Invited Review

The Role of the Thymus in the Pathogenesis of Myasthenia Gravis

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Myasthenia gravis (MG) is an autoimmune disease characterized by the production of anti-acetylcholine receptor (AchR) antibodies (Lindstrom et al. 1976). The autoantibodies impair neuromuscular transmission by blocking the function of AchR at post-synaptic sites of the neuromuscular junctions. Ocular symptoms with fluctuating diplopia and ptosis are the commonest manifestation of the disease and, in the majority of patients, MG symptoms become generalized, affecting the proximal parts of limbs, axial muscles, and bulbar muscles. Based on the findings that the removal of immunoglobulin in the blood by plasma exchange resulted in a temporary improvement of the muscle strength and the passive transfer of sera from patients to mice caused muscle weakness in the animals, the existence of autoantibodies against nicotinic muscarinic receptors was found in the blood of MG patients (Lindstrom et al. 1976).

The induction and sustained production of autoimmune antibodies is dependent on regulatory T cells, and the thymus is a critical organ for the T cell education and elimination of autoreactive T cells. Ninety percent of MG patients display thymic abnormalities, such as hyperplastic thymus (70%) and thymoma (20%), and thymectomy is an important choice for MG treatment. In Early-onset MG (EOMG), defined as presenting before the age of 40, hyperplastic thymus is commonly observed, while thymoma is more frequent in older patients (Vincent et al. 2001). Thus, the thymus has been the most attractive target of MG research and many pathological and immunological studies have been done. However, the regulatory system for the T cell-mediated antibody production in MG and the immunological difference between patients with hyperplastic thymus and patients with thymoma are not well understood. Here, I will discuss the role of the thymus in the
Epidemiology and clinical features of myasthenia gravis

Once the diagnosis of MG is made, an acetylcholinesterase inhibitor is chosen to improve the muscle weakness by increasing the acetylcholine concentration in the neuromuscular junction. Usually, immunosuppressive therapy is also employed to suppress autoreactive T cells and to reduce the production of autoantibodies. Although there are few double-blinded placebo-controlled studies for the treatment of MG, glucocorticoid has been the usual choice for immunosuppressive therapy. Thymectomy has been an important choice for MG therapy in most countries including Japan (Kawaguchi et al. 2004). For patients with thymoma, thymectomy is necessary to prevent death by tumor invasion and/or metastasis. However, no evidence-based studies for MG treatment are available and a recent study, which summarized previous reports concerning the clinical outcome after thymectomy, showed no significant benefit of thymectomy in MG patients without thymoma (Gronseth and Barohn 2000). However, many reports have found that the clinical benefit of thymectomy is greatest in EOMG. I will discuss the clinical benefits of thymectomy in MG therapy based on data obtained in Tohoku University Hospital and branch hospitals (Ohuchi et al. 2000 and unpublished data). We followed 422 cases for 1 to 25 years after therapeutic thymectomy plus glucocorticoid. Before MG therapy, 25% of the patients had only ocular symptoms and 75% of the patients showed the generalized form of MG. Thymoma was observed in 28% of the patients. More than 80% of the non-thymoma cases showed hyperplastic thymus. Generally, thymoma was observed in patients > 30 years old at a male:female ratio of 1:1, while the male:female ratio in EOMG (most of them had hyperplastic thymus) was 1:2. Thymoma-bearing MG patients were rare in cases < 30 years old.

It takes a relatively long time to achieve clinical improvement after the initiation of MG therapies. During the follow up period of > 12 months, MG patients without thymoma showed significantly better clinical courses compared with the patients with thymoma (Table 1). During the long-term observation period, approximately 30% of the non-thymoma cases could be treated without glucocorticoid, whereas glucocorticoid was successfully discontinued only in 10% of the thymoma cases. In a longer follow up period (> 5 years), approximately 90% of the thymoma cases had survived, while death in non-thymoma cases was quite rare (Ohuchi et al. 2000 and unpublished data). This could be explained by the higher average age of the thymoma cases compared to that of the non-thymoma cases. The causes of death in the patients with thymoma were invasion and/or metastasis of thymomas, stroke, ischemic heart disease, and other malignancies. Our results suggested that thymectomy in non-thymoma cases (most of them had hyperplastic thymus) was beneficial. Many previous reports described the benefit of thymectomy in EOMG based on non-blinded results (Vincent et al. 2001). However, a double-blinded study on the effect of thymectomy in MG is practically impossible and the therapeutic effects by thymectomy and glucocorticoid cannot be analyzed separately, which makes an evidence-based estimation of MG therapy quite difficult. The therapeutic effectiveness of thymectomy could be estimated by an analysis of the

