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Short title: Thymic changes in autoimmune myasthenia gravis

THYMUS INVOLVEMENT IN EARLY-ONSET MYASTHENIA GRAVIS

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Abbreviations:

AChR:	acetylcholine receptor
GC:	germinal center
EBV:	Epstein-Barr virus
IFN:	interferon
MHC:	major histocompatibility complex
MG:	myasthenia gravis
miRNA:	microRNA
Poly(I:C):	polyinosinic-polycytidylic acid
SLO:	Secondary lymphoid organ
TCR:	T-cell receptor
TEC:	thymic epithelial cell
TLO:	tertiary lymphoid organ
TLR:	toll-like receptor
TSA:	tissue-specific antigen

ABSTRACT

It has long been established that the thymus plays a central role in autoimmune myasthenia gravis (MG) either because of thymoma or thymic hyperplasia of lymphoproliferative origin. In this review, we will discuss thymic changes associated with thymic hyperplasia and their implications in the development of an autoimmune response against the acetylcholine receptor (AChR).

The hyperplastic MG thymus displays all the characteristics of tertiary lymphoid organs (TLOs): neoangiogenic processes with high endothelial venule (HEV) and lymphatic vessel development, chemokine overexpression favoring peripheral-cell recruitment, and ectopic GC development. As thymic epithelial cells or myoid cells express AChR, a specific antigen presentation can easily occur within the thymus in presence of recruited peripheral cells, such as B cells and T follicular helper cells.

How the thymus turns into a TLOs is not well-known but local inflammation seems mandatory. Interferon (IFN)- β is overexpressed in MG thymus and could orchestrate thymic changes associated with MG. Knowledge on how IFN- β is induced in MG thymus and why its expression is sustained even long after the disease onset would be of interest in the future to better understand the etiological and physiopathological mechanisms involved in autoimmune MG.

Myasthenia Gravis (MG) with anti-acetylcholine receptor (AChR) antibodies is characterized by muscle weakness and fatigability. The disease generally begins with ocular symptoms (ptosis and/or diplopia) and extends to other muscles in 80% of cases. It is a prototype autoimmune disease in which the target organ, the muscle, is distinct from the effector organ, the thymus. In MG patients with anti-AChR antibodies, functional and morphological abnormalities of the thymus are frequently observed. Patients can display a thymoma, especially after 50 years old, or B-cell infiltrations associated with thymic hyperplasia of lymphoproliferative origin in younger patients and mainly women.¹

In contrast, no thymic abnormalities are observed in MG with muscle specific kinase (MuSK) antibodies. While for MG with LRP4 antibodies, thymic hyperplasia of lymphoproliferative origin has also been observed in a few patients but not further investigated so far.² This review will be mainly focused on thymic changes occurring in early-onset AChR positive MG patients occurring usually before 45-50 years-old. Most of these MG patients present high level of anti-AChR antibodies and thymic follicular hyperplasia. Sex hormones may play a role in this form of the disease, as more than 80% of patients with follicular hyperplasia are women.

The normal thymus

The thymus is a primary lymphoid organ that provides a complex environment essential for T-cell maturation and differentiation during their migration within the cortical and medullary thymic compartments. This is orchestrated thanks to interactions between T cells and mainly thymic epithelial cells (TECs) but also other stromal cells such as dendritic cells, fibroblasts and myoid cells.^{3,4} In the cortex, in their first differentiation steps, immature T-cells become progressively double positive for CD4 and CD8 co-receptors and acquire a complete T-cell receptor (TCR). Further successful differentiation depends on the quality and the specificity of TCR interaction with major histocompatibility complex (MHC) on stromal cells. A large majority of thymocytes are eliminated because the TCR-MHC interaction is too weak (death by neglect) and only a few thymocytes pass successfully positive selection. In contrast, in the medulla, thymocytes are eliminated if TCR-MHC interactions are too strong (negative selection). This is the basis of the central tolerance process based on the ability of TECs to express a repertoire of tissue-specific antigens (TSAs) that are presented to T cells. The expression of these TSAs is monitored by the autoimmune regulator, AIRE, or the transcription factor, FEZ Family Zinc Finger 2 (Fefz2).^{5,6} In this context, TECs are able to express the different

AChR subunits whose expression is controlled by AIRE.^{7,8} Lately, AIRE expression has been demonstrated to be down-regulated by estrogen, explaining the female predisposition to autoimmunity, including MG, as detailed in this special issue by Berrih-Aknin *et al.*⁹ Thymic myoid cells that possess the antigenic characteristics of skeletal muscle cells, also express all AChR subunits and display a functional AChR.^{7,10}

TECs are also involved in the selective induction of natural regulatory T cells.¹¹ Medullary TECs promote the generation of regulatory T cells and favor their functionality.¹² Hassall's corpuscles are also observed in thymic medulla and are formed by concentrically arranged TECs that could correspond to highly differentiated TECs. Their precise function remains unclear.¹³ Altogether, this highlights that the thymus is a complex organ indispensable to set immune central tolerance and thymic dysfunction can lead to autoimmunity.

Thymic abnormalities in early-onset MG

Pathological alterations of the thymus are very often observed in AChR-MG patients with a generalized disease. Thymic hyperplasia of lymphoproliferative origin is observed in 50-60% of these patients, a thymoma is detected in approximately 15% of the patients, and in the other cases, the thymus is atrophic or involuted with mainly adipose tissue and residual areas of thymic parenchyma. As described below, the hyperplastic thymus in MG displays numerous features normally observed in secondary lymphoid organs (SLOs) and due to its inflammatory status, the hyperplastic MG thymus is even considered as a tertiary lymphoid organ (TLO).

