MiR-146a in myasthenia gravis thymus: from uncontrolled innate immunity to B-cell-mediated autoimmunity

Paola Cavalcante PhD[1], Maria Cristina Tarasco MSc[1,2], Nicola Iacomino MSc[1], Carlo Antozzi MD[1], Renato Mantegazza MD[1]

[1] Neurology 4 - Neuroimmunology and Neuromuscular Diseases Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133 Milan, Italy
[2] PhD program in Neuroscience, University of Milano-Bicocca, 20900 Monza, Italy

ABSTRACT

The thymus is the main trigger site of autoimmunity in myasthenia gravis (MG) associated with anti-acetylcholine receptor (AChR) autoantibodies, a prototypic autoimmune disease affecting the neuromuscular junction. The majority of patients with early-onset MG have follicular hyperplastic changes of the thymus that are critically implicated in the initiation and perpetuation of the autoimmune response against the AChR. Uncontrolled activation of Toll-like receptor (TLR)-mediated innate immune responses, chronic inflammation, and ectopic germinal center (GC) formation are key pathological features of the hyperplastic thymus in MG, indicating that a close link between innate immunity and B-cell-mediated autoimmunity underlies the intra-thymic pathogenesis of MG.

MiR-146a is an "immune-miR" that acts as a key modulator of both innate and adaptive immunity and is a potent inhibitor of TLR signaling pathways. It is able to prevent and avoid overstimulation of the inflammatory response by targeting the NF-κB signaling transducers IRAK1 and TRAF6. At the same time, miR-146a modulates the expression of c-REL, ICOS, and ICOSL, which are crucial regulators of B-cell function and GC response. Dysregulation of miR-146a expression is a common molecular event in several autoimmune disorders. Recent findings have found defective expression of miR-146a in follicular hyperplastic MG thymuses, associated with over-expression of its TLR- and B-cell-related target genes, which suggests that loss of regulatory functions of this miRNA may contribute to the immunopathological steps leading to MG. Of note, corticosteroids have been found to increase miR-146a expression thus suggesting that miR-146a can mediate the effects of these drugs in inducing immunosuppression and control of autoimmunity.

In this review, we discuss the role of miR-146a as a molecular bridge between innate and adaptive immunity and summarize the current knowledge on the miRNA contribution to the intra-thymic pathogenesis of MG associated with follicular hyperplastic thymus. We also highlight the role of miR-146a as a potential biomarker for therapeutic monitoring and as a target of future advanced RNA-based therapies to modulate the immune system and counteract the autoimmune response in AChR-MG.

Key Words: *autoimmunity, innate immunity, miR-146a, myasthenia gravis, thymus*

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Introduction

Myasthenia gravis (MG) is a chronic autoimmune disease characterized by fluctuating muscle weakness and fatigability of ocular, bulbar, and skeletal muscles caused by autoantibodies to neuromuscular junction (NMJ) components. In about 80% of patients the autoimmune response is directed against the acetylcholine receptor (AChR); less frequently, autoantibodies target the muscle-specific tyrosine kinase (MuSK) or the lipoprotein-related protein 4 (LRP4). Patients in which specific autoantibodies cannot be detected are currently classified as seronegative (1,2).

A consensus-based stepwise approach is recommended for treatment of MG, including symptomatic therapy with cholinesterase inhibitors, immunosuppressive (IS) therapy with corticosteroids, alone or combined with other IS agents, thymectomy in selected patients, and plasmapheresis/immunoglobulins for acute exacerbations (3). The prognosis of MG has greatly improved over the past half century. Nevertheless, up to 80% of patients fail to achieve complete stable remission and need lifelong IS treatment. Moreover, about 10% of patients are treatment refractory or intolerant to IS drugs (4,5), highlighting the importance of gaining a better understanding of the disease-specific molecular events in order to design more effective therapeutic strategies.

