regulatory T cells (T_{reg} cells).[12] T_{reg} cells are a subpopulation of T cells that act to suppress activation of other immune cells and thereby maintain immune system homeostasis and self-tolerance.[13] Current theories regarding the pathogenesis of autoimmune disease hypothesize that a functional deficiency in T_{reg} cells could result in a failure to suppress autoreactive T cell responses.[14,15]

The thymus gland is the primary source of T_{reg} cells which constitute approximately 5–10% of the peripheral $CD4^+$ T cell population, and play a crucial role in the maintenance of immune homeostasis.[13] T_{reg} cells actively mediate self-tolerance and thus control autoimmunity by suppressing the activation, proliferation and cytokine production of effector autoreactive T cells that arise de novo or escape thymic deletion.[13,16] The transcription factor forkhead box protein P3 (FOXP3) is expressed in $CD4^+$ $CD25^+$ T_{reg} cells and is the master regulator for the development and function of these cells. [17,18] FOXP3 gene transfer has been shown to convert naive $CD4^+$ $CD25^-$ T cells into a functional regulatory lymphocyte population, demonstrating the pivotal role of FOXP3 in T_{reg} cell biology.[18]

A deficiency or dysfunction of T_{reg} cells has been shown to contribute to the pathogenesis and development of many autoimmune diseases.[19–22] Functional defects have been demonstrated in T_{reg} cells isolated from patients with diverse autoimmune diseases, suggesting that in autoimmunity, T_{reg} cell suppressive capacity may be diminished. It has also recently been reported that the source of the apparent T_{reg} cell dysfunction may not necessarily be primarily intrinsic to T_{reg} cells, and that effector T cells may acquire resistance to T_{reg} cell suppression in certain autoimmune diseases. [19, 23–25]

Regulatory T cells and immune regulation in myasthenia gravis

While it is likely that T_{reg} cells exert their effects on T helper cells, thereby regulating B cell function and antibody secretion, there is also evidence that T_{reg} cells may have a direct effect on B cells.[26] Specifically, in MG, it may be hypothesized that dysfunctional T_{reg} cells permit the production of anti-AChR autoantibodies, a hypothesis that remains to be specifically tested. Investigators examining the relative frequencies and function of T_{reg} cells in the peripheral circulation and thymi of MG patients have reported conflicting results including reductions in T_{reg} cell numbers,[27–29] impaired regulatory function,[30] or no defect·[31,32] Aside from measuring T_{reg} cell numbers, and ability to suppress non-specific T cell proliferation, more detailed analyses of T_{reg} cell– intrinsic (T_{reg} phenotype, cytokine production, and suppressive function) versus extrinsic factors (T helper/effector cells, dendritic cells), and ability to suppress specific AChR-induced T proliferation have not been performed. The reported demonstration of a functional impairment of thymic T_{reg} cells in MG patients is an interesting finding and suggests an origin for T_{reg} cell dysfunction in the thymus, possibly owing to thymic pathology (hyperplasia or neoplasia). [33]

Importantly, in all of the studies referenced above, investigators utilized a single-step enrichment protocol in which T_{reg} cells were identified and isolated by high surface expression of CD25. Unfortunately, the level of expression of CD25 that defines T_{reg} cells has been inconsistently reported in the literature, and CD25 is also expressed by recently activated T cells, resulting in the possible inclusion of effector T cells in isolated T_{reg} cell populations.[34] More recent studies have shown that T_{reg} cells express low or absent levels

of the IL-7 receptor α-chain (CD127) and that this expression is inversely correlated with FOXP3 expression and with suppressive function,[35,36] and that it may provide a more precise method for the isolation of a pure T_{reg} cell population.

Impaired suppressive function of isolated MG T_{reg} cells

We have investigated the existence of a functional defect in $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ T_{reg} cells in MG patients by isolating this population of cells using flow-based sorting and co-culturing them with autologous $CD4^+$ $CD25^-$ T responder cells (T_{resp}) at different T_{reg}:T_{resp} ratios, in the presence of irradiated autologous antigen presenting cells (APCs). We performed T cell proliferation studies (CFSE dilution) stimulating the cell cultures with anti-CD3 Abs and analyzed cytokine profiles from cell culture supernatants. We found that the suppressive ability of isolated T_{reg} cells from MG patients (MG T_{reg} cells) was consistently reduced compared to age-matched controls analyzed concurrently.[37] As expected, T_{resp} cells from MG patients produced higher levels of pro-inflammatory cytokines (IL-6 and IFN-γ) and lower levels of IL-10, compared with controls. Furthermore, when isolated $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ cells (T_{reg} cells) were added to the cultures, IL-6 and IL-17 levels actually increased, without altering IL-10 levels in MG patients.[37] This result suggests not only a functional inability of MG T_{reg} cells to suppress the production of pro-inflammatory cytokines, but also the possible production of IL-6 and IL-17 by $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ cells in MG. Along these lines, it has been recently reported that peripheral T_{reg} cells may differentiate into IL-17–producing cells upon T cell receptor stimulation in the presence of IL-6.[38] Thus, enhanced T_{reg} cell plasticity in MG may underlie the observed disturbance in T_{reg} cell suppressive function in MG, through functional conversion of cells with a T_{reg} cell phenotype into pathogenic Th17 cells. This phenomenon may be associated with the level of FOXP3 expression within a T cell (see below).