Abnormal T cell functionality

In the thymus of MG patients no obvious changes are observed concerning the frequency of CD4 and CD8 T cells that are exported to the periphery.¹⁴ However, other changes have been demonstrated. Natural regulatory T cells that differentiate in the thymus are clearly less functional in the thymus of MG patients and this is also observed to a lesser degree with regulatory T cells in the periphery.^{15,16} Later on, it was demonstrated that the altered immune regulatory function observed for T cells in MG patients was not only linked to the functional defect of regulatory T cells. Indeed, effector T cells from the thymus of MG patients are also resistant to suppression by regulatory T cells and this is probably due to the inflammatory thymic environment.¹⁷ Immunoregulatory defects are thus observed in both regulatory and effector T cells in MG patients. This is associated with changes in the expression of pro-

inflammatory cytokines by MG T cells, such as an IL-17 signature in regulatory T cells, and increases in IFN- γ , IL-21, and tumor necrosis factor (TNF)- α expression in both regulatory and effector T cells.¹⁷ These data suggest that the inflammatory milieu of the MG thymus alters the function and plasticity of CD4 T cells, leading to impaired function of regulatory T cells and resistance of effector T cells to suppression.

Peripheral cell infiltrations leading to germinal center development

B cells can be detected at low levels (around 0.1-0.5% of thymocytes) in normal thymuses and there are located mainly in the medulla and perivascular spaces.¹⁸ Their precise role is not clear but medullary-B cells could be involved in negative selection while perivascular spaces contain mainly plasma cells that could confer a protection against pathogens.^{19,20}

One of the main feature characterizing the thymus in AChR MG is the presence of increased number of B cells often organized in germinal centers (GCs). GCs can sometimes be observed in normal thymuses, increasing with age,^{21,22} but this is particularly a characteristic of AChR-MG thymuses (Figure 1A).²³ The presence of thymic GCs in other autoimmune diseases has not been clearly established. There is a clear association between the thymic follicular development and the age and gender of the patients: 1) the youngest patients display the highest degree (with three or more GCs per thymic section) and the older patients the lowest degree (with less than 2 GCs per thymic section) of follicular hyperplasia, and 2) 80% of patients with thymic hyperplasia are women.¹ Recently, increased number of T follicular helper (Tfh) cells has also been described in the periphery and in the thymus of MG patients.²⁴ Tfh cells are normally located in the GCs of SLOs where they play a central role in B-cell maturation and antibody production. The MG thymus contains all components to set an immune response as in SLO and in particular an immune sensitization against AChR: 1) medullary TECs and myoid cells express AChR,^{10,7} 2) thymic B cells can produce anti-AChR antibodies,^{25,26} and 3) anti-AChR autoreactive T cells are present.²⁷ The thymus of AChR patients has been shown to contain B cells producing anti-AChR antibodies, suggesting a possible expansion of specific B cells.^{28,29} However, the polyclonality of thymic B cells and an overall increased expression of immunoglobulin genes, independent of antigenic specificity, have been demonstrated in MG patients.^{30,31,29} The clear implication of the thymus in MG is also demonstrated using immunodeficient mice that are grafted with thymic biopsies from MG patients. Indeed, almost all the animals display human anti-AChR Abs in their serum, and

50% of them develop MG-like symptoms in correlation with the loss of AChR at the muscle endplates.³²

As described by Truffault *et al.*, AChR MG patients with thymic hyperplasia have higher anti-AChR antibody titers than patients with thymoma or involuted thymuses and a clear correlation exists between the degree of thymic hyperplasia and serum levels of anti-AChR antibodies.¹ Moreover, the number of GCs is reduced in patients undergoing corticosteroid treatment.²³ All these observations support the role of the thymus in the pathogenesis of MG, and thymectomy is often advised for AChR-MG patients. A recent randomized trial in MG patients treated with prednisolone has demonstrated the benefit of thymectomy in improving MG symptoms over a 3-year follow up period.³³

Neoangiogenic processes

The development of thymic hyperplasia is supported by active neoangiogenic processes with high endothelial venule (HEV) and lymphatic endothelial vessel development. HEVs are found in SLOs and chronically inflamed tissues. They are specialized venules bearing on their luminal surface diverse chemokines and expressing high levels of peripheral node addressin carbohydrate, that allow the homing of lymphocytes and dendritic cells.³⁴ By immunohistochemistry, only a few HEVs are detected in the thymus of non-MG adults.³⁵ In contrast, in the thymus of MG patients, increased numbers of HEVs are observed around GCs (Figure 1B) and in correlation with the degree of thymic hyperplasia. Such high numbers of HEVs in hyperplastic thymuses suggest that peripheral cells enter the MG thymus through these specialized vessels. The number of thymic HEVs is reduced in patients undergoing corticosteroid treatment.³⁵

The increased expression of lymphatic markers, such as vascular endothelial growth factor receptor 3 (VEGFR3) and PROX1, has been demonstrated in hyperplastic thymuses also suggesting the expansion of the lymphatic system. Afterward lymphatic endothelial vessels expressing specifically CCL21 have been described in hyperplastic MG thymuses, as detailed below (Figure 1C).³⁶ Lymphangiogenesis occurs throughout life in homeostasis and diseases. It has been described in lymph nodes after immunization, where it was shown to be dependent on the entry of B cells.³⁷

Efficient cell recruitment via HEVs or lymphatic endothelial vessels is a multistep process and chemokines displayed on vessels are involved in the transmigration of circulating cells.³⁸

Chemokine overexpression

Chemokines play a central role in thymopoiesis through their chemotactic and chemorepulsive properties, allowing for the recruitment of pro-thymocytes, the migration of thymocytes from the cortex to the medullary region, and their export to the periphery. Chemokines are also crucial for peripheral cell recruitment in SLOs.³⁹ A transcriptome study has demonstrated that thymic chemokine expression profiles differ in MG patients in association with increased chemotactic properties of hyperplastic thymic extracts.³¹ As described below, several chemokines are increased or abnormally expressed in the MG thymus as detailed in table 1^{23,31,36,35,41,42} Altogether, these data show that chemokine profiles are strongly modified in the MG thymus. They are overexpressed in different cells and probably play a central role in peripheral cell recruitment in the MG thymus and the development of ectopic GCs.

