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# Altered expression of chemokine receptor CXCR5 on T cells of myasthenia gravis patients

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**Abstract**

Myasthenia gravis (MG) is characterized by the T cell-dependent production of anti-acetylcholine receptor (AChR) antibodies. The chemokine receptor CXCR5 regulates lymphocyte migration and is expressed on a subset of $CD4^+$ T cells named follicular helper T cells ($T_{FH}$), the key modulators of antibody production by B cells. We studied the frequency of CXCR5-positive lymphocytes in the peripheral blood of MG patients before and after therapy (thymectomy plus glucocorticoid). Before therapy, the MG patients showed a significantly higher frequency of $CXCR5^+$ $CD4^+$ T cells in the peripheral blood compared with the control group, while no significant difference in the percentages of $CXCR5^+$ $CD4^+$ T cells was observed between the patients of the hyperplasia group and those of the thymoma group. The $CXCR5^+$ $CD4^+$ T cell frequency correlated with the disease severity. The $CXCR5^+$ $CD4^+$ T cell frequency of MG patients positive for other autoantibodies together with anti-AChR antibodies was significantly higher than in those having only anti-AChR antibodies. After therapy, the $CXCR5^+$ $CD4^+$ T cell percentage decreased gradually to the control level with a significant inverse correlation between the $CXCR5^+$ $CD4^+$ T cell frequency and duration after the initiation of MG therapy. The $CXCR5^+$ $CD4^+$ T cell populations in the hyperplastic thymuses and thymomas were not significantly different from those in the control thymuses. These results suggest that $CXCR5^+$ $CD4^+$ T cells play an important role in the disease activity of MG and that some MG patients have a systemic abnormality in T cell-dependent antibody production.


*Keywords:* Myasthenia gravis; Chemokine; Helper T; CXCR5; Thymus

## 1. Introduction

Myasthenia gravis (MG) is an autoimmune disease characterized by the production of anti-acetylcholine receptor (AchR) antibodies (Drachman, 1994; Li et al., 1998; Lindstrom et al., 1976), and the induction and sustained production of autoimmune antibodies is dependent on suppressor T cells (Gomez and Richman, 1985; MacLennan et al., 1997; Vincent et al., 2001; Yi et al., 1994). A subclass of $CD4^+$ memory T cells enters the follicles of secondary lymphoid organs and regulates the differentiation and antibody production by B cells (B cell help). Th2 cells can induce proliferation and class switching in B cells. However, Th2 cells are not the critical subtype of T cells for the B cell help function, since the B cell help function occurs in mice deficient in the Th2 cytokine IL-4 (Balasa et al., 1998). Recent studies showed that non-Th1 and non-Th2 effecter T cells, which express chemokine receptor CXCR5, localize in B cell areas in secondary lymphoid organs and provide help to B cells (Moser and Ebert, 2003). A subset of CXCR5-positive $CD4^+$ T cells, called follicular B helper T cells ($T_{FH}$s), plays a pivotal role in the B cell help function in the B follicles of the secondary lymphoid organs (Moser et al., 2002; Yoshie et al., 2001). CXCR5 is also

*Abbreviations:* MG, myasthenia gravis; $T_{FH}$, follicular helper T cell; AchR, acetylcholine receptor; Ab, antibody; MGFA, The Myasthenia Gravis Foundation of America.

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expressed in the majority of B cells, whereas plasma cells do not express CXCR5 (Pevzner et al., 1999). $T_{FH}$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 chemokine 1, BCA-1), that is produced by follicular dendritic cells in B follicles (Gunn et al., 1998; Legler et al., 1998). Animals with disrupted CXCL13 gene (Gunn et al., 1998) or CXCR5 gene (Forster et al., 1996) show impaired localization of the $T_{FH}$s and B cells into B follicles and very low levels of antibody production.

The thymus is a critical organ for the T cell education and elimination of autoreactive T cells. Patients with MG display a high prevalence of thymic abnormalities and thymectomy is an important choice of treatment. However, the regulatory system for the T cell-mediated antibody production in MG is unknown. Although improvements of muscle strength and disease parameters are achieved after treatment, the effects of therapeutic thymectomy on T cell functions, particularly for the T cell subgroup with the B cell helper function, remain unknown. In order to analyze the T cell-dependent B cell function in MG, we studied the CXCR5 receptor expression on peripheral blood lymphocytes of MG patients before and after therapy, and on thymocytes with special reference to the thymic histopathology and clinical parameters.

