RESEARCH ARTICLE

The Injected Plasma of Myasthenia Gravis Patient with A Low T-reg Level Caused Clinical Myasthenic Syndromes in Swiss-Webster Mice

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Abstract

BACKGROUND: Myasthenia gravis (MG) is a rare autoimmune disease affecting neuromuscular junction involvement. The finding that T-reg level in MG patients was lower than that in normal persons leads to the idea that the primary pathology of the disease is T-reg dependent. The T-reg level of MG patients seems to be decreasing compared to that of normal persons. The study was conducted to observe the contribution of T-reg level in plasma injected into Swiss-Webster mice to develop clinically and pathologically myasthenic syndromes.

METHODS: Swiss-Webster mice were grouped into three groups: the groups received plasma with normal, low, and high T-reg levels, respectively. The T-reg levels of the mice were measured with flow cytometry analysis and a human regulatory T-cell cocktail for T-cell surface cell marker. The motor function, interleukin (IL)-2, interferon (IFN)-γ, and thymus weight of mice were measured after the injection. Histopathological examination was performed to analyze mice's muscles and thymus.

RESULTS: The result identified that the motor function (2-week treatment group: p=0.021 and 3-week treatment group: p=0.032) and muscle width (p=0.014, p=0.032 and $p\leq$0.001) were significantly lower in the low T-reg level plasma group compared to control and high T-reg level plasma groups. The thymus showed an increase in weight without an increase in the cortex-medulla ratio of the thymus, indicating hyperplasia. Both IL-2 and IFN-γ levels were lower in the low and high T-reg level groups compared with the control group, indicating the autoimmune process.

CONCLUSION: Low T-reg level was associated with lower motor function, muscle width, increased thymus weight, as well as lower IL-2 and IFN-γ levels. T-reg level contributed to clinical myasthenic syndromes but not pathological findings. This research method is expected to be a basis for the development of animal models with Swiss-Webster mice.

KEYWORDS: animal model, Myasthenia gravis, Swiss-Webster mice

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Introduction

Myasthenia gravis (MG) is a rare, T-cell-dependent autoimmune disease. Most patients have acetylcholine receptor (AChR) antibodies in the neuromuscular junction, that causes muscle weakness as the primary symptom. (1) AChR antibody is not the only antibody found in MG patients. There are muscle specific kinase (MuSK) and lipoprotein receptor-related protein-4 (LRP4) antibodies, later found as antibodies related to the MG pathophysiology. (2) The incidence of MG reaches 4-18 new cases in every one million population worldwide and this is only based on the AChR antibody finding.(3)

The histopathologic findings of thymus in MG patients can be lymphoproliferation hyperplasia (LFH) or thymoma. The pathology of the thymus is related to the production of autoreactive T-cell.(4) T-regulatory (T-reg) is involved in the pathogenesis and therapy of various autoimmune diseases. (5) In the event of immune response, T-reg cells maintain self-immunologic tolerance and negative control. In MG patients, it is found that T-reg levels are lower compared to healthy control, while patients receiving immunosuppressant therapy have higher T-reg levels compared to MG patients who are not treated with immunosuppressant.(6)

The T-reg level in MG patients is lower than in healthy persons; otherwise, some research showed that patients receiving immunosuppressant had increasing or even normal T-reg levels compared to the healthy controls. The use of T-reg as the therapy was proven to exert clinical improvement in rats with clinical myasthenic syndromes. Interestingly, the patient with thymoma who underwent thymectomy showed an improvement of T-reg levels in the peripheral blood and thymus. All findings showed that T-reg play a critical role in MG pathology and clinically aspects.(7,8)

The studies using animal models for MG mostly utilized rats injected with antibodies obtained from Torpedo Californica that showed a weakness symptom like a myasthenic patient. However, that model cannot show the immunopathology of the disease. Another animal model injectedwith rocuronium bromide had showed muscle weakness, yet it remained not be able to show the immunopathology involved in MG.(9,10) Therefore, studies focusing in MG animal models should be further conducted.

Plasma is the part of blood consisting of antibodies and other cytokines that can affect the recipient.(11) In MG patients, the plasma contains immunoglobulin. An antibody mostly related to MG pathology is the acetylcholine receptor antibody (AChR-Ab), which is found in 80% of diluted IgG in plasma. Several theories stated that the plasma of an MG patient consists of an immunology factor that is transferable, not only the antibody but also some cytokines that function as immunomodulators or provocateurs of autoimmunity in the recipient.(12) It is known the T-reg level has a role in MG pathology and distinct clinical and pathological results are exhibited with the different T-reg levels injected into the Swiss-Webster mice. This study tried to inject the plasma of MG patients with a low T-reg level to develop an animal model for MG. The objective of this study is to observe the contribution of T-reg level in plasma injected into Swiss-Webster mice to develop clinically and pathologically myasthenic syndromes.

