Editorial

Imbalance between T follicular helper and T follicular regulatory cells in myasthenia gravis

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Comment on: Wen Y, Yang B, Lu J, *et al*. Imbalance of circulating CD4(+)CXCR5(+)FOXP3(+) Tfr-like cells and CD4(+)CXCR5(+)FOXP3(-) Tfh-like cells in myasthenia gravis. Neurosci Lett 2016;630:176-82.

Received: 05 December 2016; Accepted: 09 January 2017; Published: 21 February 2017.

doi: 10.21037/jxym.2017.02.06

View this article at: http://dx.doi.org/10.21037/jxym.2017.02.06

Introduction

According to the signals they receive from the local environment, T cells can differentiate into one out of several subtypes, including Th1, Th2, Th3, Th9, Th17, T follicular helper (Tfh) and Treg cells (1). Each of these cell subsets possesses a particular function. The term "Tfh cell" first described a subset of CD4+ T cells present in human lymphoid tissues, namely tonsils, and functions primarily to provide help to B cells (2). Interestingly and similarly to B cells, Tfh cells express the homing receptor, CXCR5, whose ligand is the CXCL13, the main B cell chemoattractant chemokine. Within germinal centers (GC), Tfh cells mediate the selection and survival of B cells that differentiate into plasma cells producing high-affinity antibodies against foreign antigens, and memory B cells (3). Recently, a small subset of T follicular regulatory (Tfr) cells was described, that are Treg cells able to suppress specifically the Tfh cell function (4). Thus it is likely that a balance between Tfr and Tfh cells dictates the fate of the B cell immune response.

Myasthenia gravis (MG) is a disease mediated by antibodies. The role of B cells is not questionable (5). The involvement of CXCR5 and its ligand CXCL13 have been scarcely studied. In the thymus of MG patients, there is an increased expression of CXCR5 and its ligand CXCL13, but the involvement of Tfh and Tfr cells was not examined (6). Recently, two studies have shown an imbalance between Tfh and Tfr cells in circulating cells of MG patients, and suggest that it could play a role in the development of the disease (7,8).

This editorial aims to discuss these data in the context of the literature on MG, Tfh, and Tfr cells, and to consider how this knowledge could provide new applications for MG.

Links between Tfh, Treg and Th17 cells

In physiological conditions, naive CD4+ T cells can differentiate towards the different T cell subsets (Th1, Th2, Th9, Th17 and Treg) depending upon the local cytokine milieu. Interestingly, differentially nTregs are not very stable and can also be converted into Th1, Th2, Th17 and Tfh effector cells. That could happen when the cells are in an inflammatory milieu (9).

The conversion of Treg cells into Tfh cells was demonstrated in immune deficient mice, where Tfh were generated from Foxp3+ T cells in gut Peyer's patches. This process associated with the loss of Foxp3 expression shows that the environmental signals in gut Peyer's patches favor the differentiation of Tfh cells (10).

In addition to CXCR5 expressed at the cell membrane, the molecules specifically associated with Tfh function are mainly B-cell lymphoma-6 (Bcl-6) that is the transcription factor driving the differentiation of Tfh cells (4) and IL-21, a cytokine highly produced by Tfh cells, and that is critical for GC formation and Tfh cells (11).

Tfh cells may arise from Th17 cells. It was shown that Tfh and Th17 cells have several similarities, such as the molecular requirements for their generation

including ICOS, c-Maf, IRF4, and STAT3, production of the cytokine IL-21, and the ability to induce B cell differentiation (2,12). Interestingly, T cells with a Tfh cell phenotype can transfer disease. For example, Tfh cells from diabetic animals are highly efficient at transmitting diabetes to recipient animals (13).

However, there is also a minor subpopulation of the Tfh cells that express Foxp3, the transcription factor controlling Treg cells. This small subpopulation of cells, called Tfr cells (Tfr cells) shares features with Tfh cells (Bcl-6, CXCR5, PD1, and ICOS) and T reg cells (GITR, CTLA4, KLRG1, CD25, and Blimp-1), but differs from Tfh cells by the lack of CD40L, IL-4, and IL-21 (4).