- Expression of CXCL12 and CCL17 by ectopic high endothelial venules

Under physiological conditions, diverse chemokines are displayed on HEVs in SLOs and are involved in the transmigration of circulating cells across HEVs.³⁸ The expression of several chemokines on thymic HEVs has been investigated by Weiss *et al.* Chemokines investigated were known to be expressed by HEVs in SLOs or chronically inflamed tissues, and also known to be dysregulated in the MG thymus at that time : CCL19, CCL21, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13 and RANTES/CCL5.³⁵ Among these chemokines, only CXCL12 was found expressed on the lumen side of thymic HEVs. Moreover, antigen presenting cells such as monocytes/macrophages, dendritic cells, and B cells expressing CXCL12 receptor CXCR4 are detected inside and around thymic HEVs. Since, CCL17 has also been described on thymic HEVs and could favor the recruitment of CCR4 positive dendritic cells.⁴²

- Abnormal expression of CCL21 on lymphatic endothelial vessels

In the periphery, CCL21 is known to play a central role in immune surveillance and defense by controlling the circulation of T cells and dendritic cells within lymphoid and peripheral organs. CCL21 is also involved in naïve-B cell recruitment.³⁶ In the thymus, CCL21 but also CCL19, both interacting with the receptor CCR7, play an important role in thymopoiesis.⁴³ In MG patients, thymic hyperplasia is specifically associated with the thymic overexpression of CCL21 and CCL19. The overexpression of CCL21 in hyperplastic MG thymuses is due to lymphatic endothelial vessels (Figure 1C). Thymic overexpression of CCL21 in MG could thus play a role

in bringing naive B cells, but maybe also peripheral dendritic cells and T cells, in contact with the inflammatory environment characteristic of MG thymus, where they can be sensitized against AChR.^{44,36}

- Overexpression of CXCL13 by thymic epithelial cells

CXCL13 is the most potent chemoattractant for B cells. CXCL13 interact with cells through its receptor, CXCR5 that is also expressed on Tfh cells. In SLOs, CXCL13 participates in GC formation and it is also overexpressed at inflammatory sites characterized by ectopic GC development.⁴⁵ CXCL13 mRNA is only expressed at very low level in normal thymuses. However, in AChR MG patients, thymic CXCL13 expression is strongly increased. Even if CXCL13 is known to be produced by GCs, medullary TECs in MG patients also expressed abnormal levels of CXCL13.²³ The active recruitment of peripheral B cells but also Tfh cells via CXCL13 in MG thymuses could support the development of ectopic GCs.

- Inflammation is mandatory to reveal CXCL13 properties

As CXCL13 is overexpressed by medullary TECs in MG patients,²³ transgenic mice overexpressing CXCL13 under the control of the keratin 5 promoter were developed. The objective was to mimic thymic overexpression of CXCL13 by medullary TECs as in the MG thymus. Data demonstrate that transgenic K5-CXCL13 mice overexpress CXCL13 in their thymus but this does not induce the recruitment of B cells. However, in inflammatory conditions, induced by the injections of a molecule mimicking dsRNA from viral infection (polyinosinic-polycytidylic acid (Poly(I:C)) or the immunization with a strong adjuvant, the recruitment of B cells is detected in the thymus.⁴⁶ The classical animal mouse model of MG is induced by immunizing animals with purified AChR extracted from the electric organ of torpedo fish (T-AChR) together with complete Freund's adjuvant. If the animals produce antibodies that induce loss of AChR at the muscle endplate and dysfunction of the neuromuscular transmission, this model does not present thymic abnormalities.⁴⁷ Using the K5-CXCL13 mice, it was demonstrated that mice are more susceptible to experimental autoimmune MG with stronger clinical signs, higher titers of anti-AChR antibodies, increased thymic B cells, and the development of GC-like structures in the thymus.⁴⁶

Altogether, these data suggest that thymic inflammation is mandatory to reveal the chemotactic properties of CXCL13. Inflammation subsequent to pathogen infection appears to be a key event to optimize the recruitment of mature lymphocytes to peripheral organs and

even in the thymus.^{48,49} Interferon (IFN)-type I that is released during pathogen infection could favor cell motility.⁵⁰

Pathogen infection signature associated with toll-like receptor expression

Pathogens are major environmental-factor candidates for driving/perpetuating autoimmunity. However, since the autoimmunity onset can occur well after a possible triggering infection when the pathogen might have been cleared or the antiviral immune responses might have subsided, it is difficult to link infections with autoimmune diseases.

Nevertheless, the presence of poliovirus-infected macrophages and of Epstein-Barr virus (EBV)-infected B cells has been shown in MG thymus.^{51,52} In MG, striking evidence of chronic inflammation and emerging data on persistent viral infections in the thymus of MG patients strongly supports the hypothesis that the innate immune system may promote, exacerbate, and/or maintain the autoimmune condition.