Methods

Human Plasma Collection

Plasma was obtained from 5 healthy paramedic workers in the Dr. Kariadi General Hospital Semarang whose age between 30-50 years old, had no autoimmune or other chronic disease, had no sleep disorder (Pittsburgh Sleep Quality Index-PSQI score ≤5) (13), GAD-7 (Global Anxiety Disorder-7) score ≤5 (14). Plasma was also obtained from MG patients that visited the neurology clinic of Dr. Kariadi General Hospital Semarang. There were 5 patients with low T-reg level and 4 patients with high T-reg level. Patients who were diagnosed with MG had to fulfill 2 of 3 criteria as follows: 1. Clinically diagnosed, according to MG Foundation of America (MGFA); 2. Laboratory diagnosis, having positive AChR-Ab; 3. Based on electrophysiology examination, there was a decrement in repetitive nerve stimulation (RNS), and patients consented to get their blood taken. The protocol of this research was approved by the Ethics Committee of Dr. Kariadi General Hospital Semarang (No. 598/EC/KEPK-RSDK/2021) for the human subjects included in this study. All subjects had consented to get their vein blood taken and have signed informed consent.

T-reg Level Measurement

The T-reg level was measured with flow cytometry analysis and a human regulatory T-cell cocktail (Cat. No. 560249; BD Bioscience, Franklin Lakes, NJ, USA) for T-cell surface cell marker analysis. Blood obtained from MG subjects was put in the citrate CPT tube and homogenized. The samples were then centrifuged to separate the lymphocyte cells. PBMC were stained with anti-CD25-FITC (BD Biosciences), anti-CD4-Percp (BD Biosciences) and the Anti-CD127-PE (BD Biosciences) in accordance with the protocols from the BD Bioscience T-reg to identify $CD4^{+}CD25^{bright}CD127^{dim}$.(15) T-reg level was considered low if the level was below the average T-reg level of healthy subjects and high if the level was above the average T-reg level of healthy subjects.

Animal Model and Treatment

Forty-five female, Swiss-Webster mice (Abadi Jaya, Yogyakarta, Indonesia), 10-12 weeks old, with body weight approximately 26-to-30 grams were included in the study. The mice were acclimatized for seven days before the measurement of body weight and motor function at the initiation of the experiment. The mice were placed in a plastic solid cage, adequate for 5 mice. Daily hardwood chip bedding replacement, food (High-Provit 594; Charoen

Pokphand Indonesia, Balaraja, Indonesia), and water were given *ad libitum* with alteration light and dark conditions in 12 hours. The mice were randomly grouped into three groups. The first group was a group injected with the healthy subjects' plasma; the second group was the group that was injected with the plasma of MG subjects with low T-reg levels, and the third group was injected with the plasma of MG subjects with high T-reg levels. Each group were further divided into 3 groups, each consisting of 5 mice. The subgroup received plasma injection for 2 weeks, 3 weeks, and 4 weeks. Each subject's plasma was given to 3 mice in 3 different groups. Plasma from healthy subject A was administered to mice A from 2-week group, mice A from 3-week group, and mice A from 4-week group (Figure 1).

All mice were measured body weight and motor function on the first day before treatment. The treatment group received intraperitoneal (IP) injection of plasma from a healthy person 100 µL/day for consecutive 5 days a week and no injection for 2 days (the 6th and 7th day). Plasma injection was done before 9 AM to avoid the effect of cortisol circadian rhythm, which could affect the level of cytokine. The protocol of this research was approved by the Health Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No. KET-856/UN2.F1/ETIK/PPM.00.02/2021) for the animal use.

Motor Function Assessment

Motor function was assessed with a wire-hanging test to detect neuromuscular abnormalities. The mice were placed on the wired caged lid, with the plastic placed around the lid to prevent the mice from walking off the rim. The mice was placed on top of the lid, and then it was turned upside down. The length of the mice hanging until it fell was measured for three attempts for every mice. The cut was about 90 to 300 seconds, depending on the model.(16)

Cytokines Measurement

IL-2 and IFN-γ levels were measured in the mice's serum using enzyme-linked immunosorbent assay (ELISA) following the manufacturer's protocol. IL-2 level was