Tfr cells play a role in controlling and limiting the magnitude of normal GC responses to avoid the production of abnormally mutated or self-reactive autoimmune-associated antibodies (14). Therefore, Tfr cells have an inhibitory influence during a GC reaction. Indeed, defective Tfr development leads to elevated Tfh cells, enhanced GC responses and increased plasma cell infiltration (14,15). The mechanism of action involves TGF-β and IL-2 that contributes to the generation and stability of Treg cells, and their subsequent development into Tfr cells.

The production of Tfr cells can be promoted by several ways. First, B regs were shown to control the Tfh-cell maturation, expand Tfr cells and inhibit the Tfh-mediated antibody secretion. Thus deficient activities of B cells may impair the Tfh-dependent control of humoral immunity and may lead to the development of aberrant autoimmune responses (16). Second, enforced expression of CXCR5 in Treg cells efficiently induces Tfr cell-like properties (17). Third, in IL-21 knock-out mice, the percentage of Tfr cells was 2-fold higher compared to control mice (18). Conversely, elevated levels of IL-21 selectively enhance Tfh cell differentiation and inhibit Tfr cell commitment, impacting the regulatory function of Tfr cells on Tfh cells and B cells. These data suggest that IL-21 skews the balance from Tfr cells to Tfh cells, thus promoting autoreactive GC reactions (18).

MG, GC and follicular T cells

MG is an autoimmune disease caused by the presence of autoantibodies directed against components of the muscle membrane at the neuromuscular junction. In most cases, autoantibodies against the acetylcholine receptor (AChR) can be found (19). B cells play a significant role in the pathogenic mechanisms, and the autoantibodies represent the key pathogenic factor in MG (5). Also, treatments based on the elimination of the antibodies, and of B cells are efficient. Plasma exchanges produce a short-term clinical improvement in MG which is attributable to the removal of anti-AChR antibody (20). More recently rituximab, a monoclonal antibody against B cells was found very efficient, namely in the form of anti-MuSK antibodies (21).

MG is frequently associated with thymic pathology either a thymic follicular hyperplasia or a thymoma (22). The thymic environment is favorable for the development of GC in females before the age of 50. The degree of follicular hyperplasia is correlated with the level of antibodies to AChR (23) and the B cells in the follicular thymus are numerous and activated (24). Follicular hyperplasia with GC is reminiscent of many autoimmune diseases (25). These structures promote Ag-specific humoral responses during chronic inflammation in the diseased organ. As an example, B cell expansion and lymphoid structures are frequently found in the thyroid in Hashimoto's thyroiditis patients (25).

CXCR5, the unique receptor of CXCL13, was first described in GC, but then a small subset of T cells expressing CXCR5 was also found in the peripheral blood (26). CXCR5 is highly expressed on mature B cells as well as on a subset of T cells (2,27). It has been suggested that circulating CD4+CXCR5+ T cells derive from GCs and are presumably Tfh cells. CXCR5+ T cells are needed for the formation of GCs; they provide help to B cells, and for generating Ag-specific memory and plasma cells (2,27).

The CXCR5/CXCL13 couple has been scarcely investigated in MG disease. In the thymus, using gene chip analysis, one of the most remarkable genes upregulated in MG thymus and downregulated by corticosteroid was CXCL13 (6). Interestingly, the overexpression of CXCL13 was observed in all subgroups of thymuses, and not only in follicular hyperplasia, suggesting that B cell infiltration in the thymus is very common in MG (28). A detailed molecular analysis confirmed that CXCL13 expression was also increased in the sera of glucocorticoid-untreated patients and decreased in response to treatment in correlation with clinical improvement (6). The pathogenic role of CXCL13 was also demonstrated in a novel transgenic (Tg) mouse overexpressing CXCL13 in the thymus. Although the Tg mice did not trigger B-cell recruitment in resting conditions, B cells strongly migrated to the thymus in inflammatory conditions. Also, these Tg mice were more susceptible to EAMG and developed GC-like structures in their thymus (29).

