INFLUENCE OF THYMECTOMY ON LEUKOCYTE MIGRATION INHIBITION TEST IN MYASTHENIA GRAVIS AND ULCERATIVE COLITIS

YOSHIHIDE ODA, TOSHIO MORISANE, YOSHIO MIZUNO, HITOSHI ASAKURA and MASAHARU TSUCHIYA

Department of Internal Medicine, School of Medicine, Keio University, Tokyo

SUMMARY

Sensitized lymphocytes to muscle homogenate in myasthenia gravis and those to colonic epithelial homogenate in ulcerative colitis were assessed before and after thymectomy, using leukocyte migration inhibition test.

In myasthenia gravis, all three patients showed slight inhibition before thymectomy, and one of them showed no significant change in inhibition 2 weeks after thymectomy. After thymectomy leukocyte migration inhibition was rather slight in 4 of 11 patients, but tended to return normal in the rest of patients. 3 patients with myasthenic crises after thymectomy showed normal migration at the time of remission obtained by ACTH therapy. Thymectomy and thymolympholysis action of ACTH therapy might be effective to get rid of auto-sensitized lymphocytes to muscle in myasthenia gravis.

As for ulcerative colitis, 2 of 4 patients showed inhibition before thymectomy. One patient showed less inhibition, and another patient showed increased inhibition after thymectomy. A patient with recent onset of ulcerative colitis showed normal migration before and after thymectomy. 6 of 8 patients after thymectomy showed inhibition. The interval between thymectomy and leukocyte migration inhibition test was less than 6 months in all cases, which might be too short to get rid of sensitized lymphocytes.

INTRODUCTION

Since the thymus in myasthenia gravis (MG) and ulcerative colitis (UC) is thought to be involved by autoimmune process, the effects of thymectomy on the results of leukocyte migration inhibition test (LMIT) are discussed. In MG
thymus abnormality has long been discussed and thymectomy has been known to be quite effective measure. Armstrong stated that lymphocytes in the thymus (thymocytes) were sensitized to muscle and became cytotoxic after stimulated by mitogen. This observation might suggest that thymus is one of the organs of producing auto-sensitized lymphocytes. And our clinical results of thymectomy in the treatment of UC have been good. The facts that not only MG and other autoimmune diseases but also UC are associated with thymitis make us suggest that thymus in UC could be one of that producing auto-sensitized lymphocytes to colon epithelium.

**MATERIALS**

LMIT was carried out in MG or UC patients admitted to Keio University Hospital. Thirteen patients (11 females and 2 males, 19 to 65 years of age) had MG for 5 months to 19 years and at the time of these studies their clinical groupes according to the Osserman Classification were of IIA to III. Thymoma was found in 3 patients. Myasthenic crises were noted in 3 patients on several occasions, and they were treated with ACTH (100 units for 10 days). However, the study was done during remission in these patients.

Nine patients (7 females and 2 males, 18 to 65 years of age) had UC for 5 months to 14 years and were off corticosteroids more than 2 weeks before LMIT. In all these patients thymectomy was carried out via supra-sternal notch by Dr. H. Yoshimatsu.

Twenty three healthy medical staffs were tested for LMIT as controls.

**METHOD**

The method of LMIT used was that described by Seborg and Bendixen. Sterile procedure was carried through as far as possible. 20 to 25 ml of blood were drawn from cubital vein into a plastic syringe containing 1000 units of heparin. The blood was allowed to sediment at room temperature for 1 hour. All but the bottom 0.5 cm of leukocytes rich plasma was transferred to polyethylene tubes, and leukocytes were washed three times in Hank’s BSS by centrifugation at 1000 rpm for 5 minutes. The cells were homogenously suspended and adjusted to 10 to 15% by volume concentration with complete medium. The suspension was aspirated into capillary tubes, and was centrifuged at 1500 rpm for 10 minutes. The capillaries were cut a little below the cell-fluid interface, and were fixed on a small circular coverglass by means of silicone wax, placed in a small circular tissue culture chambers. TC 199, adjusted pH 7.3 containing 10% horse serum,
100 μg streptomycin, 100 units penicillin G, was added in each chamber with or without antigen. The cells were incubated flat at 37°C for 24 hours. The migration area was photographed and measured (Figure 1). Migration index was calculated as:

\[
MI(\%) = \frac{\text{area of migration in presence of antigen}}{\text{area of migration in absence of antigen}} \times 100
\]

Technique of thymectomy: Thymectomy was carried out by the technique described by Yoshimatsu.\(^3\) Patient under general endo-tracheal anesthesia, placed supine with the sternum elevated. A 3 to 4 cm transverse incision was made in the supra-sternal notch, the thymus stems were exposed, isolated, and the entire thymus was freed from the adjoining mediastinal tissues by careful blunt dissection, and the thymectomy was completed by severing thymus after ligation of both thymic veins and arteries. Upon thymectomy the entire area of surgical removal of the organ was thoroughly examined with a mediastinoscope to check for any hemorrhage and thymus residue.

**Leucocyte Migration Test**

(method of Søborg and Bendixen)

\[
MI = \frac{a' \times b'}{a \times b} \times 100 \ (\%)
\]

![Figure 1](image-url) Photograph and measuring of migrated area.
Fig. 2 Leukocyte migration inhibition test of skeletal muscle homogenate in myasthenia gravis. (before and after thymectomy).