## 2. Patients and methods

MG was diagnosed based on clinical and electrophysiological findings, and on the detection of anti-AchR antibodies (binding antibodies). The Myasthenia Gravis Foundation of America (MGFA) classification was used to evaluate the distribution of weakness and clinical severity in each patient (Barohn, 2003). All patients were positive for anti-AchR Abs and their thymus histology was determined at thymectomy. In the pretreatment groups, 30 patients had hyperplastic thymus and 23 patients had thymoma (Table 1). The thymoma group consisted of cases of clinical stage I, II or III according to the Masaoka's classification (Monden et al., 1988) and WHO pathology classification of type A, AB, B1, B2, or B3 thymoma. For the post-thymectomy MG groups, blood was taken from the MG patients with hyperplasia ($N=26$) and with thymoma ($N=19$) at varying times after thymectomy (Table 1). The early sampling points after thymectomy was fixed at 1 or 2 months after thymectomy. Thereafter, blood samples were taken >4 months after thymectomy. In 6 cases, blood samples were taken both before and after thymectomy and the results are shown separately (Fig. 3). All MG patients received glucocorticoid after thymectomy as soon as it was confirmed that there were no infections after operation and they were never treated with other immunosuppressants. The control group (21 healthy volunteers) and MG group had no evidence of acute infections when their blood was collected. Peripheral blood mononuclear cells (PBMC) were separated by centrifugation on a Ficoll-Hypaque (Pharmacia Biotech, Sweden) density gradient and were washed three times with phosphate buffer containing 2% bovine serum albumin (BSA) for flowcytometry.

Table 1
Populations of peripheral blood lymphocytes in myasthenia gravis patients (hyperplasia group and thymoma group) and the control subjects

| Lymphocyte population (%) | $CD4^+$ T cell | $CD8^+$ T cell | B cell |
|---|---|---|---|
| Control ($N=21$) | 45.8±8.6 | 20.9±6.4 | 11.8±3.6 |
| (male/female, 10/11) | | | |
| (mean age±SD, 42.4±15.2) | | | |
| Myasthenia gravis | | | |
| Before therapy | | | |
| Hyperplasia ($N=30$) | 43.3±12.8 | 21.9±7.7 | 13.1±5.3 |
| (12/18) | | | |
| (40.1±15.7) | | | |
| Thymoma ($N=23$) | 44.0±13.0 | 20.0±5.6 | 12.9±8.0 |
| (10/13) | | | |
| (52.2±14.0) | | | |
| After therapy | | | |
| Hyperplasia ($N=26$) | 41.7±9.7 | 20.9±6.4 | 11.8±3.6 |
| (10/16) | | | |
| (41.1±11.3) | | | |
| Thymoma ($N=19$) | 47.4±10.1 | 18.9±7.5 | 10.4±7.3 |
| (10/9) | | | |
| (52.5±13.9) | | | |
| **CXCR5-positive cells (%)** | **$CD4^+$ T cells** | **$CD8^+$ T cells** | **B cells** |
| Control | 5.1±5.6 | 2.0±1.4 | 83.4±5.1 |
| Myasthenia gravis | | | |
| Before therapy | | | |
| Hyperplasia | 12.2±4.9[a] | 3.5±4.1 | 82.2±10.4 |
| Thymoma | 10.6±3.3[b] | 2.1±1.1 | 78.5±17.5 |
| After therapy | | | |
| Hyperplasia | 8.8±6.1 | 2.6±2.5 | 79.3±11.6 |
| Thymoma | 7.7±4.6 | 1.5±0.9 | 71.6±20.3[c] |

Data are expressed as percentage of cells in lymphocytic compartments of flowcytometry analysis. The percentage of CXCR5-positive $CD4^+$ T cells (follicular B helper T cell), $CD8^+$ T cells, and B cells in peripheral blood of MG and control subjects. Data are expressed as percentage of cells in $CD4^+$ T cells, $CD8^+$ T cells, and B cells, respectively.
Mean±SD.
[a] Before treatment vs control ($p=0.00011$).
[b] Before treatment vs control ($p=0.00063$).
[c] After therapy vs control ($p=0.039$).

The chemokine receptor expression in thymocytes was studied in 10 MG patients (3 men and 7 women; 42.2±19.5) who had hyperplastic thymus and in nine patients (4 men and 5 women; 56.1±14.0) who had thymoma. None of these patients was treated with glucocorticoid preceding thymectomy and all were included in the analysis of peripheral blood taken before thymectomy as mentioned above. Seven normal thymuses (2 men and 5 women; 21.7±33.4) were obtained at the time of open cardiac surgery. Freshly excised thymic tissues were freed of fat and fibrous tissues and were then washed to remove contaminating peripheral blood. In the thymoma group, "normal" thymic tissues were removed before making the thymic cell preparations and only thymic cells in thymomas were analyzed. Single thymic cell suspensions were prepared by passing minced thymic tissue