In the peripheral blood of MG patients, higher frequency

of CXCR5+CD4+T cells was observed in untreated patients, in correlation with the disease severity. After therapy, the percentage of CXCR5+CD4+ T cells decreased gradually to the control level (30). Increased level of circulating CXCR5+ CD4+ T cells in MG compared to controls was confirmed in a more recent study (31) that shows a positive correlation between the frequency of these cells and the level of serum anti-AChR Ab in MG patients. The role of CD4+CXCR5+ was also confirmed in an experimental model of MG (EAMG) (32). An increase in CD4+CXCR5+PD-1+ cells was demonstrated in EAMG mice compared with controls, together with increased expression of Bcl-6 and IL-21. Similarly to data obtained in humans, the frequencies of CD4+CXCR5+ cells were positively correlated with the levels of anti-AChR antibodies in the serum. Interestingly, silencing Bcl-6 gene expression ameliorates the severity of EAMG, and leads to decrease in CD4+CXCR5+ cell number, IL-21 expression, as well as anti-AChR antibody levels (32). Altogether, from these studies, it appears that CD4+CXCR5+ cells are involved in MG and EAMG, but these studies did not investigate the expression of FoxP3. Therefore the CXCR5+ cell population explored included a mixture of Tfh and Tfr cells.

Since Tfh cells have a positive while Tfr cells have a regulatory action on B cell help, it is important to differentiate these two subsets. This analysis was done in two recent manuscripts that both describe a reduction in Tfr (expressing CXCR5 and FoxP3) cell number and an increase in Tfh (expressing CXCR5 and not FoxP3) frequency in the blood of untreated MG patients (7,8). Zhang *et al*. show that circulating Tfh cell percentage is increased in generalized MG patients but not in ocular patients compared with controls, while Tfr cell frequency is decreased in both subgroups of MG patients (8). The functional study of these cells shows that Tfh cells from MG patients promote B cells to produce Abs, suggesting that circulating Tfh cells may act on autoreactive B cells and contribute to the development of MG. The levels of IL-6 and IL-21 were significantly higher in MG patients (ocular and generalized) compared to healthy controls, although generalized MG group displayed higher level compared to the ocular group. Besides, Tfh cell frequency and the serum level of IL-21 were correlated with the disease activity of MG patients. A detailed analysis of Tfh cells revealed that the major subsets of Tfh secreting IL-21 were Tfh1 (CXCR3+CCR6–), and Tfh17 (CXCR3–CCR6+), but not Tfh2 (CXCR3-CCR6–), underlining the links between Tfh, Th1, and Th17 that has been previously associated with pathogenic mechanisms of MG (33). Together, these data suggest a positive correlation of Tfh cell frequency with the severity of MG.

In the study by Wen *et al*., an imbalance between circulating Tfr and Tfh cells was shown in untreated MG patients. Besides generalized MG patients exhibited significantly greater Tfh cell frequencies and lower Tfr cell frequencies compared with ocular MG patients, which is suggestive of the role of Tfh in the severity of MG (7). Interestingly and consistently with the results of others demonstrating that combined therapy (thymectomy plus glucocorticoids) can normalize CD4+CXCR5+ T cell frequency in MG patients (30) the authors showed that the patients who received glucocorticoids alone also displayed a normalized frequency of Tfh cells, in addition to attenuated disease activity. These data strengthen the hypothesis of a role of Tfh cells in MG pathogenesis.

These two manuscripts clearly highlight a link between the imbalance of the Tfh/Tfr ratio and disease manifestations in MG patients.

Tfr cells are included in the general Treg cell population (expressing FoxP3) that plays a major role in the control of both the autoimmune and the immune responses (34). Whether the number of Treg cells is altered in MG is still a matter of debate. In the thymuses of MG patients, the number of Treg cells, as defined by the CD25 and Foxp3 markers is unchanged (35,36). In the periphery, several studies did not show any change, whether the cells were evaluated using the CD25 and Foxp3 markers (37) or with the combination of CD25 and CD127 markers (38), while others including the manuscript by Wen *et al*. (7), show a decreased frequency of Treg cells, defined by the CD4+CD25+FOXP3+ phenotype. The explanation for these inconsistencies is still unclear.