Dysfunctional MG T_{reg} cells express attenuated FOXP3 levels

The level of expression of FOXP3 plays a critical role in the development and function of T_{reg} cells. A number of factors, some positive, some negative, interact to collectively drive FOXP3 gene expression and then maintain its expression in T_{reg} cells. Reduced FOXP3 expression in circulating $CD4^+$ $CD25^+$ T cells[33,39] and $CD4^+$ $CD25^+$ thymocytes[33] has been observed in MG patients. We isolated $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ T_{reg} cells from peripheral blood mononuclear cells (PBMCs) and found no alteration in the reservoir of T_{reg} cells in the peripheral blood of MG patients compared to controls. We confirmed that these cells were largely (>90%) FOXP3-expressing cells, by flow cytometry–based phenotypic analysis, and were highly suppressive in functional suppressor assays.[37] However, we observed a significant down-regulation of the relative expression of FOXP3 at the protein and mRNA levels in isolated $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ cells in MG patients, compared with healthy controls. [37]

We went on to evaluate the expression of FOXP3-associated proteins including those responsible for key features of Treg function such as cytotoxic T lymphocyte antigen-4 (CTLA-4). We have shown that a significantly lower percentage of isolated T_{reg} cells from MG patients express CTLA-4,[37] consistent with the impairment in suppressive function when these cells are used in suppressive assays. MHC II expression on human T_{reg} cells has been reported to identify a distinct population of T_{reg} cells with high FOXP3 expression and with the capability to mediate strong contact-dependent suppression.[40] HLA-DR expressing T_{reg} cells have also been hypothesized to be involved in homeostatic maintenance of T_{reg} cells *in vivo* via presentation of self- antigens,[40] so that a deficiency in this subset of T_{reg} cells may impact on tolerance to self-antigens like the AChR.

Accordingly, we have found that a reduced number of $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ cells express HLA-DR in MG patients.[37]

It should be pointed out that the demonstrated functional defect in T_{reg} cells that has been reported in MG to date appears to be a global one (non-specific) since there is a failure to normally suppress T cell proliferation in response to non-specific T cell receptor engagement (anti-CD3 antibodies). One may hypothesize that T_{reg} cell suppression of AChR-specific T cells is perturbed in MG, and that restoration of function in this population of T_{reg} cells may be a focused strategy for treatment. The first step to address this possibility would be to investigate the question of whether T_{reg} cells from MG patients have a reduced ability to suppress AChR-reactive T cells. While the number of circulating AChR-specific T cells is obviously low, previous investigators have identified in many patients with MG myasthenogenic AChR peptides that induce T cell proliferation.[41] Based on this work, we synthesized two peptides representing sequences of the human AChR-α subunit (p195–212 and p257–259), in which at least one of the two peptides have been determined to induce proliferation of peripheral blood lymphocytes in approximately 70% of patients with MG.[41] These peptides were used in suppression assay performed as described above, using AChR peptide rather than anti-CD3 stimulation. To assess T_{reg} cell suppression of AChR-stimulated T cell proliferation, previously activated $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ cells were added to the culture. T cell proliferation of 4.2–15% in response to one of the two AChR peptides was observed, none of which were significantly suppressed by the addition of T_{reg} cells.[37] Little or no proliferation in response to the AChR-α peptide was seen in the age-matched healthy control population. These studies indicate that the observed defect in suppressive function of T_{reg} cells in MG extends to AChR-specific T cell responses.

