REVIEW

Thymic Abnormalities and Autoimmune Diseases

Masaharu Tsuchiya, Hitoshi Asakura* and Hiroshi Yoshimatsu**

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

**Third Department of Internal Medicine, Niigata University*

***Second Department of Surgery, University of Occupational
and Environmental Health*

(Received for publication on September 4, 1989)

Abstract

Autoimmune diseases such as ulcerative colitis (UC) and myasthenia gravis (MG) are frequently associated with thymic abnormalities. Thymus hyperplasia and/or thymoma have been demonstrated in all cases with both of these two diseases by pneumomediastinography (PMG). In the diseases of digestive organs from which we can easily obtain the local information through the endoscopic observation and biopsy specimens, lots of immunological abnormalities have been accumulated. Antibody-dependent cell-mediated cytotoxicity mechanism has been demonstrated to play an important role in the mucosal destruction in UC. In the peripheral blood level of this disease, immunological abnormalities have been demonstrated such as the presence of lymphocytes sensitized by certain antigens, autoantibodies and disturbances of lymphocyte subpopulations. In the level of the thymus, the retrovirus has been detected in the thymus epithelial cells. The supernatant of thymus epithelial cell culture (STEC) has the capability of differentiating human bone marrow cells and of facilitating disease-specific immune abnormalities. Moreover, the serum factors (thymus growth factor) discovered in the patients with UC and MG, have been demonstrated to alter the thymic environments. Therefore, it is postulated that thymectomy is beneficial to exclude these abnormalities and it has been evaluated to be very effective in UC and MG clinically. From these observations, it is thought to be important to investigate the immunological abnormalities of autoimmune diseases from the viewpoints

土屋雅春, 朝倉 均, 吉松 博

Reprint requests to: Dr. Masaharu Tsuchiya, Department of Internal Medicine, School of Medicine, Keio University, 35, Shinanomachi, Shinjuku-ku, Tokyo 160, Japan

of three immunological levels, the level of disease-specific organ, the peripheral blood level and the level of the thymus. And it is important that the therapeutic plans should be decided in the consideration of the abnormalities in each immunological level.

Key words: thymus, autoimmune disease, ulcerative colitis, myasthenia gravis

The role of thymus has been reevaluated through the recent advances in immunology. Thymus tissue of healthy person over 15 years old is involuted and replaced by fat tissue. For the past 10 years, it has been emphasized that thymus plays an important role in the pathogenesis of myasthenia gravis (MG), multiple sclerosis and ulcerative colitis (UC). Therefore, thymectomy has been applied to these diseases with beneficial effects.^{1,2}

History of thymectomy

It was Blalock³ who had first performed thymectomy on a patient with MG in 1939. In the early decades of thymectomy for MG, myasthenic crisis caused serious states of respiratory arrest and many patients died of it just after the operation. In our group, in order to avoid consequent respiratory arrest after thymectomy, a method of suprasternal incision for thymectomy has been developed by Professor H. Yoshimatsu^{4,5} taking hints from the procedures of mediastinoscopy and pneumomediastinography.⁶ Whole thymus is pulled out and removed quite anatomically and safely through a suprasternal incision. It is also possible to take biopsy specimens from thyroid, muscle or lymph nodes in this procedure. The first thymectomy case *via* the suprasternal notch performed by us was a 18-year-old girl with MG in 1964.

Thymectomy for UC had been first performed in 1968 by Cesnik.⁷ In Japan, Dr. H. Nokita working in the Wakisaka's department of Surgery in Kurume Medical College made the first report of thymectomy for 3 patients with UC at the meeting of Japanese Society of Gastrointestinal Surgery in 1973.⁸ However, the median sternotomy was employed in all these patients, and it took about three years for them to return to usual life after thymectomy. We reported the thymus abnormalities and therapeutic effects of thymectomy in 10 patients with UC (6 females and 4 males) in 1974.⁹

Digestive diseases and thymus

In general, thymus is thought to induce differentiation of multipotential cells of bone marrow into mature T lymphocytes and to play an important role as central lymphoid tissue.¹⁰⁻¹³ The function of human thymus, however, has not been fully understood. In DiGeorge syndrome or congenital thymic aplasia, various disorders are known to occur in the digestive tract. Pathophysiological conditions of digestive system

are easily investigated using biopsy specimens under endoscopy. To clarify immunological abnormalities of the gastrointestinal diseases including inflammatory bowel diseases, the immune status at various levels such as thymus, peripheral blood and the diseased regions has been assessed. We report the immunological abnormalities in patients with MG or UC in this paper.