Toll-like receptors (TLRs) play a major role in innate immunity. TLR1-10 recognize specific microbial derived molecular structures.⁵³ The expression level of TLRs in the thymus of MG patients has been analyzed in different studies reviewed in Robinet *et al.*⁵⁴ Briefly, TLR1 to TLR9 are all expressed in control thymuses. In MG thymuses, the overexpression is described for TLR3, TLR4, TLR6, TLR7, TLR8 and TLR9 and correlations between CD19 (a B-cell marker) mRNA expression and TLR6, TLR8 and TLR9 mRNA expression is demonstrated.⁵⁴

To investigate the potential consequences of pathogen infection on the thymus, the effects of TLR agonists have been analyzed *in vitro* on human cultured TECs and *in vivo* on mouse thymus. On human TECs, among all TLR agonists, Poly(I:C), a synthetic analog of dsRNA mimicking viral infections, specifically induces the thymic overexpression of α -AChR, but not other AChR subunits or TSAs. This induction is mediated by the release of IFN- β and is completely inhibited by IFN-I receptor or IFN- β blocking antibodies.⁵⁵ In C57Bl/6 mice, injections of Poly(I:C) for one week also trigger the specific thymic expression of α -AChR. Poly(I:C) injections also induce other thymic changes, such as the overexpression of IFN-I subtypes, CXCL13 and CCL21, associated with an increased recruitment of thymic B cells. All these thymic changes disappear after one week but prolonged Poly(I:C) injections over 6-8 weeks induce the development of an anti-AChR response in the periphery with the proliferation of autoreactive B cells against AChR in lymph nodes and the production of anti-

AChR antibodies. The presence of circulating anti-AChR antibodies leads to MG symptoms with a loss of AChR on muscle diaphragm and muscle weakness.⁵⁵

In accordance with these observations on Poly(I:C) effects, dsRNA-signaling pathways are activated in MG thymuses.⁵⁵ This could be related to the thymic EBV signature observed in MG as EBV encodes small RNAs that trigger TLR3 signaling and induce IFN-I and pro-inflammatory cytokine expression, similarly to Poly(I:C).^{52,56}

Central role of interferon- β

Inflammation constitutes an essential component of the innate immune response induced by pathogens that stimulate the release of pro-inflammatory molecules. IFN-I are secreted by various cells as an anti-viral defense mechanism and depending on the context can either be considered as anti-inflammatory, as in multiple sclerosis or pro-inflammatory as in systemic lupus erythematosus.^{57,58,59} The implication of IFN-I in MG has long been suggested in various ways: 1) clinical reports demonstrate the development of MG after IFN- α - or IFN- β therapies;⁶⁰ 2) antibodies against IFN- α are found in some MG patients, mainly those with thymoma;⁶¹ and 3) IFN- β is overexpressed in MG thymuses and together with numerous IFN-I-induced genes, as detailed by Poëa-Guyon *et al.*^{55, 62,63}

Analyzing the *in vitro* effect of IFN- β in detail, it is showed that IFN-I and more particularly IFN- β induce the specific expression of α -AChR by TECs and not that of other TSAs. It also increases TEC death and the uptake by dendritic cells of TEC proteins, potentially the α -AChR that is overexpressed in IFN- β -treated TECs. In parallel, IFN- β triggers the expression of CXCL13 and CCL21 by TECs and lymphatic endothelial cells, respectively, and consequently, could favor peripheral cell recruitment in the thymus. At last, IFN- β also induces the expression of B-cell activating factor (BAFF), which favors B-cell survival, and that is overexpressed by TECs in MG thymus.⁶⁴ Similar changes are observed *in vivo*, as the injections of Poly(I:C) to C57BL/6 mice trigger a thymic overexpression of IFN- β and IFN- α 2 associated with increased expressions of CXCL13, CCL21, and BAFF, and favor the recruitment of B cells. These changes are not observed in the thymus of IFN-I receptor knockout mice injected with Poly(I:C).⁶⁴

Altogether, these observations demonstrate that IFN- β orchestrates thymic events that could lead to MG by triggering the overexpression of α -AChR, probably inducing thymic dendritic cell auto-sensitization, the abnormal recruitment of peripheral cells, and GC formation. IFN- β

can also modulate the expression of cytokines involved in the balance between regulatory T cells and proinflammatory Th17 cells (Villegas *et al.*, unpublished data). All these observations are summarized on the scheme (Figure 2). It was also demonstrated that IFN-I subtypes might play a central role in thymoma-associated MG. Huge increases of IFN-I subtypes are observed in thymoma-associated MG but not in thymomas without MG or in control thymuses. These results reinforce a specific role of IFN-I in the anti-AChR response associated with MG.⁶⁵

In MG patients, the fact that IFN- β is overexpressed even long after the disease onset suggests a persistent induction due to the presence of a pathogen agent, as discussed above with EBV, or an altered retrocontrol mechanism to repress IFN-I signaling that could affect: downregulation of IFN-I receptor from the cell surface, dephosphorylation of signaling components of the IFN-I pathway, induction of negative regulators, and induction of microRNAs (miRNAs)...

Dysregulated expression of miRNAs

miRNAs are small RNAs that are post-transcriptional regulators of gene expression. They interact specifically with mRNAs leading to their degradation or to the inhibition of their translation and consequently decreasing protein expression. Recent studies have investigated and identified circulating miRNAs as readily accessible blood biomarkers for MG patients^{66,67,68}. The differential expression of some miRNAs is also observed in peripheral blood mononuclear cells from MG patients.^{69,70}

As miRNAs play an important role in the regulation of inflammation and autoimmunity, we can envisage that altered miRNA expression could be involved in thymic changes associated with MG (Cron *et al.*, unpublished data). Besides, as miRNAs are known to be expressed in the thymus and carried out in exosomes to improve cellular communications,⁷¹ it would be interesting to investigate the modifications of thymic miRNA expression in a myasthenic context. Environmental factors such as infections are able to modify the expression of specific miRNAs and eventually alter thymic function.⁷² Moreover, miR-29a is an important regulator of the IFN-I signaling pathway by targeting IFN-I receptor in TECs and by reducing cell sensitivity to IFN-I and consequently pathogen infections.⁷³ miR-205 has also been demonstrated to be important in maintaining thymopoiesis upon inflammatory perturbations.⁷⁴ All these studies highlight the role of miRNAs in thymus homeostasis in mice

and suggest that altered miRNA expression in the human thymus could be observed in MG patients.