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<th>non-thymoma (n = 304)</th>
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<tr>
<td>Remarkable improvement</td>
<td>70</td>
<td>55 (%)</td>
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<td>Moderate improvement</td>
<td>25</td>
<td>30</td>
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duration between the onset of MG symptoms and thymectomy.

Immune pathogenesis of myasthenia gravis

Thymus

The thymus is the primary organ for T lymphocyte production. Precursor T-cells migrate from the bone marrow to the thymus throughout life and the early T cell precursors (CD3 CD4− CD8−) localize in the thymic cortex (subcapsular area). Maturating cells undergo cell division and T-cell receptor (TCR)-chain rearrangement, and develop into double-positive CD4+CD8+ thymocytes. Immature T-cells undergo either positive or negative selection and, finally, CD4+ or CD8+ single-positive T cells located in the thymic medulla leave the thymus to enter the peripheral circulation. After adolescence, the thymus undergoes a progressive reduction in size due to the reduced volume of thymic epithelial cells and a decrease in thymopoiesis, which results in the decrease in the output of newly developed T cells and reduced numbers of naive T cells in the peripheral blood. Histologically, there is a marked decrease in the volume of the thymic cortex, while the reduction in the size of the medulla is milder. This suggests that the age-related thymic involution induces a reduction of the early stage T cell development, leaving the function of the thymic medulla unchanged. However, the age-related change of the population of thymocytes is minimal (Table 2). Although the mechanism of this thymic involution is not fully understood, cytokines or bone marrow transplantation can partly increase the naive T cell production.

MG thymus. The thymus is a chimeric organ comprised of a central region, where thymocytes differentiate and proliferate in the thymic epithelial space, and of a peripheral region, where T lymphocytes migrate from the peripheral circulation into the perivascular space. Normally, the amount of thymic epithelial space decreases with age. The hyperplastic MG thymus has numerous T cells in the perivascular space (Flores et al. 1999), whereas the true epithelial space is atrophic in many MG thymes, reflecting the decreased percentage of immature double positive cells. Another important pathology of the hyperplastic thymus is the formation of germinal centers, where B cells produce anti-AchR antibodies. In most thymoma cases, the tumor cells are of thymic epithelial origin and there are no germinal centers or B cell clusters. Interestingly, the co-existence of hyperplastic thymus and thymoma is observed in some MG patients, suggesting a similar pathophysiological background.

Interleukin-2 receptor. Interleukin-2 (IL-2) is an essential cytokine for T cells. IL-2 receptor complex is formed by alpha, beta, and gamma subunits. Beta and gamma subunits are necessary for IL-2 signal transduction and the alpha subunit plays a role in increasing the affinity between IL-2 and IL-2R complex (Nakamura et al. 1994). The gamma subunit is expressed in the majority of mature T cells and beta subunit expression is a critical step for the regulation of IL-2 signaling in both control and MG thymocytes. In mice, IL-2R beta subunit-negative/high TCR cells are generated through the mainstream T cell differentiation in the thymus, while IL-2R beta subunit-positive cells are generated via an extrathymic pathway or an alternative intrathymic pathway. In normal thymus, less than 1% of thymocytes express beta subunit. Interestingly, human thymic T cells ex-

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<th>&lt; 12 years old (n = 5)</th>
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<tr>
<td>Double positive</td>
<td>77.4 ± 5.3</td>
<td>78.7 ± 5.5</td>
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<tr>
<td>CD4 single positive</td>
<td>9.0 ± 0.6</td>
<td>7.6 ± 0.7</td>
</tr>
<tr>
<td>CD8 single positive</td>
<td>3.8 ± 9.5</td>
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Mean ± s.d.
press lower levels of both alpha and beta subunits than mouse thymic T cells do. The gamma subunit has been shown to be expressed in a variety of lymphoid cells, including T, B, and natural killer (NK) cells. In human thymocytes, the gamma subunit is expressed on few double negative or double positive cells, and is expressed on the majority of single positive cells.