Potential clinical applications

In both manuscripts (7,8), the authors show an increased frequency of circulating Tfh cells in untreated MG patients and a close correlation of this increase with disease activity. Interestingly and consistently with the results of others groups, glucocorticoids or combined therapy (thymectomy plus glucocorticoids) can normalize the number of circulating CD4+CXCR5+ T cell frequency in MG patients (30). These results could be compared to the data obtained in the EAMG model. A therapy based on all-trans retinoic acid (ATRA) molecules was shown to be efficient in MG-induced rats. ATRA also alters the Th distribution in MG-induced animals

that show a reduction in Th1/Th17/Tfh cells and an increase in the number of Th2/Treg/Tfr cell types. These results suggest that ATRA reduces EAMG severity by regulating Th cell profiles and by reducing the Tfh/Tfr ratio (39).

In MG patients, it is possible that the reduction in the number of Tfh cells after corticosteroids is linked to the marked reduction in size and number of the CG in the thymus of patients treated with this drug (23). Since glucocorticoids tend to increase Treg cell function (40), it is possible the Tfr cells are increased in the thymus of corticoid-treated patients together with the whole Treg cell subset. Therefore, changes observed in the periphery could reflect the events occurring in the thymus. It would have been interesting to follow Tfh and Tfr cell populations after thymectomy. However, the data of Zhang *et al*. (8) challenges this hypothesis since Tfr cells were increased even in MG patients without abnormalities of the thymus. It is, therefore, possible that there are several sources of CXCR5+ cell production.

To find out if the combination of CXCR5 and FoxP3 markers could be relevant for routine tests to monitor disease activity and potentially preventing a myasthenic crisis, it would have been interesting to measure the progression of these cell populations in individual patients in correlation with clinical data. Furthermore, the use of these markers is difficult to use in routine tests, because the frequency of cells is quite low, less than 15% for Tfh and less 2% for Tfr cells among CD4+ cells. Also, the determination of Tfr cell percentage is made by the expression of FoxP3 and is therefore very dependent on the limit of positivity that could result in some subjectivity, making this determination difficult to be applied in the clinical analysis. A less biased analysis would be required, such as the quantification of the FoxP3 fluorescence intensity among CXCR5 cells or a molecular analysis of the expression of FoxP3 among CXCR5 positive cells. Therefore, whether these markers could serve as potential biomarkers for monitoring disease activity and potentially be a therapeutic target deserves further investigations.

Conclusions

Decreased levels of circulating Tfr cells and reduced Tfr/Tfh ratio are correlated with disease activity in MG patients. The reasons for these alterations deserve further exploration. Because immunological studies are performed in MG patients when the disease is already well established, it remains unclear whether the observed reduced Tfr cell number is a primary causal event or a result of perturbations of the immune system that occur during disease development. Data obtained in experimental MG models show that Tfr cell number is reduced when rats are immunized with AChR in the presence of adjuvant (39). It is therefore very likely that the imbalance observed between Tfh and Tfr in MG patients is the result of immunological processes. As discussed above, several pathways can regulate the Tfh/Tfr ratio, such as the Breg cells or the level of IL-21 that are both under the influence of inflammation. Acting on these intermediate pathways or more generally decreasing the level of inflammation could lead to a better balance between Tfh and Tfr and subsequently to an improvement of the disease.

Acknowledgments

Funding: The work is supported by 7th Framework Programme of the European Union FIGHT-MG (Grant No. 242210), by INSERM, and by Institute of Myology.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Journal of Xiangya Medicine*. The article did not undergo external peer review.

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jxym.2017.02.06). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/jxym.2017.02.06
Cite this article as: Berrih-Aknin S. Imbalance between T follicular helper and T follicular regulatory cells in myasthenia gravis. J Xiangya Med 2017;2:22.