Fig. 3 Leukocyte migration inhibition test of colonic epithelial homogenate in ulcerative colitis.
Preparation of muscle homogenate: Muscle were obtained aseptically from 6 months aborted fetal muscle, minced, homogenized using a mechanical teflon homogenizer in Tris buffered saline solution containing $10^{-3}$M Ca$^{++}$ ions, and Mg$^{++}$ ions, spun for 15 minutes at $250 \times g$, and the supernatante measured for protein content by the Biuret method. Muscle homogenate was added in the culture medium in the concentration of 200 $\mu$g/ml.

Preparation of colon epithelial homogenate: The mucosa of fetal colon was stripped, washed twice in Hank's BSS, homogenized by mechanical teflon homogenizer, left at 4°C for 24 hours, spun at $1000 \times g$ for 20 minutes, and the supernatante measured for protein content by the biuret method. 100 $\mu$g/ml of colon epithelial homogenate was added in the culture medium.

RESULT

The normal range (mean ± 2SD) of the migration index with muscle homogenate calculated on the basis of 14 controls was 85.6% to 106.4%, as presented in Figure 2.

In the pre-thymectomy groupe of MG, slight decrease in MI was observed in all three patients. 4 of 11 patients in the post-thymectomy groupe showed slight decrease in MI. The interval between thymectomy and LMIT in these 4 patients is 2 weeks, 6 months, 1 year and 8 months and 2 years and 6 months. One patient with interval of 1 year and 8 months was a brittle type (clinical groupe III) and received ACTH therapy (100 units, daily, 10 days). After repeated ACTH therapy, LMIT obtained normal MI. 2 of 7 patients who showed normal leukocyte migration were of brittle type (both clinical groupe III), and were at remission after ACTH therapy.

Migration indices of UC are shown in Figure 3. The normal range (mean ± 2SD) of LMIT with colon epithelial homogenate calculated on the basis of 9 patients was 85% to 117%. 2 of 4 pre-thymectomy patients showed inhibition, but with one of them MI returned to normal after thymectomy. 2 pre-thymectomy patients showed normal migration. One of them showed decrease in MI after thymectomy. Another 18 years old patient with recent onset, showed normal migration regardless of thymectomy. 6 of 8 post-thymectomy patients showed inhibition. Within 6 months after thymectomy all patients had uneventful clinical courses, if any, requiring a small dose of corticosteroids.

DISCUSSION

For the first time Weigert described the complication of MG with thymoma.
Since Blalock\textsuperscript{5} successfully treated MG by thymectomy, the procedure has been established as one of the most effective and essential treatment. Thymus in MG shows thymitis and less often thymoma. Such thymus has lymph follicles, plasma cells, and Ig containing cells. These facts may indicate the possibility that some antigen enters into the thymus in such pathological condition, and reacts with a specific antibody or causes the production of lymphocytes sensitized to auto-antigens (cytotoxic lymphocytes). In fact, study of humoral immunity in the serum of patients with MG has disclosed various antibodies, such as anti-thymus antibody, anti-muscle antibody, anti-microsome antibody, etc. As for cellular immunity, Armstrong\textsuperscript{1} described that thymus contained lymphocytes sensitized to muscle or thymic antigens and that these lymphocytes responded to certain mitogens and became cytotoxic to muscle cells in tissue culture.

Alpert\textsuperscript{6} described that peripheral lymphocytes were sensitized to muscle in MG. Mori\textsuperscript{7} described that peripheral lymphocytes in MG destroyed autologous thymus in tissue culture. In conclusion, lymphocytes sensitized to muscle or thymus are found in the thymus and in the peripheral blood, and are cytotoxic to the muscle or the thymus. As thymectomy is effective in the treatment of MG, we conclude that thymus might be a site where such abnormal lymphocytes are produced.

Shimabukuro\textsuperscript{8} observed that anti-microsome antibody concentration in chronic thyroiditis associated with MG decreased after thymectomy. Kurita\textsuperscript{9} reported the disappearance of anti-thymus antibody in the patients with MG after thymectomy.

Concerning the changes in cellular immunity after thymectomy, Alpert\textsuperscript{6} observed remission and improvement of migration inhibition following thymectomy in one patient. Goust\textsuperscript{10} described that 5 patients continued to show abnormal migration inhibition even after thymectomy and that thymectomy had no relation with improvement in migration inhibition.

In our study, however, migration inhibition is frequently abnormal before thymectomy, and tends to return to normal after thymectomy. In addition, migration inhibition was abnormal during myasthenic crisis in a thymectomized patient and it returned to normal at remission obtained by ACTH therapy. This fact suggests thymo-lympholysis action of ACTH, which may be effective to get rid of abnormal lymphocytes from the thymus residue, peripheral blood or other tissues.

As for ulcerative colitis, various abnormalities have been observed in humoral and cellular immunity, and we have found thymitis in this disease.

Bendixen\textsuperscript{11} found sensitized lymphocytes to colonic epithelial homogenate in
UC using LMIT, and Shorter observed that lymphocytes in UC patients were cytotoxic to colonic epithelium. These lymphocytes in their observation and the thymus observed in our study may indicate that thymus in UC may also be a place of producing abnormal lymphocytes as in the case of MG.

In fact, patients with UC are well controlled after thymectomy. We also observed sensitized lymphocytes to colonic epithelial homogenate. 6 of 8 patients after thymectomy still show migration inhibition. Since the interval between thymectomy and LMIT is less than 6 months in all cases, we should consider that 6 months may not be enough long to get rid of sensitized lymphocytes from the body. We have no clear explanation why a 18 years old girl with typical clinical signs, showed normal value, and that one patient aged 40 years showed decreased MI after thymectomy.

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REFERENCES