There are no significant differences in the population of thymocytes (double positive cells, CD4+CD8- single positive cells, and CD4+CD8+ single positive cells) expressing alpha, beta, or gamma subunits between control and MG thymuses. Thymocytes obtained from MG patients show higher sensitivity to IL-2 treatment than those from controls (Kaminsky et al. 1993). These findings could be explained by the fact that the MG thymus has a greater number of single positive cells, which exhibit more IL-2 receptor complex and can react more strongly to exogenous IL-2 than double positive cells. Less than 4% of double positive cells from normal and MG thymuses express the gamma subunit.

**Thymic expression of genes associated with apoptosis.** Our systematic microarray and polymerase chain reaction (PCR) analysis showed that the messenger RNA (mRNA) levels of apoptosis-associated molecules, such as bcl2, bcl-xL, bad, and caspase-3, in MG thymuses were similar to those in the control thymus (Nagata et al. 2001 and unpublished data). Thus, the basic function of positive and negative selection for thymocytes is not abnormal in the MG thymus. However, the mRNA levels for c-myc and max were markedly decreased in the MG thymuses compared with the control thymuses (Nagata et al. 2001). In contrast, the mRNA levels of mad-1, max’s another heterodimerization partner, were unaltered in MG thymuses. In the myc family genes, transcriptional activation is mediated exclusively by Myc:Max heterodimers, whereas Max:Max and Mad:Max complexes mediate transcription repression through identical binding sites. The expression of c-myc is associated with cell proliferation since c-myc expression increases in proliferating cells and decreases with terminal differentiation. The intracellular signal transduction molecule signal-transducing adaptor molecule (STAM), which transduces IL-2 receptor-mediated signals, has been reported to induce c-myc expression (Takeshita et al. 1997). The STAM mRNA expression in the MG thymus was similar to that in the control thymus. Interleukin-7, an important cytokine for T-cell differentiation, is produced by thymic epithelial cells and can induce c-myc mRNA expression in thymocytes. However, we failed to observe a difference in the level of IL-7R mRNA between control and MG thymuses. The NFkappaB family of transcription factors regulates a number of genes, such as c-myc and p53. However, the mRNA levels of c-rel and p65, subunits of NFkappaB, were unaltered in MG thymuses. The interpretation of the reduced level of max mRNA in MG thymuses is rather complicated. Unlike c-myc, the steady-state level of max is not affected by proliferation or differentiation stimuli. Treatment of human myeloid leukemia cells with protein phosphatase inhibitors resulted in the down-regulation of both c-myc and max gene expression followed by apoptosis. Many putative target genes of c-myc have been reported, such as ornithine decarboxylase, cdc25A, prothymosin-alpha, p53, and cdc2. Prothymosin-alpha is known as a thymic hormone whose function is related to cell proliferation. However, the mRNA levels of prothymosin-alpha in control and MG thymuses were similar.

**Chemokines**

**Chemokine receptor CXCR3 (Fig. 1)**

Chemokines are a group of structurally-related heparin binding cytokines essential in the immunological processes because they recruit selective subsets of lymphocytes to the target compartments. Chemokines and their receptors play critical roles in T cell activation, immune surveillance, and tolerance. Moreover, distinct chemokine receptors are associated with the polarization of T-helper (Th) 1 and Th2 cells. The chemokine receptors are differentially expressed on T cells according to their functional polarization. Chemokine receptors CXCR3 and CCR5 are preferentially expressed by Th1 cells, while CCR3 and CCR4 are expressed by a subset of
Th2 cells (Sallusto et al. 1998; Imai et al. 1999). CCR2 is expressed on activated and memory T cells polarized to both Th1 and Th2 subclasses.