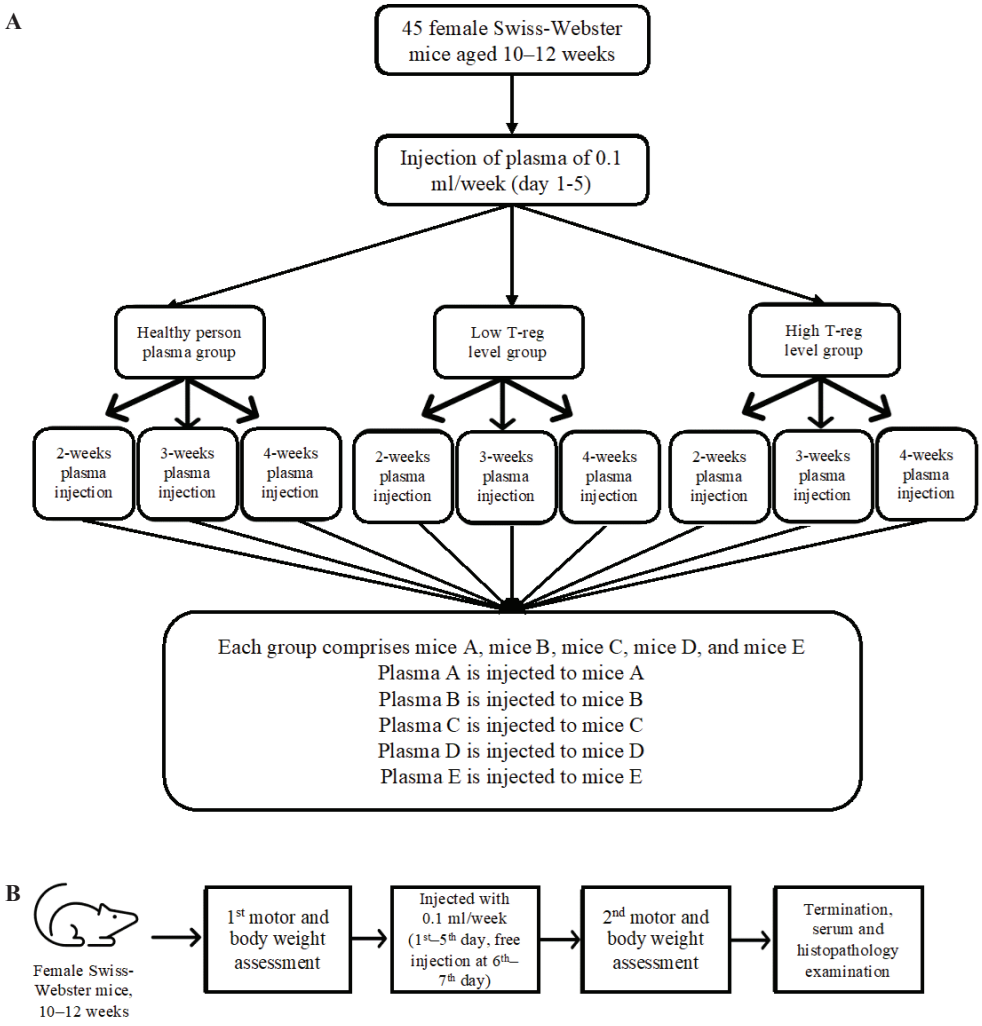

Figure 1. The study flowchart. Three large groups, comprising healthy person plasma, low T-reg level, and high T-reg level groups. Each group was divided into three small groups, receiving plasma injections for 2, 3, and 4 weeks. All groups contained five mice that received different plasma injections. For example, mice A from each group of 2-week, 3-week, and 4-week plasma injections of the healthy person plasma group received the same plasma A of a healthy subject.

measured using Rat IL-2 (Interleukin 2) ELISA Kit (Cat. No. E-El-R0013; Elabscience, Wuhan, China), and IFN-γ level was measured using Rat IFN-γ (Interferon Gamma) ELISA Kit (Cat. No. E-EL-R0009; Elabscience). The cytokine levels were quantified by reference to standard curves, and the results were presented in pg/mL. The serum was obtained from the blood drawn from the mice's retinal artery after anesthesia with diethyl ether for one minute. The blood was centrifuged for 20 minutes to obtain the serum for cytokine measurement.

Histopathology Analysis

Mice had undergone anesthesia and terminated by cervical dislocation before the organs (muscle, thymic, heart, and lung) were taken to be weighed and kept at -80°C. The right hindlimb and thymic muscles were put in the 10% buffer formalin solution. Trimming, dehydration, clearing, impregnation, embedding, cutting, and Hematoxylin-eosin (HE) staining protocols were provided by the Pathology Anatomy Department, Faculty of Medicine, Universitas Diponegoro.(17) The width of the muscle and thymus were analyzed with a microscope with 400x magnification. The thymus was analyzed, and the normal proportion between cortex and medulla was approximately 2:1 in ratio.(18) The ratio between the cortex and medulla was calculated with the Indocroview application. The cross-sectional area of muscle fibers was counted with the mean of five muscle fibers (Figure 2).

The samples were prepared at room temperature. A total of 100 µL of standard was added and then it was incubated for 2.5 hours at room temperature. A total of 100 µL of biotin antibody preparation was added, and it was incubated at room temperature. Afterwards, 100 µL of Streptavidin solution was added, followed by incubation at room temperature. A total of 100 µL of TMB One-Step Substrate was added, followed by incubation at room temperature for 30 minutes. Finally, 50 µL of Stop Solution was added, and the absorbance level was read at 450 nm wavelength directly.