Defects in *in vitro* T_{reg} cell suppression in autoimmune disease may potentially be explained by T_{reg} cell–intrinsic defects, resistance of T_{resp} cells, or the pro-inflammatory properties of antigen presenting cells (APCs).[23–25] Our data showing reduced cellular expression of FOXP3 in MG T_{reg} cells argues for a T_{reg} cell–intrinsic defect. To further test this, we have co-cultured MG T_{reg} cells from individuals with T_{resp} from healthy controls, and have also performed mirror image experiments, determining the degree of *in vitro* suppression of both of polyclonal and AChR-stimulated T cell proliferation. Both polyclonal and AChR-activated T_{resp} cells from MG patients could be effectively suppressed using T_{reg} cells isolated from healthy controls, while polyclonal-activated T_{resp} cells from controls were not effectively suppressed using T_{reg} cells isolated from MG patients, which strongly suggests [37] a primary intrinsic defect in isolated T_{reg} cells in MG.[37]

Imbalanced homeostatic composition of circulating T_{reg} cells in MG

While the existing data suggests that the relative numbers of circulating T_{reg} cells is unchanged in MG patients, the observed T_{reg} cell dysfunction may be linked to an imbalanced homeostatic composition of circulating T_{reg} cell subsets. There are two major types of $CD4^+$ $FOXP3^+$ T_{reg} cells.[42] Natural T_{reg} cells (nT_{reg} cells) constitute a stable subset derived from the thymus and are thought to control reactivity to self-antigens. Induced or adaptive T_{reg} cells (iT_{reg} cells) are less stable, being derived from $CD4^+$ $CD25^-$ T cells in the periphery upon antigen exposure. There is currently no good method for isolating viable nT_{reg} cells for functional studies, since the most reliable method relies on the presence of an epigenetic signature. However, cells recently exiting the thymus, including nT_{reg} cells, may be identified by expression of CD45RA and CD31 (recent thymic emigrants). In addition, important biological differences between naive ($CD45RA^+$) and memory ($CD45RO^+$) $CD4^+$ $CD25^{high}$ T_{reg} cells have been described.[43,44] Differences in T_{reg} cell subsets in patients with autoimmune disease (SLE, T1D) compared to controls have also been reported when CD45RA is used to differentiate T_{reg} cell subsets,[45] suggesting

that alterations in the relative composition of T_{reg} cell subsets may underlie autoimmunity. Therefore, we have analyzed the frequency of circulating recent thymic emigrant ($CD31^+$) and naive ($CD45RA^+$) $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ T_{reg} cells in MG patients. We have found that the prevalence of recent thymic emigrant and naive T_{reg} cells is significantly decreased in MG patients compared to healthy controls. (37)

This finding raises the question of whether T_{reg} cells may exhibit multiple differences from healthy controls that are likely to play roles in the complex pathogenesis of MG, including reduced thymic output of naive T_{reg} cells, impaired naive T_{reg} cell suppression, and distinct impairments in specific subsets of memory T_{reg} cells. The reduced percentage of circulating naive and RTE T_{reg} cells is particularly intriguing given the high incidence of thymic pathology (hyperplasia and neoplasia) in MG. Further studies correlating T_{reg} cell subset phenotype and function with thymic pathology will help to better characterize the relationship of circulating T_{reg} cell alterations and thymic T_{reg} cell development.

Are $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ T_{reg} cells affected by immunotherapy?

Most MG patients in our study were treated with prednisone or other immunosuppressive drugs, and it is conceivable that these drugs may have an effect on T_{reg} cell function. Subset analyses of T_{reg} cell suppressive function and FOXP3 expression in MG patients treated with immunosuppressants (IS) compared to those that were immunsuppression-naive at the time of blood collection, showed that T_{reg} cells from MG patients treated with IS more effectively suppressed T cell proliferation and expressed higher levels of FOXP3 (Fig. 1). In addition, a correlation was also found between manual muscle testing (MMT) scores and T_{reg} cell suppressive function.(37) These findings agree with those of other investigators,(46,47) and suggest that the observed T_{reg} cell defects are clinically relevant and that the beneficial effects of IS therapy are at least partly mediated through enhancement of T_{reg} cell function.

GM-CSF enhances T_{reg} cell function in MG

The expansion of T_{reg} cells for cellular therapy is a common experimental strategy applied to a number of autoimmune conditions.(48,49) Unfortunately, expansion of dysfunctional MG T_{reg} cells is unlikely to provide a therapeutic benefit, and the identification of agents that enhance the function of T_{reg} cells would be a more appropriate strategy. GM-CSF is an important hematopoietic growth factor and immune modulator that has a profound effect on various circulating immune cells.(50) *In vivo* administration of GM-CSF has been shown to prevent or attenuate autoimmunity in a number of mouse models of autoimmune disease by expanding dendritic cells and inducing an expansion of T_{reg} cells.(51–55) Furthermore, we have used the murine experimental model of MG to demonstrate that the therapeutic administration of GM-CSF produces clinical improvement, an expansion of the numbers of splenic $FOXP3^+$ cells, a suppression of AChR-induced T cell proliferation, diminished production of anti-AChR antibodies, and a reduction of the deposition of IgG and complement at the muscle endplate, resulting in preservation of the normal postsynaptic endplate morphology and functional AChRs.(53,54) More importantly, adoptively transferred GM-CSF-induced Tregs potently suppressed ongoing MG in recipient mice, confirming their critical role in GM-CSF's effects.(55)