Th1 signals have been reported to be critical in inducing experimental autoimmune myasthenia gravis (EAMG). Interferon (IFN)-γ (Th1 cytokine) expression in the neuromuscular junction induces a MG-like syndrome (Gu et al. 1995) and IFN-γ deficient mice but not IL-4 (Th2 cytokine) deficient mice are resistant to EAMG induction (Balasa et al. 1997). However, in human MG, AChR-specific CD4+ cells of patients could produce both Th1-type and Th2-type cytokines (Yi et al. 1994). We observed a significant decrease in the frequency of CD4+ T cells expressing Th1-type chemokine receptor CXCR3 in the peripheral blood of untreated MG patients (Onodera et al. 2003). Importantly, there was a significant inverse correlation between the percentage of CXCR3-positive CD4+ T cells of untreated patients and the disease severity. As mentioned above, MG patients with thymoma are more resistant to treatment compared with patients with hyperplastic thymus. Since the frequency of CXCR3-positive CD4+ T cells of the thymoma group was lower than that of the hyperplasia group, it may be attractive to hypothesize that CXCR3-mediated signaling is closely associated with the pathophysiology of MG, particularly for those with thymoma. Interestingly, the frequencies of CCR5-positive CD4+ cells were normal in the hyperplastic thymus and the thymoma before and after MG therapy. The CXCR3-positive CD4+ cells can accumulate at sites of Th1-type inflammation and produce IFN-γ, irrespective of the presence or absence of CCR5 expression. In rheumatoid arthritis, a well-known Th1-type disease, infiltrating T cells in synovial fluid are highly positive for CXCR3 and much less frequently positive for CCR5 (Suzuki et al. 1999). The proportion of CXCR3-positive CD4+ T cells is increased in the bronchoalveolar lavage fluid of patients with sarcoidosis (Katchar et al. 2003). Thus, the thymus or neuromuscular junction may be an important target tissue, which could explain the reduced frequency of CXCR3-positive peripheral blood CD4+ cells in MG. Since the frequency of CXCR3-positive CD4+ single positive thymocytes from thymomas is higher than that from hyperplastic thymus, thymoma may be one of the targets of CXCR3-mediated immune reactions. Another target of the CXCR3-positive T cells is the neuromuscular junction, where lymphocytes and immune-complex accumulate. The muscle may contribute to the disease progression of MG by producing soluble factors that influence the ac-
tivities of the immune system. The chemokine signaling of T cells in MG patients could be influenced by chemokines produced in the muscle. To identify the chemokines important for the disease activity of MG, it is necessary to study the expression of CXCR3 ligands, as well as the frequency of CXCR3-positive CD4+ T cells, in the neuromuscular junction. The frequency of CXCR3-positive CD4+ T cells in the peripheral blood returns toward the control level long after therapy. The percentage of CXCR3-positive CD4+ T cells and the elapsed months after thymectomy were found to be significantly correlated. CXCR3 in the peripheral blood could be used as a marker of the disease activity. We observed no significant changes of the Th2-type receptors CCR3 and CCR4 in MG. Thus, the signals mediated by the Th1-type chemokine receptor may play a critical role in the immune dysfunction of MG. Myasthenic symptoms are sometimes worsened after infections, and chemokines are tightly integrated in both innate immunity and adaptive immunity. Modulation of the biased CXCR3 signaling may be a promising candidate for future MG treatment, particularly for patients with thymoma.

**Chemokine receptor CCR7**

CCR7 is essential for the localization of naive T cells and central memory T cells to the thymus and secondary lymphoid organs. The homeostatic chemokines CCL19 and CCL21 use CCR7 as their specific receptor and play distinct roles in lymphocyte migration in the thymus (Yoshie et al. 2002). Mice with targeted disruption of CCR7 show morphological abnormalities in the lymphoid organs, with severely impaired homing of lymphocytes and dendritic cells to these organs. Mature dendritic cells also migrate into lymphoid organs with the guidance of CCR7 ligands and present antigens to T cells. The ectopic expression of CCL21 in mice can induce the infiltration of T cells and dendritic cells, which is followed by the development of secondary lymphoid organ-like structures, including germinal centers. In peripheral blood, > 80% of T cells are positive for CCR7.