The thymus is the main site of autoimmunity development in MG associated with anti-AChR antibodies. AChR-MG patients frequently present morphological and functional changes of the thymus including follicular hyperplasia and thymoma (6,7). Follicular hyperplasia is the most common alteration in early-onset (< 50 years) MG patients. It is characterized by an expanded thymic medulla containing germinal centers (GCs) forming follicles, as observed in secondary lymphoid organs (6). Thymectomy improves the clinical outcome in a considerable proportion of patients with hyperplastic thymus (8), thus supporting a role for this organ in sustaining the autoimmune reaction against the AChR.

The hyperplastic MG thymus may be considered a

prototypic autoimmune organ, since it encompasses a number of immunological alterations commonly observed in target organs of autoimmune disorders, including chronic inflammation, abnormal T- and B-cell activation, B-cell dysfunction, and GC formation (6,9). Experimental data over the past two decades have pointed to a critical role for uncontrolled Toll-like receptor (TLR)-mediated innate immune responses to pathogenic infections in driving and perpetuating the inflammatory autoimmune process in this organ (10-15). However, factors that cause persistence of innate immunity and inflammation, and ultimately chronicity of the autoimmune response in MG thymus still remain to be determined.

The innate immune system consists of a variety of factors that control and participate in all aspects of inflammation and immunity. The innate immune system is the body's first line of defense from invading pathogens, but its improper activation may lead to autoimmunity (16). In normal conditions, innate immune pathways are kept under control by fine-tuning mechanisms to avoid hyper-activation of immune cells and autoimmune phenomena (16). Thus, identification of the molecular events underlying the loss of regulation of innate immunity is an important field of research in MG and other autoimmune diseases in which a dangerous link between innate and adaptive autoimmunity has been demonstrated. A deeper understanding of these molecular events could promote the design of new targeted therapies.

MicroRNAs (miRNAs) modulate many biological processes, including innate and adaptive immune responses (17). MiR-146a-5p (hereinafter called miR-146a) is one of the most important miRNAs known to orchestrate TLR-mediated innate immune signaling, as well as T- and B-cell function, including GC response (18-20). This regulatory property makes this miRNA a good candidate to play a role in the intra-thymic pathogenesis of MG associated with thymic hyperplastic changes and a target for innovative therapeutic interventions to treat long-term inflammation and autoimmunity.

We review the key role of miR-146a in modulating innate and adaptive immune responses and discuss its contribution to AChR-MG by highlighting its biomarker and therapeutic potential.

Innate autoimmune mechanisms in follicular hyperplastic MG thymus

The hyperplastic MG thymus provides a complex microenvironment where the anti-AChR autoimmune reaction can develop and perpetuate. The presence of thymic epithelial cells (TECs) and myoid cells expressing the autoantigen, along with antigen-presenting cells, favors specific antigen presentation/cross-presentation, leading to intra-thymic T- and B-cell autosensitization (9). AChR-specific T- and B-cells and autoantibody-producing plasma cells are present in hyperplastic thymuses of MG patients (21,22). Moreover, abnormal neoangiogenic processes, consisting of high endothelial venule development and over-expression of chemokines (e.g. CXCL13 and CCL21) promoting peripheral cell recruitment into the thymus have been described (6,9), indicating that autoimmunity can be triggered and then perpetuated.

Chronic inflammation, with over-expression of pro-inflammatory cytokines, including interleukin-6 (IL-6), IL-17, and type I Interferons (IFN-I), and up-regulation of TLRs (i.e. TLR3, TLR4, TLR7, TLR9), is likely to play a role in inducing thymic hyperplastic changes and intra-thymic anti-AChR sensitization in MG patients (6-15). Cufi and colleagues demonstrated that TLR3 signaling selectively increased the expression of the AChR-α subunit in TECs via IFN-β (14). Moreover, stimulation of both TLR3 and TLR4, via a combination of Poli(I:C) and lipopolysaccharide (LPS) induced thymic hyperplasia, anti-AChR antibody production, and MG symptoms in mice without immunization, suggesting that lymphoid neogenesis and anti-AChR autoreactivity could result from dysregulated TLR signaling in the thymus (15). These events can be mediated by TLR-induced production of the antiviral mediator IFN-β. Indeed, IFN-β can increase AChR-α expression and apoptosis in TECs, thereby favoring protein uptake by dendritic cells (DCs) and antigen presentation, at the same time increasing CXCL13, CCL21, and BAFF expression, that result in peripheral immune system cell recruitment and enhanced survival of B-cells, including autoreactive cells (23-25).