Conclusion

Chronically inflamed tissues can turn into TLOs that possess numerous specific characteristics of SLOs, such as the development of a vascular system, the infiltration of leukocytes, the presence of GCs, sustained by the overexpression of chemokines and inflammatory cytokines. The hyperplastic MG thymus displays all the characteristics of TLOs. Recent data suggest that IFN- β could initiate and orchestrate these thymic changes and the intrathymic autoimmune response to AChR. Future investigations are needed to decipher the upstream events, what is triggering thymic inflammation and why this inflammation is sustained over time.

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Conflict of interest

The authors have declared that no conflict of interest exists.

References

1. Truffault, F., V. de Montpreville, B. Eymard, *et al.* 2017. Thymic Germinal Centers and Corticosteroids in Myasthenia Gravis: an Immunopathological Study in 1035 Cases and a Critical Review. *Clin. Rev. Allergy Immunol.* 52: 108-124.
2. Zisimopoulou, P., P. Evangelakou, J. Tzartos, *et al.* 2013. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. *J. Autoimmun.*
3. Kondo, K., K. Takada & Y. Takahama. 2017. Antigen processing and presentation in the thymus: implications for T cell repertoire selection. *Curr. Opin. Immunol.* 46: 53-57.
4. Le Panse, R. & S. Berrih-Aknin. 2005. Thymic myoid cells protect thymocytes from apoptosis and modulate their differentiation: implication of the ERK and Akt signaling pathways. *Cell Death Differ.* 12: 463-472.
5. Anderson, M.S., E.S. Venanzi, L. Klein, *et al.* 2002. Projection of an immunological self shadow within the thymus by the aire protein. *Science.* 298: 1395-1401.
6. Takaba, H., Y. Morishita, Y. Tomofuji, *et al.* 2015. Fezf2 Orchestrates a Thymic Program of Self-Antigen Expression for Immune Tolerance. *Cell.* 163: 975-987.
7. Mesnard-Rouiller, L., J. Bismuth, A. Wakkach, *et al.* 2004. Thymic myoid cells express high levels of muscle genes. *J. Neuroimmunol.* 148: 97-105.
8. Giraud, M., R. Taubert, C. Vandiedonck, *et al.* 2007. An IRF8-binding promoter variant and AIRE control CHRNA1 promiscuous expression in thymus. *Nature.* 448: 934-937.
9. Dragin, N., J. Bismuth, G. Cizeron-Clairac, *et al.* 2016. Estrogen-mediated downregulation of AIRE influences sexual dimorphism in autoimmune diseases. *J. Clin. Invest.* 126: 1525-1537.
10. Wakkach, A., S. Poeta, E. Chastre, *et al.* 1999. Establishment of a human thymic myoid cell line. Phenotypic and functional characteristics. *Am. J. Pathol.* 155: 1229-1240.
11. Cowan, J.E., S.M. Parnell, K. Nakamura, *et al.* 2013. The thymic medulla is required for Foxp3+ regulatory but not conventional CD4+ thymocyte development. *J. Exp. Med.* 210: 675-681.
12. Nazzari, D., A. Gradolatto, F. Truffault, *et al.* 2014. Human thymus medullary epithelial cells promote regulatory T-cell generation by stimulating interleukin-2 production via ICOS ligand. *Cell death & disease.* 5: e1420.

13. Watanabe, N., Y.H. Wang, H.K. Lee, *et al.* 2005. Hassall's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. *Nature*. 436: 1181-1185.
14. Berrih-Aknin, S., E. Morel, F. Raimond, *et al.* 1987. The role of the thymus in myasthenia gravis: immunohistological and immunological studies in 115 cases. *Ann. N. Y. Acad. Sci.* 505: 50-70.
15. Balandina, A., S. Lecart, P. Dartevelle, *et al.* 2005. Functional defect of regulatory CD4(+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. *Blood*. 105: 735-741.
16. Thiruppathi, M., J. Rowin, B. Ganesh, *et al.* 2012. Impaired regulatory function in circulating CD4(+)CD25(high)CD127(low/-) T cells in patients with myasthenia gravis. *Clin. Immunol.* 145: 209-223.
17. Gradolatto, A., D. Nazzal, F. Truffault, *et al.* 2014. Both Treg cells and Tconv cells are defective in the Myasthenia gravis thymus: roles of IL-17 and TNF-alpha. *J. Autoimmun.* 52: 53-63.
18. Isaacson, P.G., A.J. Norton & B.J. Addis. 1987. The human thymus contains a novel population of B lymphocytes. *Lancet*. 2: 1488-1491.
19. Yamano, T., J. Nedjic, M. Hinterberger, *et al.* 2015. Thymic B Cells Are Licensed to Present Self Antigens for Central T Cell Tolerance Induction. *Immunity*. 42: 1048-1061.
20. Nunez, S., C. Moore, B. Gao, *et al.* 2016. The human thymus perivascular space is a functional niche for viral-specific plasma cells. *Science immunology*. 1.
21. Bradfield, J.W. 1973. Altered venules in the stimulated human thymus as evidence of lymphocyte recirculation. *Clin. Exp. Immunol.* 13: 243-252.
22. Flores, K.G., J. Li & L.P. Hale. 2001. B cells in epithelial and perivascular compartments of human adult thymus. *Hum. Pathol.* 32: 926-934.
23. Méraouna, A., G. Cizeron-Clairac, R. Le Panse, *et al.* 2006. The chemokine CXCL13 is a key molecule in autoimmune Myasthenia Gravis. *Blood*. 108: 432-440.
24. Zhang, X., S. Liu, T. Chang, *et al.* 2016. Intrathymic Tfh/B Cells Interaction Leads to Ectopic GCs Formation and Anti-AChR Antibody Production: Central Role in Triggering MG Occurrence. *Mol. Neurobiol.* 53: 120-131.
25. Safar, D., S. Berrih-Aknin & E. Morel. 1987. In vitro anti-acetylcholine receptor antibody synthesis by myasthenia gravis patient lymphocytes: correlations with thymic histology and thymic epithelial-cell interactions. *J. Clin. Immunol.* 7: 225-234.