Based on these preclinical studies, we have investigated the effects of GM-CSF treatment in a case of refractory myasthenic crisis.(56) Clinical improvement in this case was associated with expansion in the circulating numbers of $FOXP3^+$ cells, increase in FOXP3 expression levels in $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ T_{reg} cells, early improvement in T_{reg} cell suppressive capacity for AChR-α–induced T cell proliferation, and subsequent enhancement in T_{reg} cell suppression of polyclonal T cell proliferation.(56) Although the use of multiple

immunosuppressive drugs may have contributed to these findings, the observed effects on T_{reg} cells are consistent with preclinical studies.

We have also performed studies examining the *in vitro* effects of GM-CSF in T cell proliferation/suppression assays from MG patients and healthy controls. Total $CD4^+$ and/or $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ T_{reg} cells depleted $CD4^+$ T cells were cultured with autologus APCs in the presence of anti-CD3 and or anti-CD3 plus GM-CSF. We have found that the addition of GM-CSF to lymphocyte cultures ameliorates the suppressive defect in MG T_{reg} cells (Fig. 2A and 2B), and markedly enhances expression of FOXP3 in MG T_{reg} cells at the mRNA and protein level (Fig. 2C and 2D). This suggests that GM-CSF may be effectively used as an enhancer of T_{reg} cell suppressive function, as has been reported for a number of other agents (i.e., rapamycin, vitamin D analogues).[57,58] Additional studies are needed to confirm these findings, define the requirements for GM- CSF's *in vitro* effects, and also determine its actions on non-T_{reg} cells.

Conclusions

Defective immune regulation is present in patients with MG, characterized by defects in suppressive function and expression of FOXP3 in $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ cells. T_{reg} cell–mediated suppression of both polyclonal and autoantigen (AChR)-specific T cell responses is impaired and may correlate with disease severity, being notably less severe in the subgroup of patients treated with immunosuppressive drugs. This defect primarily resides within the isolated population of $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ cells which display reduced or unstable FOXP3 expression. While it has been generally thought that FOXP3 expression serves as an on-off switch endowing T lymphocytes with suppressive ability, emerging evidence, including our own work showing an association between attenuated FOXP3 expression in T_{reg} cells and human MG, suggests a paradigm in which alterations in FOXP3 expression in lymphocytes may lead to loss of immune tolerance in a dose-dependent manner (Fig. 3). Furthermore, GM-CSF has a profound *in vitro* beneficial effect on T_{reg} cell function as well as FOXP3 expression, and further investigations of the mechanisms responsible for these effects are warranted.

In sum, investigation of the nature and subset localization of the T_{reg} cell defect in MG may reveal critical factors that regulate immune responses in MG and other autoimmune diseases, leading to the identification of novel therapeutic targets.

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References

1. Meriggioli MN, Sanders DB. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. Lancet Neurol. 2009; 8:475–490. [PubMed: 19375665]
2. Berrih S, Morel E, Gaud C, et al. Anti-AChR antibodies, thymic histology, and T cell subsets in myasthenia gravis. Neurology. 1984; 34:66–71. [PubMed: 6228745]
3. Roxanis I, Micklem K, Willcox N. True epithelial hyperplasia in the thymus of early-onset myasthenia gravis: implications for immunopathogenesis. J Neuroimmunol. 2001; 112:163–173. [PubMed: 11108945]