Viral infections are likely the main trigger for abnormal TLR activation and IFN-I production in hyperplastic MG thymuses, although a role for endogenous molecules, such as nucleic acids (25), is also plausible. Poliovirus persistence was demonstrated in TLR4-positive macrophages in the thymus of some MG patients, suggesting a viral contribution to persistent TLR4 activation and inflammation (26). However, since TLR4 over-expression, but not poliovirus, was common in MG thymuses, it is plausible that in some cases autoimmunity might become clinically apparent when the triggering pathogen has already been cleared by the thymus ("hit-and-run" hypothesis), or viruses other than poliovirus can trigger dangerous TLR4 hyper-activation. Epstein-Barr virus (EBV), a highly B-cell-tropic virus, has been associated with several autoimmune disorders. EBV persistence and reactivation was found to be a common pathological feature of hyperplastic MG thymuses, suggesting a contribution of the virus to abnormal TLR and B-cell activation in the inflamed MG thymic milieu (10,12). EBV nucleic acids can stimulate TLR3, TLR7, and TLR9, with the last two being over-expressed in intra-thymic MG B-cells positive for EBV proteins (12). Since TLR7 and TLR9 can act as co-stimulatory signals for proliferation and survival of B-cells, including autoreactive B-cells, their EBV-driven signals could well participate in perpetuation of autoimmunity in MG thymuses (12,13). Dysregulated

TLR pathways can also affect the balance between effector (Teff) and regulatory T-cells (Treg), in favor of Teffs, as demonstrated for TLR4 pathways (11), thus supporting a TLR contribution to T-cell dysfunction and autoreactive T-cell responses in MG thymuses (11).

The overall data in the literature strongly indicate that a dangerous link between innate immunity and autoimmunity underlies intra-thymic MG pathogenesis. Nevertheless, the reasons why TLR-mediated responses are not properly regulated and turned off in hyperplastic MG thymuses to avoid sustained activation and chronicity of the inflammatory cascade, ultimately leading to autoimmunity, remain to be elucidated.

MiR-146a role in modulation of innate and adaptive immune response

MiR-146a is one of the most important "immune-miRs" capable of regulating TLR signaling and the inflammatory response, and its dysregulated expression has been associated with several autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis, and multiple sclerosis (MS) (27-30). MiR-146a acts as a dominant, potent inhibitor of MyD88-dependent TLR pathways via suppression of two recognized target genes, the tumor necrosis factor (TNF) receptor associated factor 6 (TRAF6) and the interleukin 1 receptor associated kinase 1 (IRAK1), which are key components of the TLR pathways functioning as NF-κB signaling transducers (18).

The gene encoding miR-146a is located within the *MIR3142HG* host gene on chromosome 5 (5q33.3) and has a promoter locus with binding sites for NF-κB, IRF3/7, and c-myc transcription factors (18,31-33). Regulation of TLR signaling via the miRNA occurs through a negative feedback loop: miR-146a is induced by NF-κB in response to TLR stimulation, and it then targets TRAF6 and IRAK1, thus inhibiting TLR signaling to dampen the magnitude of the immune response and guarantee maintenance of immunological tolerance (32,33). Indeed, mice lacking the miR-146a gene have several immune defects and spontaneously develop autoimmunity, pointing once again to miR-146a function as an effective control on autoimmune processes (33).