Statistical Analysis

Statistical analysis was performed with SPSS 28.0 version (IBM Corporation, Armonk, NY, USA). Independent t-test or Mann-Whitney test were used to analyze the difference of variables (motor function, muscle width, cortex-medulla ratio, weight of thymus, level of IL-2 and IFN-γ) between the low T-reg and high T-reg level groups with control group. Analysis of data within low T-reg, high T-reg, and normal T-reg level groups was performed with One-way ANOVA or Kruskal-Wallis tests. The correlation between two numerical scales (IL-2 and IFN-γ level) was analyzed with the Spearman test.

Results

Characteristics of the Subject

Five healthy subjects (1 male and 4 females) were included in the normal subjects, 5 MG subjects (1 male and 4 females) with low T-reg level were included in the low T-reg group, and 4 MG subjects (1 male and 3 females) with high T-reg level were included in the high T-reg group. To determine the T reg level, flowcytometry analysis was performed and an example of the results were presented as follows (Figure 3). Due to limitations on the availability of plasma from MG subjects with high T-reg levels, only 4 mice (A, B, C, and D) underwent the study. Each mice received plasma from 4 different subjects.

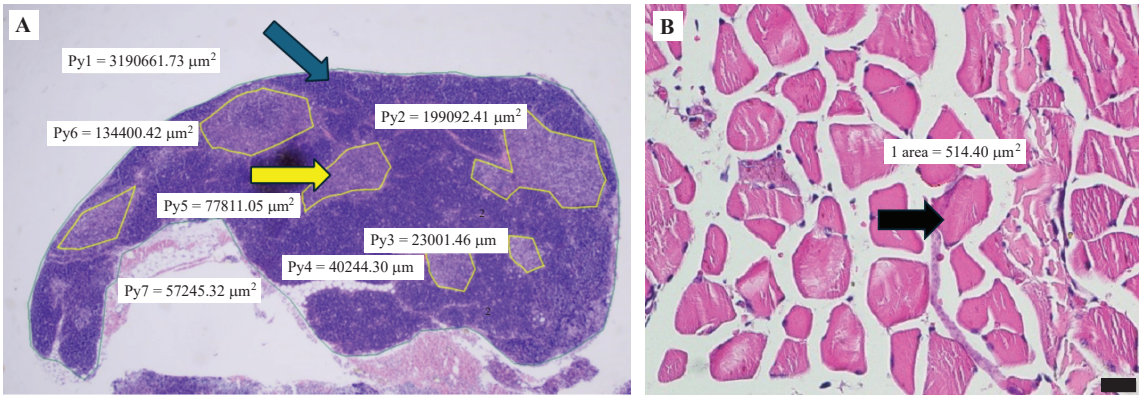

Figure 2. Calculation of the cortex-medulla ratio of the thymus and the muscle width. A: Cortex-medulla ratio of the thymus (1:200 µM) was counted by comparing the cortex area (all cortex area-blue arrow [Py1] subtracted by all medulla area-yellow arrow [Py2-Py6]). B: The width of the muscles was counted as the mean of five wide muscle fibers (black arrow) (1:20 µM). White bar: 20 µM.

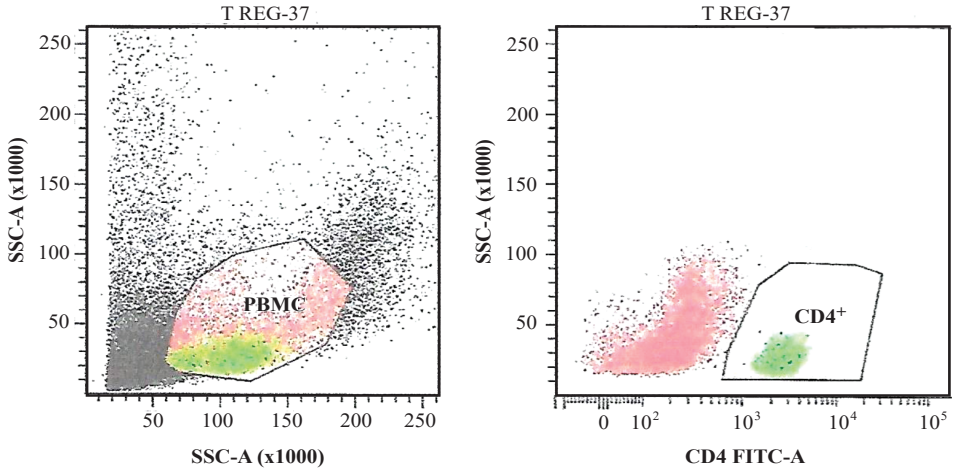

Figure 3. The results of flowcytometry examination. Grey spots: all events (100%); orange spots: PBMC (43.8%); green spots: CD4+ (10.6%); blue spots: Treg (0.3%).