The CCL21 mRNA levels in hyperplastic MG thymi are markedly higher than those in the control thymuses, while those in the thymomas are within a normal range. Accordingly, very strong CCL21 immunoreactivity is observed in stromal cells of the hyperplastic thymuses, whereas other structures, such as lymphocytes, follicular dendritic cells, or dendritic cells, lack CCL21 immunoreactivity. In contrast, the CCL19 mRNA levels and CCL19 immunostaining intensities are similar in the control and hyperplastic thymuses. Thus, in the hyperplastic thymus, CCL21 is selectively overexpressed by a specific type of stromal cell. The total number of dendritic cells is similar between hyperplastic thymus and thymoma. However, the CD83-positive mature dendritic cell density is significantly higher in hyperplastic thymus than in thymoma. The frequencies of single positive T cells are similar in hyperplastic thymuses and thymomas and more than 90% of single positive cells expressed CCR7 in both hyperplastic thymuses and thymomas. However, thymic cells separated from hyperplastic thymuses efficiently migrate to CCL21 in a concentration dependent manner, while those from thymoma migrate poorly to CCL21.

Although CCL21 and CCL19 share CCR7 as their chemotactic receptor, their roles in T cell migration are different. Normally, CCL21 guides the migration of developing T cells from the cortex to the medulla, while CCL19 guides the emigration of mature T cells out of the thymus. CCL19 localizes in the medullary vessels, whereas CCL21 localizes in the epithelial cells surrounding these vessels. Thus, the extraordinarily high levels of CCL21 and the normal levels of CCL19 in the hyperplastic thymus may prevent the emigration of mature T cells and keep them within the thymus. In addition, recirculating T cells can home to secondary lymphoid organs guided by CCR7 and cell adhesion molecules. Thus, peripherally circulating CCR7-positive T cells (naive and central memory T cells) may also migrate into the hyperplastic thymus. Since CCL21 overexpression alone can induce an abnormal accumulation of T cells and dendritic cells that eventually lead to the development of secondary lymphoid organ-like structures, many
pathologic features of the hyperplastic thymus could be explained by the CCL21 overexpression. The thymic myoid cells express acetylcholine receptors and hyperplastic thymus shows an abnormal distribution and faster degeneration of myoid cells, which may increase the level of autoantigens available for dendritic cells. Thus, naive and central memory T cells in the hyperplastic thymus could have a higher chance of being activated by mature dendritic cells, which also accumulate because of the elevated levels of CCL21 and potentially present autoantigens pathogenic for MG.

**Chemokine receptor CXCR5 (Fig. 2)**

Non-Th1 and non-Th2 effector T cells, which express chemokine receptor CXCR5, localize in B cell areas in secondary lymphoid organs and provide help to B cells. These CXCR5-positive CD4⁺ T cells, called follicular B helper T cells (T<sub>FH</sub>s), play a pivotal role in the B cell help function in the B follicles of the secondary lymphoid organs (Moser et al. 2002). CXCR5 is also expressed in the majority of B cells, whereas mature B cells differentiated in the B follicles and plasma cells do not express CXCR5. T<sub>FH</sub>s and B cells localize to the B follicles of the secondary lymphoid organs attracted by the CXCR5’s only ligand, CXCL13 (also known as B cell-attracting chemo-

Fig. 2. The percentages of CXCR5-positive CD4⁺ T cells in peripheral blood of the MG patients and the control subjects. (A) The patients at severer disease stages showed higher percentages of CXCR5-positive CD4⁺ T cells. Bars indicate mean values. Hyperplastic thymus (open circles), thymoma (filled circles). (B) The frequency of CXCR5-positive CD4⁺ T cells decreased after therapy. (C) The percentages of CXCR5xCD4⁺ T cells in the peripheral blood of the MG patients were compared between the MG patients having other autoantibodies (either anti-nuclear antibodies, anti-thyroglobulin antibodies, antiperoxysome antibodies, anti-thyroglobulin antibodies, or rheumatoid factor) together with anti-AChR antibodies (Abs) and those having only anti-AChR autoantibodies. The former group had significantly higher TFH percentages.
Myasthenia Gravis and Chemokines