Normally, suppression of TRAF6 and IRAK1 via miR-146a leads to reduced expression of NF-κB target genes, such as IL-6, IL-8, IL-1β, TNF-alpha, IFN-I, and inflammatory chemokines (18,32), the excessive production of which may favor an autoimmune response in a susceptible background. In SLE, reduced miR-146a levels correlate with higher levels of inflammatory molecules and IFN-I, and with worse clinical manifestations; contrariwise, introduction of the miR-146a into patients' PBMCs alleviates the activation of the IFN-I pathway (34). Along with TRAF6 and IRAK1, miR-146a has been shown to target the signal transducer and activator transcription 1 (STAT-1) and interferon regulatory factor 5 (IRF-5) to control the antiviral IFN-I response (18). Since STAT-1 is a transcription factor required for Teff differentiation, its repression via miR-146a is also important for the suppressive function of Tregs. Indeed, miR-146a is highly expressed in Tregs, and its knock-out expression in these cells leads to a fatal tolerance breakdown in mice which results in CD4+ T helper lymphocyte-mediated immunopathology (35). MiR-146a has also been demonstrated to block the autocrine IL-6- and IL-21-induced Th17 differentiation pathways in autoreactive CD4+ T-cells. In this regard, miR-146a-deficient mice developed a more severe experimental autoimmune encephalomyelitis (EAE), an animal model of MS, associated with increased differentiation of T-cells into Th17 cells (36). There is considerable evidence of miR-146a involvement in the control of adaptive immunity by modulating not only T- but also B-cell functions, particularly the GC response (36). Indeed, miR-146a deficiency promotes activation of c-Rel, an NF-κB subunit implicated in B-cell proliferation and differentiation (36). Moreover, miR-146a limits the accumulation of follicular T helper (Tfh) cells and GC B-cells by targeting the inducible T-cell costimulator (ICOS) and its ligand (ICOSL), as demonstrated in mice by Pratama and colleagues (19). Additionally, increased miR-146a expression was associated with down-regulation of Fas cell surface death receptor (FAS) in naïve B-cells, which disrupts lymphocyte homeostasis and leads to hyper-lymphoproliferation and GC formation (20).

Taken together, the aforementioned studies strongly point to an extensive role for miR-146a as a critical negative regulator of innate and adaptive immune reactions (Table 1), highlighting its deficiency as harmful, and its normalization as a potential therapeutic approach for treating inflammatory autoimmune disorders. However, determination of the optimal miR-146a dosage, as well as identification of the optimal target cells, would be of outmost importance for its use as a therapeutic agent, since superabundant miR-146a expression can lead to imbalanced immune homeostasis and side effects (e.g. spleen and lymph node enlargement) (20).

MiR-146a in MG associated with follicular hyperplastic thymus

Despite the critical involvement of miR-146a in modulation of the innate and adaptive immune system, its possible contribution to intra-thymic MG pathogenesis has only recently been investigated. Defective miR-146a expression was found to be a key alteration in hyperplastic thymuses from early-onset (< 50 years) MG patients, with a profound impact on the expression of genes involved in TLR signaling, as well as genes controlling B-cell function and GC formation (37).

Table 1. Main target genes of miR-146a involved in innate and adaptive immune response

	Symbol	Function	MiR-146a effect	References
Innate immunity	TRAF6, IRAK1	Key mediators of MyD88-dependent TLR signaling pathways	***Down-regulation:*** Inhibition of MyD88-dependent TLR signaling pathways and suppression of the inflammatory response	18, 32, 33
	TLR4	TLR family member for recognition of LPS, and other bacterial and viral components whose signaling leads to NF-kB activation and pro-inflammatory gene expression	***Down-regulation:*** Inhibition of TLR4 signaling pathways and suppression of inflammatory response	38
Adaptive immunity	STAT-1	Transcription factor required for Teff differentiation	***Down-regulation:*** Reduced Teff differentiation and increased Treg function	35
	IRF-5	Transcription factor for IFN-I pathway activation	***Down-regulation:*** Inhibition of IFN-inducible gene expression and IFN-I-mediated antiviral response	18
	c-REL	NF-kB subunit implicated in B-cell proliferation and differentiation	***Down-regulation:*** Negative regulation of B-cell proliferation and differentiation	36
	ICOS	Inducible T-cell costimulator acting as a T-cell response activator and positive regulator of Tfh cell differentiation	***Down-regulation:*** Inhibition of Tfh cell accumulation and GC formation	19
	ICOSL	Cell surface antigen acting as ICOS ligand to activate T-cell response and positively regulate Tfh cell differentiation	***Down-regulation:*** Inhibition of Tfh cell accumulation and GC formation	19
	FAS	Cell death receptor leading to apoptosis pathway by Fas ligand	***Down-regulation:*** Interference with Fas-mediated apoptosis; increase of B-cell survival, activation and GC response	20, 42