4. Giraud M, Vandiedonck C, Garchon HJ. Genetic factors in autoimmune myasthenia gravis. Ann NY Acad Sci. 2008; 1132:180–192. [PubMed: 18567868]
5. Landoure G, Knight MA, Stanescu H, et al. A candidate gene for autoimmune myasthenia gravis. Neurology. 2012; 79:342–347. [PubMed: 22744667]
6. Maniaol AH, Elsais A, Lorentzen AR, et al. Late onset myasthenia gravis is associated with HLA DRB1*15:01 in the Norwegian population. PLoS One. 2012; 7(5):e36603. Epub 2012 May 9. [PubMed: 22590574]
7. Protti MP, Manfredi AA, Straub C, et al. Immunodominant regions for T helper-cell sensitization on the human nicotinic receptor alpha subunit in myasthenia gravis. Proc Natl Acad Sci USA. 1990; 87:7792–7796. [PubMed: 2145582]
8. Wang ZY, Okita DK, Howard J Jr, et al. T-cell recognition of muscleacetylcholine receptor subunits in generalized and ocular myasthenia gravis. Neurology. 1998; 50:1045–1054. [PubMed: 9566393]
9. Abramsky O, Aharonov A, Webb C, Fuchs S. Cellular immune response to acetylcholine receptor rich-fraction, in patients with myasthenia gravis. Clin Exp Immunol. 1975; 19:11–16. [PubMed: 1239345]
10. Richman DP, Antel JP, Patrick JW, Arnason BGW. Cellular immunity to acetylcholine receptor in myasthenia gravis: relationship to histocompatibility type and antigenic site. Neurology. 1979; 29:291–296. [PubMed: 571972]
11. Hohlfeld R, Kalies I, Kohleisen B, et al. Myasthenia gravis: stimulation of anti-receptor autoantibodies by autoreactive T cell lines. Neurology. 1986; 36:618–621. [PubMed: 3486379]
12. Bouneaud C, Kourilsky P, Buosso P. Impact of negative selection on the T cell repertoire reactive to a self peptide: a large fraction of T cell clones escapes clonal deletion. Immunity. 2000; 13:829–840. [PubMed: 11163198]
13. Workman CJ, Szymczak-Workman AL, Collison LW, et al. The development and function of regulatory T cells. Cell Mol Life Sci. 2009; 66:2603–2622. [PubMed: 19390784]
14. Baecher-Allan C, Hafler DA. Human regulatory T cells and their role in autoimmune disease. Immunol Rev. 2006; 212:203–216. [PubMed: 16903916]
15. Torgerson TR. Regulatory T cells in human autoimmune diseases. Springer Semin Immunopathol. 2006; 28:63–76. [PubMed: 16902772]
16. Sakaguchi S, Miyara M, Costantino CM, et al. FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol. 2010; 10:490–500. [PubMed: 20559327]
17. Fontenot JD, Gavin MA, Rudensky AY. FOXP3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol. 2003; 4:330–336. [PubMed: 12612578]
18. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor FOXP3. Science. 2003; 299:1057–1061. [PubMed: 12522256]
19. Buckner JH. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. Nat Rev Immunol. 2010; 10:849–859. [PubMed: 21107346]
20. Moes N, Rieux-Laucat F, Begue B, et al. Reduced expression of FOXP3 and regulatory T-cell function in severe forms of early-onset autoimmune enteropathy. Gastroenterology. 2010; 139:770–778. [PubMed: 20537998]
21. Valencia XC, Yarboro G, Illei P, et al. Deficient $CD4^{+}CD25^{high}$ T regulatory cell function in patients with active systemic lupus erythematosus. J Immunol. 2007; 178:2579–2588. [PubMed: 17277168]
22. Venken K, Hellings N, Thewissen M, et al. Compromised $CD4^{+}CD25^{high}$ regulatory T-cell function in patients with relapsing-remitting multiple sclerosis is correlated with a reduced frequency of FOXP3-positive cells and reducedFOXP3 expression at the single-cell level. Immunology. 2008; 123:79–89. [PubMed: 17897326]
23. Venigalla RK, Tretter T, Krienke S, et al. Reduced CD4+, CD25− T cell sensitivity to the suppressive function of CD4+, CD25high, CD127−/low regulatory T cells in patients with active systemic lupus erythematosus. Arthritis Rheum. 2008; 58:2120–2130. [PubMed: 18576316]
24. Vargas-Rojas MI, Crispín JC, Richaud-Patin Y, et al. Quantitative and qualitative normal regulatory T cells are not capable of inducing suppression in SLE patients due to T-cell resistance. Lupus. 2008; 17:289–294. [PubMed: 18413409]