Three subjects had undergone thymectomy in the high T-reg group and accidentally none had undergone thymectomy in the low T-reg group. All MG subjects took acetylcholine esterase inhibitor (AChE-I) medication to maintain the symptoms of the disease. Two subjects in the high T-reg group and 1 in the low T-reg group took steroid (methylprednisolone) along with the symptomatic medication (AChE-I) (Table 1).

Decrease in Motor Function and Muscle Width in The Group Injected with MG Subject's Plasma

The motor function of mice was analyzed with Mann-Whitney test (Figure 4). Comparison of the low T-reg group to the healthy subject group after 2 weeks of treatment showed a significant decrease in low T-reg group (p=0.021). There was a significantly decreasing motor function in the low T-reg group compared to the healthy subject group after 3 weeks of treatment (p=0.032). The high T-reg group did not show any decreases.

Muscle width difference was analyzed statistically (Figure 5). Muscle width significantly decreased in the group injected with low T-reg level plasma compared to the group injected with healthy subject plasma after 2 weeks of treatment (p=0.014). There was a significant decrease of the muscle width in the group injected with low T-reg level plasma compared to the group injected with the healthy subject plasma after 3 weeks of treatment (p=0.042). There was a significantly decreasing muscle width in groups injected with low T-reg level plasma compared to the group injected with healthy subject plasma after 4 weeks treatment ($p\leq$0.001). There was a significantly decreasing muscle width in groups injected with high T-reg level plasma compared to groups injected with normal plasma ($p\leq$0.001).

Thymus Alteration in The Group Injected with The MG Subjects's Plasma

Analysis with an Independent T-test was performed to compare thymus weight between MG subject groups and healthy subject group (Figure 6). Comparison of low T-reg level group to the healthy subject group after 2 weeks treatment showed a significant increase of the thymus weight (p=0.032). There was a significant increase of the thymus weight in the group injected with low T-reg level plasma compared to the group injected with the healthy subject plasma after 3 weeks of treatment (p=0.015). There was an increase of thymus weight in group injected with high T-reg level plasma compared to normal group injected with healthy subject plasma after 2 weeks of treatment (p=0.020). There was an increase of thymus weight in group injected with high T-reg level plasma compared to normal group injected with healthy subject plasma after 3 weeks of treatment (p=0.029). There was an increase of thymus

Table 1. Characteristics of the subject.

Plasma	Gender (M/F)	Age (Range)	AChR	T-reg (%)	Treatment		
					Steroid	AChE-I	Thymectomy
Normal	1/4	26-32	-	15.8±1.6	-	-	-
MG low T-reg level	1/4	35-60	26.6±14.5	3.7±0.8	1	5	0
MG high T-reg level	1/3	22-55	17.8±3.0	24.3±4.7	2	4	3

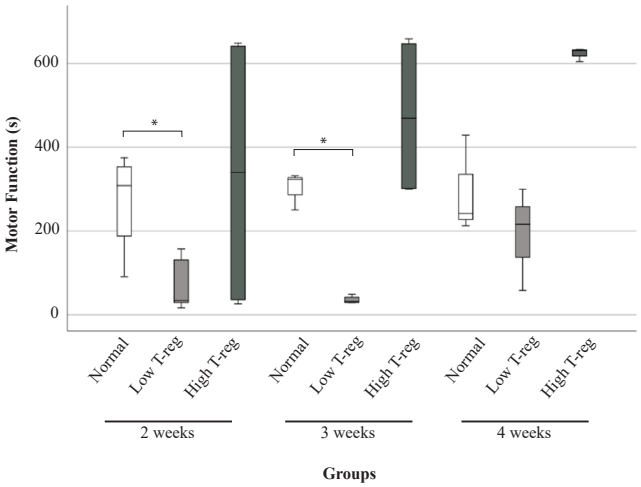

Figure 4. Motor function of the mice after intervention. *Tested with Independent T-test, significant if $p<0.05$.

weight in group injected with high T-reg level plasma compared to normal group injected with health subject plasma after 4 weeks of treatment ($p=0.042$).

There was no significant difference of cortex-medulla ratio between the group injected with low T-reg level plasma and high T-reg level plasma with the group injected with normal plasma (Figure 7).

Cytokines Alteration as The Result of MG Subject's Plasma Injection

There were alterations in the level of cytokines in both experimental groups injected with low and high T-reg level plasma compared to those injected with normal plasma. IL-2 and IFN-γ were decreased in groups injected with MG subject's plasma. The levels of IL-2 in both groups (low and high T-reg groups) were significant difference compared to the normal plasma group. Analysis with independent T-test for low and high T-reg level groups compared to normal plasma injection showed significant differences (Figure 8A). There were significantly different levels of IL-2 in the group injected with low T-reg level plasma compared to healthy plasma after 2 weeks, 3 weeks, and 4 weeks of treatment ($p=0.047$, $p=0.005$, and $p=0.041$, respectively). There were also significant differences of IL-2 in the group injected with high T-reg level plasma compared to healthy plasma after 2 weeks, 3 weeks, and 4 weeks of treatment ($p=0.022$, $p=0.029$, and $p=0.041$).