FH

With higher percentages of CXCR5+ T cells, patients with MG have systemic abnormalities in the antibody production system mediated by T\textsubscript{FH}. A follow-up study will be able to answer the question of whether the MG patients with higher percentages of CXCR5+CD4+ T cells in the peripheral blood have a higher risk to develop other autoimmune diseases.

It is of particular interest that, compared with the pretreatment group, a significant decrease of the T\textsubscript{FH} frequency was observed relatively long after initiation of MG therapy. There was a significant inverse correlation between the percentage of CXCR5+CD4+ T cells and the duration after therapeutic thymectomy. The gradual normalization of the T\textsubscript{FH} frequency after therapy may reflect a slow correction of the immune dysfunction, and may also relate to the slow decline of the anti-AChR antibody titer and the gradual recovery from muscle weakness.

Natural killer T cells and Natural killer cell (Fig. 3)

The disease activity of MG is frequently associated with various types of stress and infection. NK cells and NKT cells participate in the innate immune responses and contribute to combating viral and non-viral infections. Immune cells with NK markers can influence both Th1 and Th2 functions. NK cells activate Th1 helper effector cells by secreting IFN-gamma. NKT cells can promote Th2 development by secreting a large amount of IFN-4 (Yoshimoto and Paul 1994), and the suppression of NKT cell function could induce Th1-type diseases. Human NK cells can be divided into two subsets CD16\textsuperscript{bright} NK cells, and CD16\textsuperscript{dim} NK cells (Cooper et al. 2001). CD16\textsuperscript{dim} NK cells are more cytotoxic, whereas the CD16\textsuperscript{bright} subset can secrete cytokines but has lower cytotoxic activity. We analyzed the subpopulations of NK and NKT cells in the peripheral blood and thymuses of MG patients. Mature NKT cells are defined as those with the TCRV\textsubscript{alpha}24+CD161\textsuperscript{bright} phenotype and immature NKT cells as those with the TCRV\textsubscript{alpha}24+CD161\textsuperscript{dim} phenotype. The population of mature NKT cells in the peripheral blood mononuclear cell (PBMC) of the normal subjects was very low, comprised only 0.2%. Before MG therapy, the percentages of mature NKT cells in the blood of the patients with hyperplastic thymus were significantly elevated in comparison with those of the control subjects (Suzuki et al. 2005).
In contrast, the patients with thymoma showed normal frequencies of mature NKT cells. The frequencies of immature NKT cells were similar among the control subjects, the MG patients with hyperplastic thymus, and the MG patients with thymoma. The percentages of both NKT cell subpopulations (TCR^\(\alpha_24^+\)CD161\^bright\) NKT cells and TCR^\(\alpha_24^+\)CD161\^dim\) cells) in the control and MG thymuses were below the limit of detection. The thymic origin of NKT cells is proved by the fact that NKT cells develop in fetal thymus organ culture and that NKT cells are completely absent in athymic nude mice (Habu et al. 1986). However, NKT cell maturation and activation occur extrathymically. Thus, unaltered percentages of NKT cells in MG thymuses and thymomas suggest that the thymic contribution to the NKT cell function is minimal. Since NKT cell maturation occurs after emigration from the thymus (Habu et al. 1986), the frequencies of NKT cells of MG patients with hyperplastic thymus could be determined by factors outside the thymus.