25. Walker LS. Regulatory T cells overturned: the effectors fight back. Immunology. 2009; 126:466–474. [PubMed: 19278420]
26. Iikuni N, Lourenco EV, Hahn BH, et al. Regulatory T cells directly suppress B cells in systemic lupus erythematosis. J Immunol. 2009; 183:1518–1522. [PubMed: 19570829]
27. Fattorossi A, Battaglia A, Buzzonetti A, et al. Circulating and thymic CD4+CD25+ T regulatory cells in myasthenia gravis: effect of immunosuppressive treatment. Immunol. 2005; 116:134–141.
28. Li X, Xiao BG, Xi JY, et al. Decrease of Foxp3+ regulatory T cells and elevation of CD19+BAFF-R+ B cells and soluble ICAM-1 in myasthenia gravis. Clin Immunol. 2008; 126:180–188. [PubMed: 18054287]
29. Masuda M, Matsumoto M, Tanaka S, et al. Clinical implication of peripheral CD4+ CD25+ regulatory T cells and Th17 cells in myasthenia gravis patients. J Neuroimmunol. 2010; 225:123–131. [PubMed: 20472307]
30. Zhang Y, Wang H, Chi L, et al. The role of FoxP3+CD4+CD25hi Tregs in the pathogenesis of myasthenia gravis. Immunol Lett. 2009; 122:52–57. [PubMed: 19111574]
31. Huang YM, Pirskanen R, Giscombe R, et al. Circulating CD4+CD25+ and CD4+CD25+T cells in myasthenia gravis and in relation to thymectomy. Scand J Immunol. 2004; 59:408–414. [PubMed: 15049785]
32. Matsui N, Nakane S, Saito F, et al. Undiminished regulatory T cells in the thymus of patients with myasthenia gravis. Neurology. 2010; 74:816–20. [PubMed: 20211905]
33. Balandina A, Lécart S, Dartevelle P, et al. Functional defect of regulatory CD4(+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. Blood. 2005; 105:735–741. [PubMed: 15454488]
34. Lim AY, Price P, Beilharz MW, et al. Cell surface markers of regulatory T cells are not associated with increased forkheadbox p3 expression in blood CD4+ T cells from HIV-infected patients responding to antiretroviral therapy. Immunol Cell Biol. 2006; 84:530–536. [PubMed: 16956389]
35. Seddiki N, Santner-Nanan B, Martinson J, et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med. 2006; 203:1693–1700. [PubMed: 16818676]
36. Liu W, Putnam AL, Xu-Yu Z, Szot GL, et al. CD127 expression inversely correlates with FOXP3 and suppressive function of human CD4+ T reg cells. J Exp Med. 2006; 203:1701–1711. [PubMed: 16818678]
37. Thiruppathi M, Rowin J, Ganesh B, et al. Impaired Regulatory Function in Circulating $CD4^{+}CD25^{high}CD127^{low/-}$ T cells in Patients with Myasthenia Gravis. Clin Immunol. 2012 (In Press). 10.1016/j.clim2012.09.012
38. Voo KS, Wang YH, Santori FR, et al. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. Proc Natl Acad Sci USA. 2009; 106:4793–4798. [PubMed: 19273860]
39. Xu WH, Zhang AM, Ren MS, et al. Changes of Treg-associated molecules on CD4(+)CD25(+) Treg cells in myasthenia gravis and effects of immunosuppressants. J Clin Immunol. 2012; 32:975–983. [PubMed: 22467037]
40. Baecher-Allan C, Wolf E, Hafler DA. MHC class II expression identifies functionally distinct human regulatory T cells. J Immunol. 2006; 176:4622–4631. [PubMed: 16585553]
41. Brocke S, Brautbar C, Steinman L, et al. In vitro proliferative responses and antibody titers specific to human acetylcholine receptor synthetic peptides in patients with myasthenia gravis and relation to HLA class II genes. J Clin Invest. 1988; 82:1894–1900. [PubMed: 2461962]
42. Miyara M, Yoshioka Y, Kitoh A, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity. 2009; 30:899–911. [PubMed: 19464196]
43. Sakaguchi S, Yamaguchi T, Nomura T, et al. Regulatory T cells and immune tolerance. Cell. 2008; 133:775–787. [PubMed: 18510923]
44. Hoffmann P, Eder R, Boeld TJ, et al. Only the CD45RA+ subpopulation of CD4+CD25high T cells gives rise to homogeneous regulatory T-cell lines upon in vitro expansion. Blood. 2006; 108:4260–4267. [PubMed: 16917003]