26. Leprince, C., S. Cohen-Kaminsky, S. Berrih-Aknin, *et al.* 1990. Thymic B cells from myasthenia gravis patients are activated B cells. Phenotypic and functional analysis. *J. Immunol.* 145: 2115-2122.
27. Melms, A., B.C. Schalke, T. Kirchner, *et al.* 1988. Thymus in myasthenia gravis. Isolation of T-lymphocyte lines specific for the nicotinic acetylcholine receptor from thymuses of myasthenic patients. *J. Clin. Invest.* 81: 902-908.
28. McLachlan, S.M., L.V. Nicholson, G. Venables, *et al.* 1981. Acetylcholine receptor antibody synthesis in lymphocyte cultures. *J. Clin. Lab. Immunol.* 5: 137-142.
29. Vrolix, K., J. Fraussen, M. Losen, *et al.* 2014. Clonal heterogeneity of thymic B cells from early-onset myasthenia gravis patients with antibodies against the acetylcholine receptor. *J. Autoimmun.* 52: 101-112.
30. Guigou, V., D. Emilie, S. Berrih-Aknin, *et al.* 1991. Individual germinal centres of myasthenia gravis human thymuses contain polyclonal activated B cells that express all the Vh and Vk families. *Clin. Exp. Immunol.* 83: 262-266.
31. Le Panse, R., G. Cizeron-Clairac, J. Bismuth, *et al.* 2006. Microarrays reveal distinct gene signatures in the thymus of seropositive and seronegative myasthenia gravis patients and the role of CC chemokine ligand 21 in thymic hyperplasia. *J. Immunol.* 177: 7868-7879.
32. Sudres, M., M. Maurer, M. Robinet, *et al.* 2017. Preconditioned mesenchymal stem cells treat myasthenia gravis in a humanized preclinical model. *JCI insight.* 2: e89665.
33. Wolfe, G.I., H.J. Kaminski, I.B. Aban, *et al.* 2016. Randomized Trial of Thymectomy in Myasthenia Gravis. *N. Engl. J. Med.* 375: 511-522.
34. von Andrian, U.H. & T.R. Mempel. 2003. Homing and cellular traffic in lymph nodes. *Nat. Rev. Immunol.* 3: 867-878.
35. Weiss, J.M., P. Cufi, J. Bismuth, *et al.* 2013. SDF-1/CXCL12 recruits B cells and antigen-presenting cells to the thymus of autoimmune myasthenia gravis patients. *Immunobiology.* 218: 373-381.
36. Berrih-Aknin, S., N. Ruhlmann, J. Bismuth, *et al.* 2009. CCL21 overexpressed on lymphatic vessels drives thymic hyperplasia in myasthenia. *Ann. Neurol.* 66: 521-531.
37. Angeli, V., F. Ginhoux, J. Llodra, *et al.* 2006. B cell-driven lymphangiogenesis in inflamed lymph nodes enhances dendritic cell mobilization. *Immunity.* 24: 203-215.
38. Miyasaka, M. & T. Tanaka. 2004. Lymphocyte trafficking across high endothelial venules: dogmas and enigmas. *Nat Rev Immunol.* 4: 360-370.