Pneumomediastinography

One of the striking properties of normal thymus is the age involution. Around the age of 40, most of the thymus tissue is replaced by fat tissue.^{14,15} In the thymus of patients with MG or UC, however, such involution has not been observed. Pneumomediastinography (PMG) was used to detect such persistent thymus.^{6,16} The instruments for PMG are readily available at any medical institution (Fig. 1). A needle with a slight curving at the tip was inserted between the lower pole of thyroid and the upper end of the sternum and proceeded along the posterior surface of the sternum. 200–250 ml of oxygen sterilized *via* a flask containing distilled water is then slowly injected into the anterior mediastinum. The figure of the thymus with a density similar to that of the heart is clearly revealed when lateral tomographies are taken (Fig. 2).

Fig. 1 The instruments for pneumomediastinography, which are readily available at any medical institutions.

Fig. 2 The typical X-ray photograph of pneumomediastinography. The left photograph shows the lateral view of chest X-ray film of the patient with ulcerative colitis. The right one shows the pneumomediastinogram after injection of oxygen into the anterior mediastinum of the same patient. The density of the thymus is clearly seen in the superior and anterior aspect of the heart.

Professor Y. Mizuno summarized various shapes of the thymus observed by PMG in patients with autoimmune diseases.^{17,18} Once this technique is applied, latent thymoma which is detected only by exploratory operation in patients with MG can be demonstrated. No thymoma has been found out in patients with UC so far.

Therapeutic effects of thymectomy

Thymectomy was performed in 202 patients with MG, 40 of whom had thymoma. Thymectomy was also performed in 78 patients with UC (Table 1). The therapeutic effects of thymectomy was evaluated as either "unchanged" or "improved" in patients with MG. According to the follow-up study for more than 5 years during the approximately 20 year period between 1964 and 1985, improvement has been achieved in 60% of MG patients with thymoma, whereas improvement was noted in as many as 90% of MG patients with hyperplastic thymus (Fig. 3).

The effects of thymectomy in patients with UC were also examined. When percent remission was calculated by "patient-month" method in patients with UC, the percent remission was 78% on the conventional treatment alone. However, in patients in whom it was difficult to achieve complete cure by conventional treatment alone, percent remission was only 60.6% before thymectomy. Even in such refractory patients, percent remission reached to 87.1% by thymectomy (Fig. 4).

Peripheral immune abnormalities in patients with MG and UC

Therapeutic effect of thymectomy was thus revealed to be favorable in both MG and UC. This would suggest the important role of thymus in the pathogenesis of these two diseases. Our investigation has been focussed on the immunological backgrounds of these phenomena. Peripheral immune system was at first surveyed. No constant abnormalities were noted in peripheral non-specific immune function in either

Table 1 The Summary of the Cases of Autoimmune Diseases Treated with Thymectomy So Far in Our Institutions

Myasthenia gravis	202
(with thymoma	40)
(without thymoma	162)
Ulcerative colitis	78
Behçet's disease	56
Thyroid diseases	20
SLE	10
Primary myxedema	4
AIHA	3
Sjögren's syndrome	3
Burger's disease	3
PRCA	2
Lupoid hepatitis	2
Others	8
Total	391

Totally 391 patients were thymectomized.

Fig. 3 The summary of the therapeutic results of thymectomy in patients with myasthenia gravis. According to the follow-up observation for more than 5 years during the approximately 20 year period between 1964 and 1985, improvement was noted especially in patients with hyperplastic thymus.

Fig. 4 The summary of the effects of thymectomy in patients with ulcerative colitis. Thymectomy was performed in patients resistant to drug treatment and percent remission of 87.1% was achieved.