45. d'Hennezel E, Yurchenko E, Sgouroudis E, et al. Single-cell analysis of the human T regulatory population uncovers functional heterogeneity and instability within FOXP3+ cells. J Immunol. 2011; 186:6788–6797. [PubMed: 21576508]
46. Luther C, Adamopoulou E, Stoeckle C, et al. Prednisolone treatment induces tolerogenic dendritic cells and a regulatory milieu in myasthenia gravis patients. J Immunol. 2009; 183:841–848. [PubMed: 19542375]
47. Kessel A, Ammuri H, Peri R, et al. Intravenous immunoglobulin therapy affects T regulatory cells by increasing their suppressive function. J Immunol. 2007; 179:5571–5575. [PubMed: 17911644]
48. Hoffman P, Eder R, Kunz-Schughart LA, et al. Large-scale in vitro expansion of polyclonal human CD4+CD25high regulatory T cells. Blood. 2004; 104:895–903. [PubMed: 15090447]
49. Earle KE, Tang Q, Zhou X, et al. In vitro expanded human CD4+CD25+ regulatory T cells suppress effector T cell proliferation. Clin Immunol. 2005; 115:3–9. [PubMed: 15870014]
50. Hamilton JA. GM-CSF in inflammation and autoimmunity. Trends Immunol. 2002; 23:403–408. [PubMed: 12133803]
51. Ganesh BB, Cheatem DM, Sheng JR, et al. GMCSF-induced CD11c+CD8a- -dendritic cells facilitate FOXP3+ and IL-10+ regulatory T cell expansion resulting in suppression of autoimmune thyroiditis. Int Immunol. 2009; 21:269–282. [PubMed: 19174473]
52. Gaudreau S, Guindi C, Menard M, et al. Granulocyte-macrophage colony stimulating factor prevents diabetes development in NOD mice by inducing tolerogenic dendritic cells that sustain the suppressive function of regulatory T cells. J Immunol. 2007; 179:3638–3647. [PubMed: 17785799]
53. Sheng JR, Li LC, Ganesh BB, et al. Suppression of experimental autoimmune myasthenia gravis (EAMG) by granulocyte-macrophage colony-stimulating factor (GMCSF) is associated with an expansion of FOXP3+ regulatory T cells. J Immunol. 2006; 177:5296–5306. [PubMed: 17015715]
54. Sheng LC JR, Li BB, Ganesh, et al. Regulatory T cells induced by GM-CSF suppress ongoing experimental myasthenia. Clin Immunol. 2008; 128:172–180. [PubMed: 18502693]
55. Sheng JR, Muthusamy T, Prabhakar BS, et al. GM-CSF-induced regulatory T cells selectively inhibit anti-acetylcholine receptor-specific immune responses in experimental myasthenia gravis. J Neuroimmunol. 2011; 240–241:65–73.
56. Rowin J, Thiruppathi M, Arhebamen E, et al. Granulocyte macrophage colony-stimulating factor treatment of a patient in myasthenic crisis: Effects on regulatory T cells. Muscle Nerve. 2012; 46:449–453. [PubMed: 22907239]
57. Adorini L, Penna G. Dendritic cell tolerogenicity: a key mechanism in immunomodulation by vitamin D receptor agonists. Hum Immunol. 2009; 70:345–352. [PubMed: 19405173]
58. Battaglia M, Stabilini A, Migliavacca B, et al. Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. J Immunol. 2006; 177:8338–8347. [PubMed: 17142730]

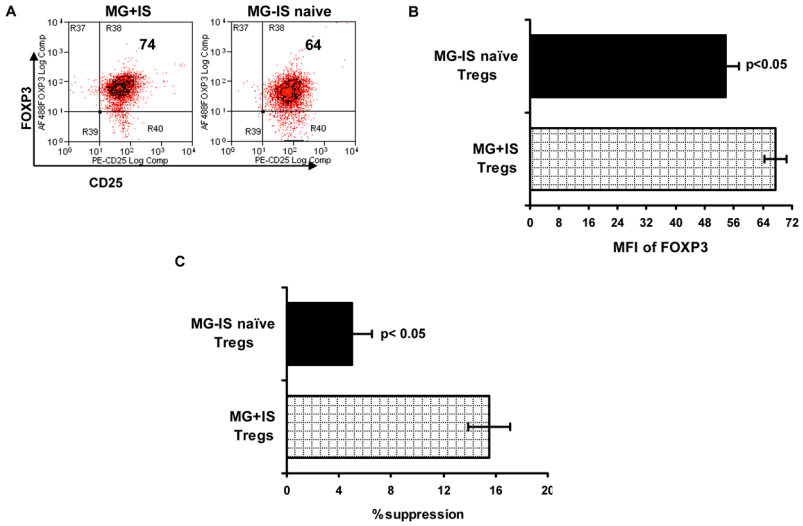

Figure 1.

FOXP3 expression and suppressive function of T_{reg} cells in treated versus untreated MG patients. Intensity of FOXP3 expression (MFI = mean fluorescent intensity) in $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ T_{reg} cells. (A) Representative data for immunosuppressive naive (MG - IS naive) and immunosuppressed (MG + IS) subjects. (B) MFI of FOXP3 expression in isolated $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ cells. (C) Percent suppression of T_{resp} cell proliferation by T_{reg} cells. Data in (B) and (C) represent $n = 5$ for MG-IS naive subjects and $n = 12$ for MG-IS subjects. Results are expressed as mean ± SEM.