IFN-γ levels were significantly different only in 3 weeks and 4 weeks treatment groups. Groups injected with MG patients' plasma showed a significantly decreasing level of IFN-γ compared to the normal groups after 3 weeks and 4 weeks treatment (Figure 8B). The correlation between the IFN-γ and IL-2 levels was analyzed using the Spearman test and showed a significant strong and positive correlation ($r=0.781$; $p<0.001$). There were significantly different levels of IFN-γ in the group injected with low T-reg level plasma compared to healthy plasma after 3 weeks and 4 weeks of treatment ($p=0.007$, $p\leq0.001$, respectively). There were significantly different levels of IFN-γ in the group injected with high T-reg level plasma compared to healthy plasma after 3 weeks and 4 weeks of treatment ($p=0.005$, $p\leq0.001$, respectively).

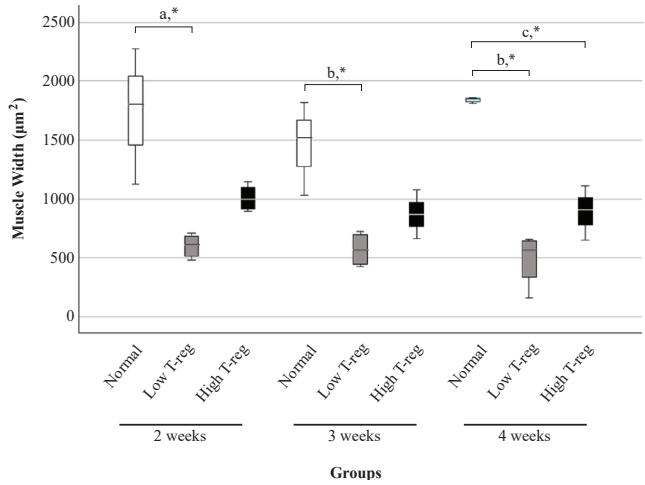

Figure 5. Muscle width of the mice after intervention. [a]Tested with Mann-Whitney test, [b]Tested with Independent T-test, [c]Tested with One-way ANOVA; *significant if $p<0.05$

Discussion

Autoimmunity is the imbalance between effector cells and regulatory cells, in which one of the autoimmune diseases is MG. There is an imbalance between the effector (autoantibody) and T-reg cells as the regulatory cells in MG patients. Autoantibody should be dismissed during the innate immune responses in the thymus because, for some causes, B cells produce autoantibody. On the other hand, in MG patients, it was found that the T-reg levels were lower compared to the healthy person.(1,19)

Epidemiology data found that MG mainly affects women more than men. The ratio of incidence of early-onset MG in men and women is 1:3. In late-onset MG, the incidence ratio in men and women is 2:3.(2,20). In this research, we injected one man's plasma and four women's plasma into mice. In MG patients, the T-reg level has no significant association with the severity of the disease. Still, many studies showed that the T-reg level was related to the patient's prognosis, therapy responses, and disease

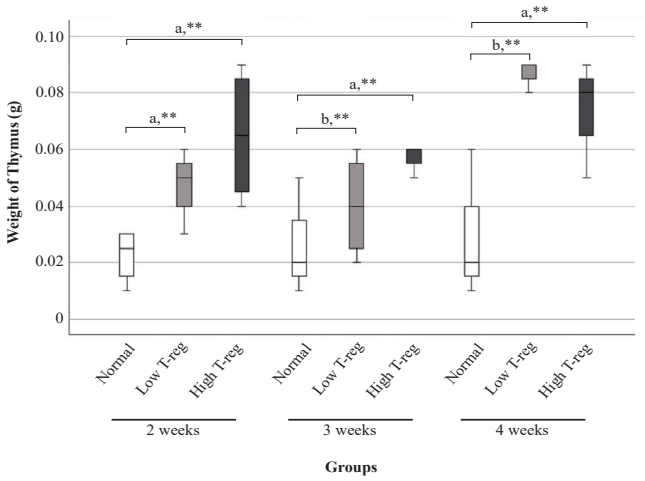

Figure 6. The weight of the thymus of the mice after intervention. [a]Tested with Mann-Whitney test, [b]Tested with Independent T-test; *significant if $p<0.05$.

development. The T-reg level in MG patients was lower than in healthy control. Other studies showed that the T-reg level of MG patients was higher in patients who received immunosuppressant compared to the group that did not receive immunosuppressant agents. T-reg level was found to be higher in patients who underwent thymectomy compared to patients who had not undergone thymectomy. T-reg levels play a significant role in the pathology of the disease and are related to clinical symptoms.(5,21,22) This study showed that groups with low T-reg levels had lower motor function. The decreasing motor function presented as the clinical manifestation of MG, namely muscle weakness, while the high T-reg level group did not show the same result. The decrease in muscle width could be found in all groups injected with low T-reg levels compared to the healthy plasma group (Figure 4, Figure 5).

