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Analysis of CD4⁺CD25⁺ Cell Population in the Thymus from Myasthenia Gravis Patients

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Abstract

The present study is aimed at exploring the regulatory CD4⁺CD25⁺ T cells in the thymus from myasthenia gravis (MG) patients. In early-onset MG, the thymus is hyperplastic and contains autoreactive activated T cells. Preliminary studies indicate that these CD4⁺CD25⁺ cells include activated autoreactive T cells. Studies to characterize the phenotype and suppressive capacity of these cells will be discussed.

Keywords

CD4⁺CD25⁺ T cells; myasthenia gravis; thymus; thymocyte population

Myasthenia gravis (MG) is mediated by autoantibodies to the acetylcholine receptor (AChR) at the neuromuscular junction. The high titer of anti-AChR antibodies is associated with thymic abnormalities. In early-onset MG, the thymus is hyperplastic and contains autoreactive activated T cells, being the effector site of the autoimmune anti-AChR response.

CD4⁺CD25⁺ T cells, which express the α -chain of the IL-2 receptor, have emerged as an important immunoregulatory T cell subset. These regulatory T cells are generated in the thymus and prevent the induction of autoimmune diseases in a number of experimental animal models.¹ Such T cells are able to suppress the proliferation of CD4⁺CD25⁻ cells and represent about 10% of CD4⁺ cells. This subpopulation has also been described in the thymus and peripheral blood of healthy humans.² Induction of MG can be associated with the deficiency in immunoregulatory cells. We analyzed the number of CD25⁺ cells and their distribution between the different thymocyte populations in the thymus from MG patients.

METHODS

Thymic tissue was obtained at thymectomy of the patients selected according to thymic histology. Patients with thymomas or those on steroid treatment were excluded from the study. Control thymuses were obtained from children aged from 11 days to 8 months who were undergoing heart surgery. Thymocytes were retrieved by gentle scraping of fresh thymic tissue, filtering, and washing them once in Hanks' balanced solution (HBSS). They were stained with mAbs to CD4, CD8, and CD25 and analyzed by flow cytometry.

RESULTS

Figure 1 shows an analysis of CD25 expression in one representative MG patient with positive anti-AChR antibody titer and in one healthy control. CD25 expression is limited mostly to CD4^{hi}CD8⁻ T cells in both patients and controls; however, the percentage of CD25⁺ cells among CD4⁺ cells is augmented in patients, 20% compared to 10% in controls. Our data on five thymuses show that CD25 expression is increased in CD4⁺ and CD4⁻CD8⁻ cells from patients with positive titer of anti-AChR antibodies, from 12% to 35% of CD4⁺ cells, whereas CD25⁺ cells comprise 10–16% of CD4⁺ thymocytes in control subjects. The increased expression of CD25 in CD4⁻CD8⁻ cells in MG patients could be related to the activated state described in the B cell population of MG thymus.³ In contrast, there is no significant difference in the numbers of CD4⁺CD25⁺ cells between seronegative patients and controls. This finding correlates with the results that the proportion of cells with high levels of Fas expression is increased in all thymocyte populations from MG patients with high titers of anti-AChR antibodies.⁴ CD4⁺Fas^{hi} thymocytes had been shown to be enriched in AChR-reactive activated cells. Since CD25 is transiently expressed upon activation of naïve CD4⁺ T cells, CD4⁺CD25⁺ cells from MG thymus must contain both the activated pathogenic cells and the resting or activated regulatory cells. Even in healthy adults, the T cell population should be enriched in activated memory cells as they encounter the foreign antigens during the course of life. In conclusion, CD25 does not represent a perfect marker for regulatory human CD4 T cells. The analysis of other cell markers in combination with CD25 will be necessary to discriminate between activated and regulatory cells.

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FIGURE 1.

Analysis of CD25 expression in the thymus of an MG patient with anti-AChR antibody titer of 11 nmol/L and in a healthy newborn control. Freshly isolated thymocytes were stained with mAbs to CD8-FITC, CD4-PerCP, and CD25-PE and analyzed by flow cytometry. Expression of CD25 in different populations is shown with a solid line, and the staining with an isotype IgG2 control with a dotted line.