MG or UC.¹⁹⁻²¹

Since there was no tendency in the non-specific immune system, our attention was focussed on specific immune system. When some lymphocytes sensitized by a specific antigen are stimulated by the same antigen, these lymphocytes release a humoral factor called lymphocyte migration inhibition factor (LMIF), which inhibits the *in vitro* migration of white blood cells. Utilizing this phenomenon, leukocyte migration inhibition test (LMIT) has been performed. The muscle homogenate was used as an antigen in patients with MG.²² Prior to thymectomy, leukocyte migration in these patients was inhibited more than 20% on exposure to this antigen. This would indicate the presence of lymphocytes sensitized by certain antigen found in the muscle homogenate. After thymectomy, the number of the sensitized lymphocytes decreased and inhibition of leukocyte migration was completely disappeared in 5 years (Fig. 5). In UC, LMIT using homogenate of colon mucosa demonstrated the presence of lymphocytes sensitized by a colon specific antigen in peripheral blood.²³

Various kinds of specific antibodies were present in sera of the patients with MG or UC. Anti-thymus antibody was demonstrated in sera of patients with MG firstly by an indirect fluorescence antibody method using thymus tissue.²⁴⁻²⁶ In UC, anti-thymus antibody was analyzed by fluorescence activated cell sorter (FACS) using isolated thymic lymphocytes (thymocytes) of infant. It revealed that anti-thymus antibody was present in sera of almost all patients.²⁷

Fig. 5 The effects of thymectomy on leukocyte migration inhibition test of skeletal muscle homogenate in myasthenia gravis. Prior to thymectomy, migration of the leukocytes from the patient was decreased on exposure to this antigen. After thymectomy, such sensitized lymphocytes were decreased or even disappeared and the results of this test also approached to 100%.

Fig. 6 Anti-acetylcholine receptor antibody (AChR-Ab) titer in sera of patients with myasthenia gravis among Japan, Finland and Sweden. The antibody titer in sera of the patients prior to thymectomy is shown with open columns. The antibody titers after thymectomy are shown with two columns marked "Tx". The titer in patients with hyperplastic thymus is depicted as "H", and the titer in patients with thymoma is depicted as "T". "X-ray" indicates the cases treated with cobalt irradiation.

In MG, anti-acetylcholine receptor antibody (AChR-Ab) has been noted as one of the pathogenetic factors of the disease since 1970. Pirskanen in Sweden collaborated with us in early 70's compared AChR-Ab titer in sera of patients with MG among Japan, Sweden and Finland.²⁸ In patients with hyperplastic thymus, AChR-Ab titer decreased after thymectomy. On the contrary, antibody titer in patients with thymoma increased after thymectomy but clinical symptoms were mostly improved. In patients subjected to cobalt irradiation, the decrease of the titer was most pronounced (Fig. 6). It would indicate that AChR-Ab titer did not correlate with clinical symptoms. It is suggested that the antibody titer did not decrease after thymectomy in the patients with thymoma, because only tumorous portion of thymus was removed and that non-tumorous portion of thymus remained to play an important role in the production of the antibody. In case of irradiation therapy, non-tumorous portion was also destroyed and then the antibody titer decreased. These data lead us to conclude that hyperplastic non-tumorous portion of the thymus is most important in the pathogenesis of this disease.

Anti-colon antibody (colon-Ab) is autoantibody in UC like AChR-Ab in MG. Colon-Ab was detected about 20% in the sera of patients with UC by using an indirect immunofluorescence method, while about 78% in the sera of the patients by using FACS.²⁹ After thymectomy, the titer of this antibody has decreased in accordance with the rise of the remission rate.³⁰ It is postulated that thymectomy would correct the maturation defect of immune-regulating T cells which caused the decrease of the number of suppressor T cell and the increase of antibody production.³¹

Among other autoantibodies detected in the sera of patients with UC, lymphocytophilic antibody is noted. FACS study demonstrated that there was the antibody which could bind with peripheral lymphocytes in sera of those patients.^{32,33} Similar antibody was also noted in some patients with MG. These antibodies also bound with thymic lymphocytes of the patients, although the reactivity was different between in the case of MG and the case of UC. It is indicated that thymic lymphocytes would be different in each disease which is discussed in later paragraph.