Abbreviations: TRAF6: tumor necrosis factor receptor associated factor 6; IRAK1: interleukin 1 receptor associated kinase 1; MyD88: Myeloid differentiation primary response 88; TLR: Toll-like receptor; TLR4: Toll-like receptor 4; LPS: lipopolysaccharide; NF-kB: Nuclear factor kappa-light-chain-enhancer of activated B-cells; STAT-1: signal transducer and activator transcription 1; Teff: effector T-cells; Treg: regulatory T-cells; IRF-5: interferon regulatory factor 5; IFN-I: type I interferon; c-REL: proto-oncogene c-REL; ICOS: inducible T-cell costimulator; Tfh: follicular T helper; GC: germinal center; ICOSL: inducible T-cell costimulator ligand; FAS: Fas cell surface death receptor

Defective control of innate immune response

The expression of miR-146a and its TLR-related target genes was recently assessed in follicular hyperplastic thymuses from early-onset AChR-MG patients and normal control thymuses from patients without autoimmune diseases (37). MiR-146a levels were significantly lower in hyperplastic MG compared to control thymuses, whereas the expression levels of the miRNA targets TRAF6 and IRAK1 were increased (37). No significant difference in intrathymic miRNA levels was found between male and female patients. In view of the crucial miR-146a inhibitory role discussed above, this finding pointed out the lack of efficient control of innate immune responses and inflammation in hyperplastic MG thymuses (37).

MiR-146a is a key regulator of MyD88-dependent TLR signaling pathways, including those of TLR4, known to be over-expressed in hyperplastic MG thymuses (11). A close relationship between defective miR-146a expression and TLR4 up-regulation in MG thymic tissues can be postulated. Indeed, an interaction between TLR4 and miR-146a has been demonstrated via a consensus bioinformatics approach, and decreased expression of the miRNA was found to be concomitant with TLR4 up-regulation in macrophages. Conversely, TLR4 down-regulation was accompanied by over-expression of miR-146a (38). In line with these observations, double immunofluorescence analyses disclosed increased expression of IRAK1 in macrophages and myeloid DCs (mDCs), known to over-express TLR4 (11), in hyperplastic MG compared to control thymuses (37). This links miR-146a deficiency with increased TLR activation and pro-inflammatory cytokine production via these cells, that in turn may contribute to chronic inflammation. TLR7 and TLR9 were also found to be up-regulated in hyperplastic MG thymuses, likely due to active EBV infection (10,12). Of note, macrophages and mDCs were found to over-express TLR7, the expression levels of which were correlated with those of IFN-β (12), suggesting a relationship among low miR-146a levels, TLR7 over-activation, and IFN-β over-expression in the above-mentioned cells. Of note, EBV proteins are able to modulate miR-146a expression and function: EBV nuclear antigen 2 (EBNA2), expressed in newly infected naïve B-cells, down-regulates miR-146a, thus increasing IRAK1 and antiviral IFN-I expression (39). On the contrary, latent membrane protein 1 (LMP1), expressed in latently infected cells, induces miR-146a expression to decrease the intensity or duration of IFN-I response in a negative feedback loop for latency maintenance (40). Thus, defective expression of miR-146a in chronically inflamed hyperplastic MG thymus, characterized by active EBV infection, might be a critical factor contributing to the loss of regulation of IFN-I pathways, that in turn promote anti-AChR autosensitization (23,24).