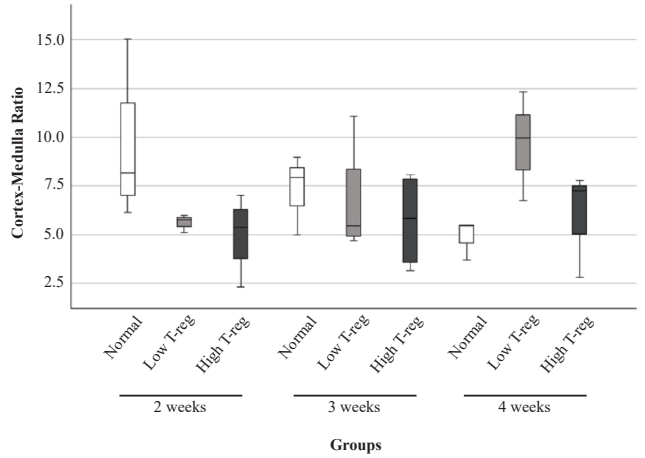

Figure 7. The ratio of cortex and medulla of the mice after intervention.

Many diseases show muscle weakness as a symptom related to the loss of muscle mass. The hypothesis is that muscle contractility problems can lead to muscle mass loss.(23) Disorders in the neuromuscular junction can lead to muscle mass wasting, hence maintaining normal signaling in the neuromuscular junction can prevent muscle mass loss.(24) The interesting finding is that the decrease in muscle width in groups injected with low T-reg levels was statistically significant. These theories also explained the findings that mice injected with high and low T-reg level plasma had smaller muscle widths compared to those injected with normal plasma.

The low T-reg level in MG is related to thymus pathology, which is usually found in MG patients. The thymus could be enlarged in MG patients, whether because of thymoma or LFH. Both abnormalities led to activated intra-thymic autoreactivity and the B-cell releasing autoantibodies in the peripheral immune system.(4,25) This study found no significant differences in the cortex-medulla

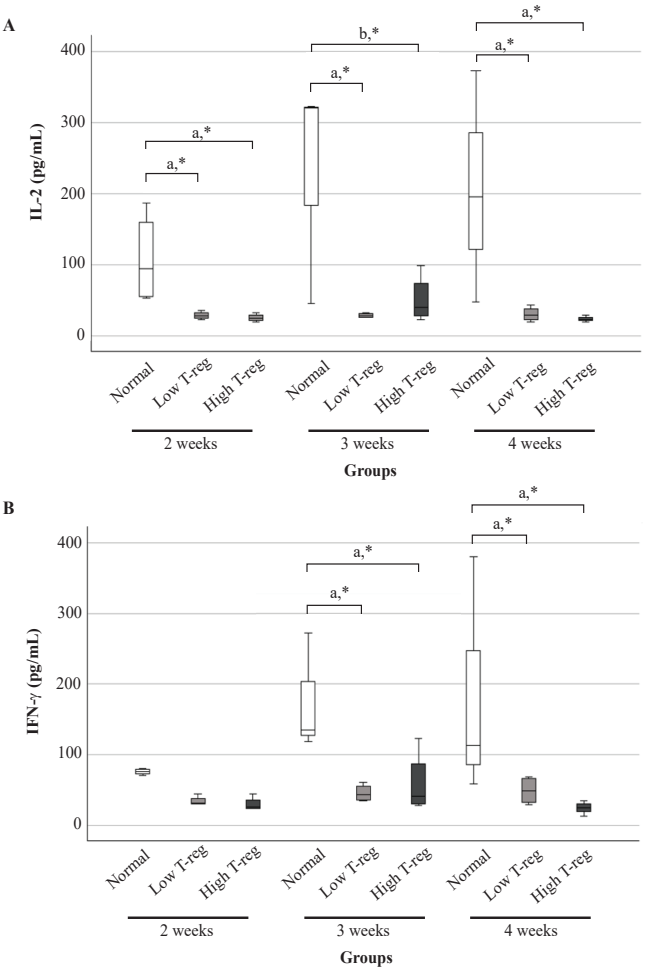

Figure 8. IL-2 and IFN-γ levels of the mice after intervention. A: IL-2 level. B: IFN-γ level. [a]Tested with Mann-Whitney test, [b]Tested with Independent T-test; *significant if $p<0.05$.

ratio of the thymus between groups (Figure 7). Still, the weight of the thymus showed a substantial increase in groups injected with MG patient's plasma (Figure 6). This result showed thymus hypertrophy in groups injected with MG patient's plasma.