We classified the NK cells into CD3^-CD16^+CD56^dim\) NK cells and CD3^-CD16^-CD56^bright\) NK cells. The CD16^-CD56^dim\) NK cells comprise the majority of peripheral blood NK cells. Before MG therapy, the percentages of CD16+CD56dim NK cells in the peripheral blood of the patients with thymoma were significantly lower than those of the control subjects (Suzuki et al. 2005), while those in the patients with hyperplastic thymus were also lower, though not significantly. The percentages of CD3^-CD16^+CD56^dim\) NK cells in the patients with thymoma returned to normal after therapy. Both patient groups showed normal percentage ranges of CD3^-CD16^-CD56^bright\) NK cells before and after treatment. Although CD3^-CD16^-CD56^dim\) NK cells are the major NK subclass in the blood, the majority of NK cells in the thymuses were CD3^-CD16^-CD56^bright\) NK cells in the control thymuses, hyperplastic thymuses, and thymomas. There

Fig. 3. Natural killer cell subsets in blood of myasthenia gravis patients with thymic hyperplasia or thymoma and in healthy controls. Data are expressed as the percentages of peripheral blood mononuclear cells.
were no significant differences in the frequencies of the two NK subclasses in the hyperplastic thymus and thymomas compared with the control thymus. With their poor proliferative response and higher cytotoxicity, CD16^+CD56^{dim} NK cells are believed to be more mature than CD16^-CD56^{bright} NK cells. As CD16^+CD56^{dim} NK cells have strong antibody-dependent cellular cytotoxicity (Cooper et al. 2001), the present result is consistent with a previous one showing that the NK activity in the blood of MG patients was lower than that of controls (Kott et al. 1990). The development of NK cells occurs outside the thymus and NK cells are present in nude mice. Circulating NK cells survey pathogens and home into such areas as those having inflammation. Thus, the decrease of CD16^+CD56^{dim} NK cells in the blood of patients with thymoma may reflect the mobilization of these cells into the neuromuscular junction, where immune cells accumulate.

It is necessary to compare the NK cell density in the neuromuscular junction between patients with hyperplastic thymuses and those with thymomas. Since patients with thymoma are more resistant to treatment than those with hyperplastic thymus, the disease activity of patients with thymoma may be modulated by NK cells, particularly when their conditions are exacerbated by infection.

The pathophysiology of MG with hyperplastic thymus is becoming clearer and the intrathyamic antibody production, increased number of activated dendritic cells, and enhanced production of autoreactive T cells can explain why thymectomy has a therapeutic effect in EOMG (Fig. 4). However, the MG pathomechanism of thymoma-bearing patients remains to be clarified. Altered intercellular signaling via chemokine receptors can explain many immune abnormalities in MG.

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![Diagram](https://example.com/diagram.png)

**Fig. 4.** Pathogenic model for myasthenia gravis with hyperplastic thymus. The extraordinarily high levels of CCL21 and the normal levels of CCL19 in the hyperplastic thymus may prevent the emigration of mature T cells and keep them within the thymus. Furthermore, recirculating T cells can home to secondary lymphoid organs attracted by CCL21. Thus, peripherally circulating CCR7-positive T cells (naive and central memory T cells) may also migrate into the hyperplastic thymus, attracted by the high CCL21 concentration. The thymic myoid cells express acetylcholine receptors and the higher rate of myoid cell degeneration in the hyperplastic thymus can increase the level of autoantigens available for dendritic cells. Taken together, T cells in the hyperplastic thymus may have a higher chance to present antigens of the neuromuscular junction. The increased number of CXCR5-positive CD4^+ T cells may enhance the antibody production by B cells. Altered Th1-type chemokine signaling via CXCR3 influences the cellular autoimmunity in the neuromuscular junction.
Biased chemokine signaling has been observed in many autoimmune diseases and specific chemokine receptors, such as CXCR3, are used as HIV co-receptors. The modification of chemokine signals is an attractive target for the treatment of various kinds of diseases and many potential approaches, such as the use of monoclonal antibodies, chemokine receptor agonists and antagonists, are being studied. MG is one of the best examples of autoimmunity to a specific antigen, and understanding of the MG pathomechanism will provide important information concerning the etiology and therapy of many autoimmune diseases.

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References


Takeshita, T., Arita, T., Higuchi, M., Asao, H., Endo, K., Kuroda, H., Tanaka, N., Murata, K., Ishii, N. & Sugamura,