Impact on B-cell function and GC response

The expression of B-cell-related miR-146a target genes was assessed in hyperplastic MG thymuses characterized by reduced levels of the miRNA (37). Transcriptional levels of c-Rel, an NF-κB subunit implicated in proliferation and differentiation of B-cells and GC formation (36), were significantly increased in MG pathological tissues compared to controls, suggesting that miR-146a deficiency may favor intra-thymic B-cell dysregulation via c-REL in MG patients. Indeed, the miRNA and target mRNA levels were negatively correlated, supporting a functional relationship with each other (36). At the protein level, c-REL was markedly expressed in both GCs and infiltrating B-cells of the MG thymic medulla (37). Similarly, the expression of ICOS, another recognized miR-146a target implicated in GC formation (19), was significantly increased in hyperplastic MG versus control thymuses, further supporting a link between low miRNA levels and GC development in the thymus of MG patients (37). This idea is based on considerable data that show miR-146a ability to repress ICOS, which is expressed in Tfh cells, and ICOSL, which is expressed in GC cells (19). Interestingly, Cho and colleagues demonstrated that specific miR-146a deletion in T-cells can increase Tfh cell number, strongly enhancing GC reactions (41). Thus, it is reasonable that the miRNA decrease observed in MG thymuses (37) can promote accumulation of Tfh and GC B-cells. The relationship between miR-146a deficiency and the presence of GCs was explored by laser-capture microdissection experiments, showing that the miRNA was expressed in GCs, whereas its levels were defective in the thymic medulla surrounding the GCs in MG thymic tissues (37). Of note, FAS mRNA levels were reduced in miR-146a-positive GCs compared to the surrounding medulla, in line with data in the literature that indicate miR-146a ability to induce GC formation via inhibition of FAS (20). The importance of FAS in GC formation was supported by data showing that B-cell-specific FAS-deficient mice develop fatal lymphoproliferation due to B-cell activation, and ablation of FAS specifically in GC B-cells may reproduce lymphoproliferation (42).

In summary, a critical role for miR-146a in B-cell dysfunction and GC response in MG thymuses can be postulated: on the one hand its defective expression in Tfh can increase the Tfh cell number, hence enhancing GC formation via the ICOS/ICOSL axis; on the other, the miRNA is expressed in B-cells and can promote GC response by targeting FAS (37).

MiR-146a in MG animal models

MiR-146a involvement in MG immune responses has been investigated in experimental autoimmune MG (EAMG) models. Zhang and colleagues proved that miR-146a is up-regulated in activated B-cells in response to

the AChRα-subunit R97-116 peptide in EAMG mice, and this up-regulation was significantly attenuated by the antagomiR-146a (43). Silencing of the miRNA in B-cells led to decreased total IgG levels *in vitro* and to significant improvement of symptoms in mice with ongoing disease (43). In a subsequent study, miR-146a expression was found to be significantly different between EAMG and control rats in immune organs, including the thymus, lymph nodes, and spleen (44). MiR-146a levels were decreased in the EAMG thymus and drainage lymph nodes compared with those in the same organs of the control animals in line with data obtained in thymuses from MG patients (37); contrariwise, in splenic tissue, higher levels of miR-146a were observed in EAMG compared to control animals (44). Since the thymus and drainage lymph nodes are enriched by T-cells, while the spleen is composed mainly of B-cells, differential expression of miR-146a in these tissues could be related to the cell content. Indeed, miRNA levels were down-regulated in Th17 and Treg cells and up-regulated in B-cells, of EAMG compared to control rats (44). Decreased miR-146a levels in T-cells from EAMG animals (44) was in line with the contribution of defective miR-146a expression to pathogenic T-cell function (35).

To assess the therapeutic effects of miR-146a in EAMG, Yin and colleagues (45) produced exosomes from miR-146a overexpressing DCs and observed that they suppressed ongoing disease in mice, altering the Th cell profiles from Th1/Th17 to Th2/Tregs both in serum and spleen. These therapeutic effects were antigen-specific and partly dose dependent (45).

MiR-146a as mediator of corticosteroid effects, treatment monitoring biomarker, and new therapeutic target for MG