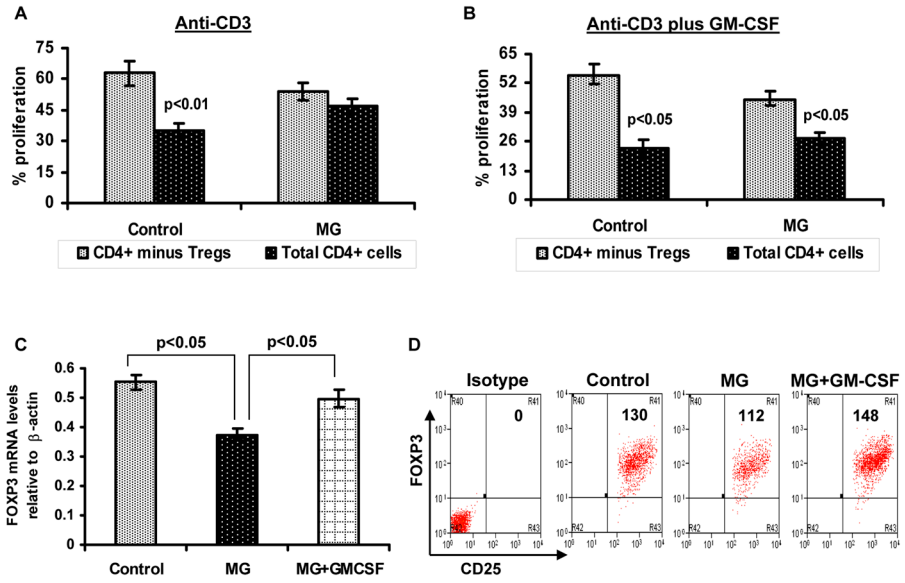

Figure 2.

In vitro GM-CSF treatment to lymphocytes enhances FOXP3 expression and suppressive function of T_{reg} cells in MG. CFSE-labeled total $CD4^+$ T cells and T_{reg} cell–depleted $CD4^+$ T cells were stimulated with human anti-CD3 alone or anti-CD3 plus GM-CSF in the presence of autologus irradiated APCs. Cultures consisting of total $CD4^+$ cells (black bars) and $CD4^+$ cells after removal of $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ cells (grey bars), were compared to evaluate suppressive function of T_{reg} cells. After 5 days of culture, percentage of T cell proliferation was analyzed based on CFSE dilution. The bar diagram represents percentage T_{resp} cell proliferation in response to anti-CD3 alone (A) and anit-CD3 plus GM-CSF (B) for four MG patients and four healthy controls.(C) FOXP3 mRNA expression was determined by multiplex PCR using isolated $CD4^+$ $CD25^{high}$ $CD127^{low/-}T_{reg}$ cells obtained from a control subject, MG patient and an MG patient's PBMCs treated with GM-CSF. Results are expressed as relative FOXP3 mRNA expression. Results are expressed as mean ± SEM. (D) Representative flow cytometry plots illustrate mean florescent intensity of FOXP3 expression within isolated $CD4^+$ $CD25^{high}$ $CD127^{low/-}$ T_{reg} cells.

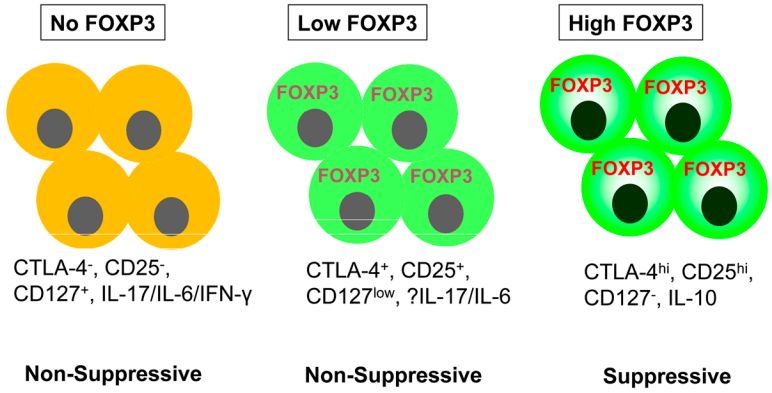

Figure 3.

Hypothetical model linking T_{reg} cell suppressive function to FOXP3 expression level. In this model, high expression of FOXP3 in T cells endows them with enhanced suppressive capacity and a characteristic phenotype. Absence of FOXP3 expression is associated with no suppressive capacity, but reduced FOXP3 expression may result in an intermediate phenotype without suppressive function, and possibly a tendency for enhanced functional plasticity.