39. Cyster, J.G. 1999. Chemokines and cell migration in secondary lymphoid organs. *Science*. 286: 2098-2102.
40. Feferman, T., P.K. Maiti, S. Berrih-Aknin, *et al.* 2005. Overexpression of IFN-induced protein 10 and its receptor CXCR3 in myasthenia gravis. *J. Immunol.* 174: 5324-5331.
41. Colombara, M., V. Antonini, A.P. Riviera, *et al.* 2005. Constitutive activation of p38 and ERK1/2 MAPKs in epithelial cells of myasthenic thymus leads to IL-6 and RANTES overexpression: effects on survival and migration of peripheral T and B cells. *J. Immunol.* 175: 7021-7028.
42. Cordiglieri, C., R. Marolda, S. Franzi, *et al.* 2014. Innate immunity in myasthenia gravis thymus: pathogenic effects of Toll-like receptor 4 signaling on autoimmunity. *J. Autoimmun.* 52: 74-89.
43. Annunziato, F., P. Romagnani, L. Cosmi, *et al.* 2001. Chemokines and lymphopoiesis in human thymus. *Trends Immunol.* 22: 277-281.
44. Nagane, Y., K. Utsugisawa, D. Obara, *et al.* 2003. Dendritic cells in hyperplastic thymuses from patients with myasthenia gravis. *Muscle Nerve.* 27: 582-589.
45. Legler, D.F., M. Loetscher, R.S. Roos, *et al.* 1998. B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. *J. Exp. Med.* 187: 655-660.
46. Weiss, J.M., M. Robinet, R. Aricha, *et al.* 2016. Novel CXCL13 transgenic mouse: inflammation drives pathogenic effect of CXCL13 in experimental myasthenia gravis. *Oncotarget.* 7: 7550-7562.
47. Meinl, E., W.E. Klinkert & H. Wekerle. 1991. The thymus in myasthenia gravis. Changes typical for the human disease are absent in experimental autoimmune myasthenia gravis of the Lewis rat. *Am. J. Pathol.* 139: 995-1008.
48. Katzman, S.D. & D.J. Fowell. 2008. Pathogen-imposed skewing of mouse chemokine and cytokine expression at the infected tissue site. *J. Clin. Invest.* 118: 801-811.
49. Hodge, D.L., D. Reynolds, F.M. Cerban, *et al.* 2012. MCP-1/CCR2 interactions direct migration of peripheral B and T lymphocytes to the thymus during acute infectious/inflammatory processes. *Eur. J. Immunol.* 42: 2644-2654.
50. Foster, G.R., S.H. Masri, R. David, *et al.* 2004. IFN-alpha subtypes differentially affect human T cell motility. *J. Immunol.* 173: 1663-1670.

51. Cavalcante, P., M. Barberis, M. Cannone, *et al.* 2010. Detection of poliovirus-infected macrophages in thymus of patients with myasthenia gravis. *Neurology*. 74: 1118-1126.
52. Cavalcante, P., B. Serafini, B. Rosicarelli, *et al.* 2010. Epstein-Barr virus persistence and reactivation in myasthenia gravis thymus. *Ann. Neurol.* 67: 726-738.
53. Kawai, T. & S. Akira. 2010. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11: 373-384.
54. Robinet, M., S. Maillard, M.A. Cron, *et al.* 2017. Review on Toll-Like Receptor Activation in Myasthenia Gravis: Application to the Development of New Experimental Models. *Clin. Rev. Allergy Immunol.* 52: 133-147.
55. Cufi, P., N. Dragin, J.M. Weiss, *et al.* 2013. Implication of double-stranded RNA signaling in the etiology of autoimmune myasthenia gravis. *Ann. Neurol.* 73: 281-293.
56. Iwakiri, D., L. Zhou, M. Samanta, *et al.* 2009. Epstein-Barr virus (EBV)-encoded small RNA is released from EBV-infected cells and activates signaling from Toll-like receptor 3. *J. Exp. Med.* 206: 2091-2099.
57. Kasper, L.H. & A.T. Reder. 2014. Immunomodulatory activity of interferon-beta. *Ann Clin Transl Neurol.* 1: 622-631.
58. Bennett, L., A.K. Palucka, E. Arce, *et al.* 2003. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J. Exp. Med.* 197: 711-723.
59. Rauch, I., M. Muller & T. Decker. 2013. The regulation of inflammation by interferons and their STATs. *Jak-Stat.* 2: e23820.
60. Stubgen, J.P. 2009. Interferon alpha and neuromuscular disorders. *J. Neuroimmunol.* 207: 3-17.
61. Meager, A., M. Wadhwa, P. Dilger, *et al.* 2003. Anti-cytokine autoantibodies in autoimmunity: preponderance of neutralizing autoantibodies against interferon-alpha, interferon-omega and interleukin-12 in patients with thymoma and/or myasthenia gravis. *Clin. Exp. Immunol.* 132: 128-136.
62. Poeta-Guyon, S., P. Christadoss, R. Le Panse, *et al.* 2005. Effects of cytokines on acetylcholine receptor expression: implications for myasthenia gravis. *J. Immunol.* 174: 5941-5949.
63. Le Panse, R., G. Cizeron-Clairac, M. Cuvelier, *et al.* 2008. Regulatory and pathogenic mechanisms in human autoimmune myasthenia gravis. *Ann. N. Y. Acad. Sci.* 1132: 135-142.

64. Cufi, P., N. Dragin, N. Ruhlmann, *et al.* 2014. Central role of interferon-beta in thymic events leading to myasthenia gravis. *J. Autoimmun.* 52: 44-52.
65. Cufi, P., P. Soussan, F. Truffault, *et al.* 2014. Thymoma-associated myasthenia gravis: On the search for a pathogen signature. *J. Autoimmun.* 52: 29-35.
66. Punga, T., R. Le Panse, M. Andersson, *et al.* 2014. Circulating miRNAs in myasthenia gravis: miR-150-5p as a new potential biomarker. *Ann Clin Transl Neurol.* 1: 49-58.
67. Nogales-Gadea, G., A. Ramos-Fransi, X. Suarez-Calvet, *et al.* 2014. Analysis of serum miRNA profiles of myasthenia gravis patients. *PloS one.* 9: e91927.
68. Chunjie, N., N. Huijuan, Y. Zhao, *et al.* 2015. Disease-specific signature of serum miR-20b and its targets IL-8 and IL-25, in myasthenia gravis patients. *Eur. Cytokine Netw.* 26: 61-66.
69. Cheng, Z., S. Qiu, L. Jiang, *et al.* 2013. MiR-320a is downregulated in patients with myasthenia gravis and modulates inflammatory cytokines production by targeting mitogen-activated protein kinase 1. *J. Clin. Immunol.* 33: 567-576.
70. Barzago, C., J. Lum, P. Cavalcante, *et al.* 2016. A novel infection- and inflammation-associated molecular signature in peripheral blood of myasthenia gravis patients. *Immunobiology.* 221: 1227-1236.
71. Skogberg, G., J. Gudmundsdottir, S. van der Post, *et al.* 2013. Characterization of human thymic exosomes. *PloS one.* 8: e67554.
72. Linhares-Lacerda, L., C.C. Palu, M. Ribeiro-Alves, *et al.* 2015. Differential Expression of microRNAs in Thymic Epithelial Cells from Trypanosoma cruzi Acutely Infected Mice: Putative Role in Thymic Atrophy. *Frontiers in immunology.* 6: 428.
73. Papadopoulou, A.S., J. Dooley, M.A. Linterman, *et al.* 2012. The thymic epithelial microRNA network elevates the threshold for infection-associated thymic involution via miR-29a mediated suppression of the IFN-[alpha] receptor. *Nat. Immunol.* 13: 181-187.
74. Hoover, A.R., I. Dozmorov, J. MacLeod, *et al.* 2016. MicroRNA-205 Maintains T Cell Development following Stress by Regulating Forkhead Box N1 and Selected Chemokines. *J. Biol. Chem.* 291: 23237-23247.