Palagani and colleagues (46) demonstrated that glucocorticoids can regulate the expression of multiple genes involved in cell cycle control, cell organization, cell death, and immune response, as well as a number of miRNAs, termed glucocorticoid-inducible miRNAs, including miR-146a. In line with these observations, defective expression of miR-146a was found in hyperplastic thymuses from corticosteroid-naïve but not corticosteroid-treated MG patients, suggesting that IS treatment before thymectomy could have normalized/restored miRNA levels (37). MiRNA normalization in the thymus of treated patients was accompanied by down-regulation of TRAF6, IRAK1, c-REL, and ICOS genes, thus supporting a link between anti-inflammatory and IS effects of corticosteroids and miR-146a induction (37). *In vitro* studies strengthened this idea, since treatment with prednisone enhanced miRNA expression in peripheral blood cells (37). Considering the key role of the miR-146a/target gene axis in the regulation of GC formation, restoration of miR-146a levels by corticosteroids could partially explain the previously demonstrated ability of these drugs to reduce thymic GCs in MG patients (47). According to data obtained in the thymus, significant down-regulation of miR-146a was also observed in serum of corticosteroid-naïve AChR-MG patients compared to controls, whereas in corticosteroid-treated patients, serum miR-146a levels were normal (37), supporting a role of the miRNA as a therapeutic monitoring biomarker in AChR-MG patients. Based on overall findings, we suggest that miR-146a may mediate the effects of corticosteroids and that its levels in individual patients can affect, or be related to, the therapeutic responses to these drugs. Indeed, sensitivity and specificity performances of serum miR-146a discriminated AChR-MG patients from healthy controls (AUC: 0.78, P=0.027) (37). The potential role of the miRNA as a biomarker to predict or monitor AChR-MG patients' response to IS drugs deserves further study.

The ability of miR-146a to control both innate and adaptive immune response strongly highlights its modulation as a prospective molecular option to counteract autoimmunity in MG and potentially other autoimmune diseases. However, due to the multifaceted functions of miR-146a in different immune system cells, its therapeutic manipulation could result in beneficial or detrimental effects in a cell-dependent manner. Silencing of miR-146a in B-cells improves MG symptoms in the EAMG animal model (43), as described above. Metformin improves EAMG by reversing the expression of miR-146a in AChR specific B- and Th17 cells, partially inhibiting the pathogenic functions of these cells: beneficial effects were associated with decreased expression of miR-146a in B-cells and its increase in Th17 cells (44). Over-expression MiR-146a in DCs inhibits their maturation and leads to generation of exosomes able to reduce T-cell proliferation and polarize them toward an anti-inflammatory phenotype in EAMG animals and suppressing the ongoing disease (45).

The overall data indicate that miR-146a may serve as a potential therapeutic target for MG, but the challenge will be to design miRNA-modulating cell-specific therapies based on advanced delivery vehicles for administration of RNA therapy.

Conclusions

MiR-146a is a regulator of innate and adaptive immune responses implicated in the pathogenesis of several autoimmune conditions, including intra-thymic MG pathogenesis. A model of miR-146a as a molecular bridge linking innate and adaptive autoimmunity in hyperplastic MG thymus is shown in Figure 1. Based on literature data, miR-146a offers an important resource for innovative strategies to modulate immune system cells in the context of MG, and restore immune regulation. Thus, a deeper understanding of the miRNA mimicking/inhibition impact on specific cell types (e.g. dendritic cells, T- and B-lymphocytes) could prospectively pave the way to development of advanced molecular strategies to disrupt the link between innate immune activation and adaptive autoimmune response in MG.