IL-2 levels were higher in patients with myositis, but on the other hand, the serum IL-2 levels were lower in patients with Guillain-Barre Syndrome.(26,27) IL-2 is a cytokine related to the development and expansion of T-effector cells in peripheral blood. There is a deficient IL-2 level in autoimmune disease which showed the relation between T-reg and IL-2 levels in autoimmune disease.(28,29) A previous study found that the IL-2 level in MG patients was lower compared to healthy persons. Still, the IL-2 level decreased significantly in MG patients with thymoma who had undergone thymectomy surgery. Therefore, it showed that the IL-2 level is related to the pathology of the thymus.(30) IL-2-knockout mice showed that the progression in autoimmunity was more severe compared to the control group, indicating the association between IL-2 and the disease progression.(31) This study showed that the groups injected with low and high T-reg levels had low IL-2 levels compared to the group injected with normal plasma, indicating that the T-reg level did not affect the pathology of IL-2 in autoimmune disease.

IL-2 is reported to be linked to experimental autoimmune MG. IL-2 is secreted by activated Th1 related to the immune responses towards AChR. The autoimmunity towards AChR, as seen in MG, was proven to upregulate the production of IL-2 along with IFN-γ.(32) A study in human subjects reported an increase in serum IL-2 levels in patients with generalized MG compared to normal subjects. IL-2 can recover T-reg homeostasis in MG patients who had suffered from T-reg impairment.(33)

The role of IFN-γ in the innate immunity system due to the proliferation of T-cells in the thymus and autoimmunity is related to the severity of the disease. However, some studies showed contradictory results. Previous study showed that the pathology in MG was related to interferonopathies, especially in those with thymoma.(34) In this study, we found that the level of IFN-γ was lower in the group injected with the plasma of MG patients (both low and high T-reg levels) compared to control groups, indicating that there was a role of autoimmunity.

IFN-γ and IL-2 levels are both related to the proliferation of T-cells in the thymus. The maturation and proliferation of T-cells cause an elevation of the serum IFN-γ and IL-2 levels, respectively.(35,36) Both cytokines belong to Th-1 groups that were produced in the peripheral immune system related to inflammation. In patients with chronic infection, the elevation of IFN-γ co-stimulated with IL-2, but IL-2 will increase alone during the treatment.(37) Another study showed the opposite result: an elevation of both IL-2 and IFN-γ levels in MG mice compared to the control.(38)

IFN-γ, produced in the neuromuscular junction of transgenic mice, upregulates the experimental autoimmune MG, which leads to the manifestation of MG-like syndrome. IFN-γ is thought to destroy AChR in the postsynaptic membrane.(32) A previous study in mice induced with autoimmune MG concluded that IFN-γ is necessary for the production of the anti-AChR immune response. The IFN-γ knockout mice had normal proliferation of lymph node cells which were primed with AChR compared to wild-type mice. The wild-type mice subsequently suffered from muscle weakness and died while the IFN-γ knockout mice did not show any clinical signs of MG. This indicated the mice's susceptibility to clinical experimental autoimmune MG related to IFN-γ.(39) Similarly, another study inducing experimental autoimmune MG in C57BL/6 mice showed an increase in IFN-γ level of AChR- and LRP4-immunized mice. This indicated the important role of IFN-γ in experimental MG related to AChR.(40)

The difference with this study is we used mice injected with healthy control plasma, while in the previous study, they used animals that had not been given any treatment as the control. The limitation of this study was that it did not measure the cytokine level of the patient's plasma which was injected into the mice. Further studies are warranted to prove that the changes in cytokine levels and motoric status of the mice are due to the autoimmunity process. Changes in antibody levels before and after intervention should be examined. To avoid bias of plasma difference being injected, the patient's plasma can be mixed and injected into the mice. The interleukin level of the patient's plasma should also be measured.

Conclusion

Low T-reg level was associated with lower motor function, muscle width, increased thymus weight, as well as lower IL-2 and IFN-γ levels. T-reg level affects the motor function of Swiss-Webster mice, which is a clinical finding of MG, and the cytokine level, while the animal model injected with plasma patient MG did not show the pathology of the thymus. T-reg level contributed to clinical myasthenic syndromes but not pathological findings. The findings of

this study could be used to develop an animal model using Swiss-Webster mice.

Acknowledgments

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Authors Contribution

EDP and EHP were involved in the study conception and design. EDP performed the data collection. EDP, EHP, JSP, and RR was involved in the data analysis and interpretation of results. EDP, EHP, JSP, RR, and FO prepared the manuscript draft. All authors reviewed the results and approved the final version of the manuscript.

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