Figure legends

Figure 1: Thymic changes in MG patients.

Thymic sections from MG patients were labelled (A) in red with an anti-keratin antibody (Clone MNF116, Dako) and in green with an anti-CD21 antibody (ref 555421, BD Biosciences) for B cells and follicular dendritic cells to localize GCs, (B) in red for HEVs with an anti peripheral node addressin (PNA_d) (MECA 79, 553863, BD Biosciences) and a biotinylated anti-rat IgM (349023; BD Biosciences) followed by an alexa-Fluor 594 streptavidin and in green with an anti-CD19 (48-0199-42; eBiosciences) antibody for B cells, (C) in red for HEVs and in green for CCL21 with an anti-human CCL21 (AF366; R&D Systems) followed by an alexa-Fluor 488 (A21222; Invitrogen). Reprinted with permission of Annals of Neurology from Berrih-Aknin et al. (Berrih-Aknin et al, 2009).

Figure 2: Central role of IFN- β in thymic changes associated with MG

IFN- β induces the expression in thymic epithelial cells (TECs) of α -AChR, the main autoantigen in MG. This effect is very specific α -AChR, as IFN- β does not induce the expression of other AChR subunits or other tissue-specific antigens. IFN- β also induces TEC death and the uptake of TEC proteins by dendritic cells, suggesting a potential sensitization of dendritic cells to the α -AChR. In parallel, IFN- β increases the expression of the chemokines CXCL13 and CCL21 by TECs and lymphatic endothelial cells, respectively. These two chemokines are involved in GC development and are overexpressed in MG with follicular hyperplasia. We also demonstrated that the B-cell activating factor (BAFF), which favors autoreactive B-cells, was overexpressed by TECs in MG thymus and was also induced by IFN- β . Altogether, these results demonstrate that IFN- β plays a central role in thymic events leading to MG by triggering the overexpression of α -AChR probably leading to thymic dendritic cells autosensitization against AChR, and the abnormal recruitment of peripheral cells involved in GC formation. Reprinted with permission of Clinical reviews in allergy & immunology from Robinet et al. ⁵⁴.

Chemokine	Receptor(s)	Expression in MG thymuses
CCL17	CCR4	Increased expression in Hassall's corpuscle and surrounding cells. ⁴² Expressed on HEVs in MG thymus. ⁴²
CCL19	CCR7	Upregulated in MG. ^{31,42}
CCL21	CCR7	Upregulated in MG thymus. ^{31,42}
CCL22	CCR4	Increased expression in Hassall's corpuscle and surrounding cells. ⁴²
CXCL9	CXCR3	Upregulated in MG thymus. ³¹
CXCL10	CXCR3	Upregulated in MG thymus. ⁴⁰
CXCL11	CXCR2/CXCR7	Upregulated in MG thymus. ³¹
CXCL12	CXCR4/CXCR7	Expressed on HEVs in MG thymus. ³⁵
CXCL13	CXCR5	Upregulated in MG thymus. ^{23,31}
RANTES/CCL5	CCR1/CCR3/CCR5	Overexpressed in thymic epithelial cells in MG. ⁴¹

Table 1: List of the chemokines that are abnormally expressed in thymus of early-onset MG patients

Figure 1

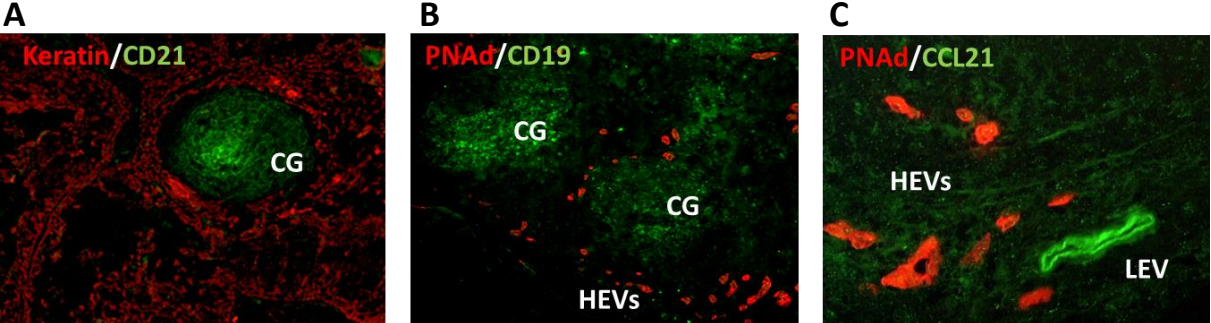


Figure 2