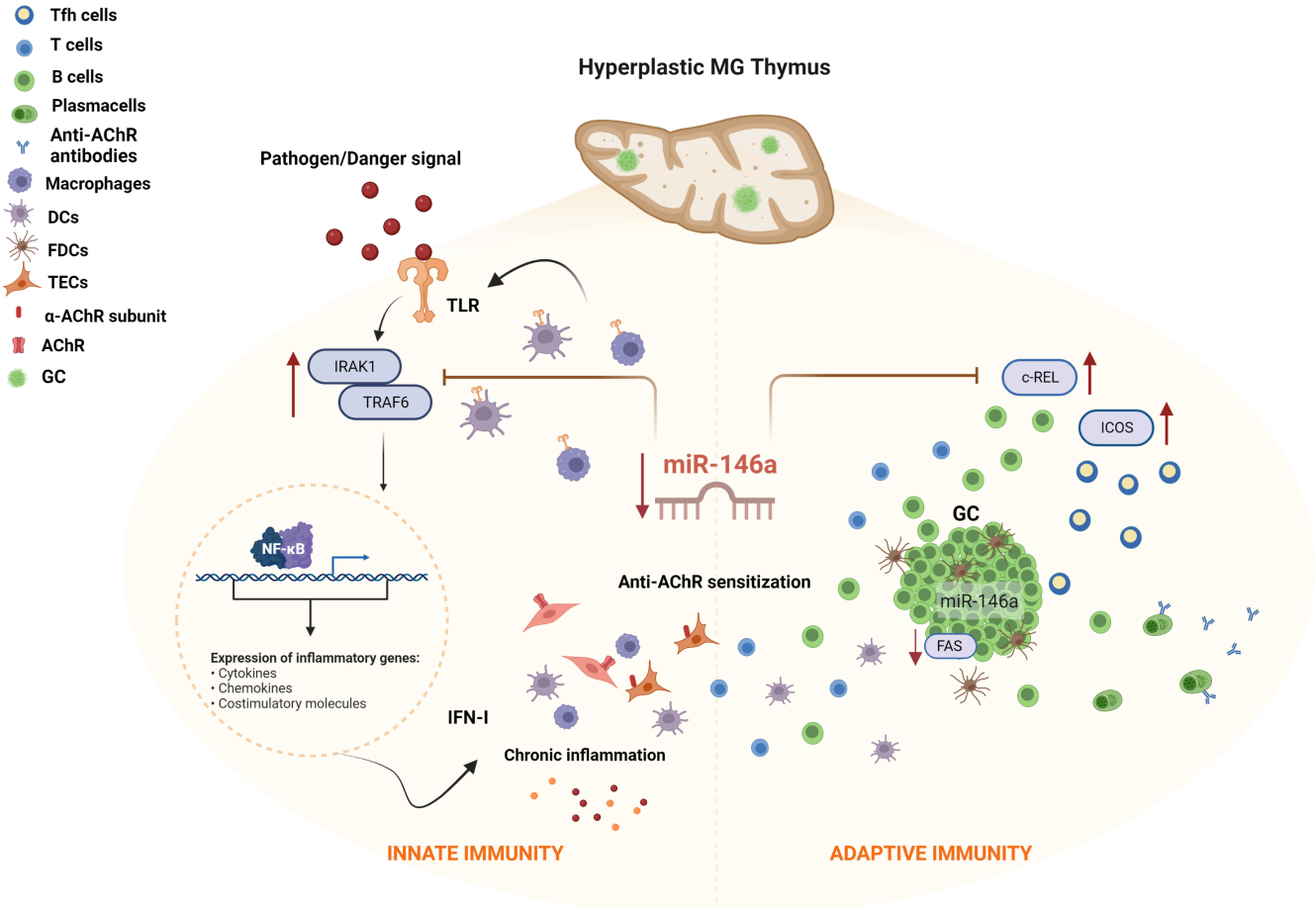

Figure 1. Model of miR-146a involvement in intra-thymic pathogenesis of MG associated with follicular hyperplastic thymus. Defective expression of miR-146a in innate immune system cells (e.g. macrophages, dendritic cells) of the thymus contributes to uncontrolled activation of pathogen-stimulated MyD88-dependent Toll-like receptor (TLR) signaling pathways due to loss of the miRNA inhibitory/regulatory effects on IRAK1 and TRAF6 expression. IRAK1 and TRAF6 increases cause sustained NF-kB activation and hence over-expression of pro-inflammatory cytokines, chemokines, and type I interferons (IFN-I), in turn promoting intra-thymic chronic inflammation. MiR-146a deficiency also contributes to over-expression of c-REL and ICOS, favoring B-cell proliferation and differentiation, and accumulation of follicular T-helper (Tfh) cells that, along with follicular dendritic cells (FDCs), promote germinal center (GC) formation. Decreased expression of Fas via miR-146a allows GC maintenance. IFN-I production in the inflamed thymic milieu, favorable to B-cell activation and survival, ultimately leads to autosensitization to the locally expressed acetylcholine receptor (AChR), and perpetuation of autoimmunity in the context of genetic backgrounds prone to MG. Figure created with BioRender.com.

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