Thymic abnormalities of the patients with MG and UC

It was demonstrated that the thymic lymphocyte populations were different between patients with UC and patients with MG. Thymic lymphocytes were isolated from the patients and were analyzed by FACS using monoclonal antibodies, Leu2a (against suppressor/cytotoxic T cell) and Leu3a (against helper T cell).³¹ In patients with MG, the subsets of thymic lymphocytes were scarcely different from those of thymic lymphocytes of children whose thymus was estimated as normal control. However, in patients with UC, both numbers of Leu2a- and Leu3a-positive thymic lymphocytes decreased, especially the number of Leu2a-positive cells was remarkably lower than that of normal (Fig. 7), while OKT1a1-positive cells increased.^{31,32} These data indicate

Fig. 7 Analysis of thymic lymphocytes using monoclonal antibodies, Leu-2a and Leu-3a. Thymocytes were isolated from the thymus of the patients with myasthenia gravis and ulcerative colitis and were made to react with Leu-2a or Leu-3a monoclonal antibodies.

that disturbed maturation of lymphocytes within the thymus is suggested in patients with UC but no such disturbance occurred in patients with MG. In MG, as there is some abnormality in peripheral blood but no difference in thymic lymphocyte population in subsets, there may be no maturation disturbance but abnormality of lymphocyte itself.

One of other characteristic abnormalities in the thymus is the formation of lymphoid follicles which was seen in 80% of patients with MG and 40% of patients with UC.³⁴ It was indicated that lymphoid follicle formation represented the focus of B cell proliferation in response to some antigenic stimulation and this formation in the thymus is frequently found out in patients with autoimmune diseases. Even if lymphoid follicle was not found out, it was demonstrated that some B cells were present in the area of cortico-medullary junction of the thymus, which was revealed by immunohistochemistry using an OKB1 monoclonal antibody.³⁵

Retrovirus in human thymus cells

Since B cells were present within the thymus tissue from the patient with MG or UC, *in vitro* experiment was designed to elucidate these phenomena. Thymus epithelial cells of the patient and a human B cell line were co-cultured in the culture medium. These cells were pelleted and observed under electron microscopy. Retrovirus-like particles were demonstrated on the cell surface that indicated a budding formation

Fig. 8 Reactivity of sera from patients with myasthenia gravis and ulcerative colitis with KK-1 cells.

which was characteristic of retrovirus. The activity of reverse transcriptase was demonstrated in the culture supernatant.³⁰ Human T cell line was added to the mixed culture in order to infect this retrovirus into the T cells, which was designated as KK-1. From the culture supernatant of KK-1, the fraction of virus was collected. Then this fraction was used as the antigen of the virus and serum of the patient with UC or MG was examined for the reactivity with this antigen by enzyme-linked immunosorbent assay (ELISA). It was revealed that there were antibodies to react with this virus in the sera of patients with UC and MG³⁷ (Fig. 8). Recently, molecular biological studies of this virus are being achieved.

Supernatant of thymus epithelial cell culture (STEC)

In order to test the release of some humoral factor from the thymus epithelium of the patients, the surgically removed thymus was minced and cultured in the medium supplemented with 20% fetal calf serum. Thymic epithelial cells gradually proliferated in monolayer after 1–2 weeks. We collected this culture supernatant (supernatant of thymus epithelial cell culture (STEC)) and various properties of this supernatant were studied.^{38,39}

Humoral substances such as thymosin,⁴⁰ thymine,⁴¹ thymic humoral factor (THF),⁴² and facteur thymique serique (FTS)⁴³ secreted by the thymus have been so far studied

in the world. All these substances, however, were obtained from animals except FTS which was obtained from human serum as well as guinea pig serum. STEC, therefore appears to be important as a humoral substance obtained from epithelial component of human thymus, especially disease-specific thymus.

One of the properties of STEC was the ability to induce differentiation of bone marrow cells into E-rosette forming cells.⁴⁴ Moreover, STEC has an effect on lymphocytes functions. It was demonstrated that STEC has a suppressive effect on ConA induced suppressor T cell function (Fig. 9). STEC stimulated a specific antibody production *in vitro* but did not stimulate non-specific antibody production. For example, STEC from the patients with MG (MG-STEC) increased *in vitro* production of AChR-Ab assayed using the procedure developed by Dr. Kobayashi at Kitasato Institute, but not of total IgG amount (Fig. 10).

The biological functions of MG-STEC was compared with those of thymosin fraction 5, FTS and STEC from patients with UC (UC-STEC). While MG-STEC as well as thymosin fraction 5 has a property of differentiating multipotential stem cells into E-rosette forming cells, UC-STEC does not possess such a property⁴⁵ (Fig. 11). The thymic epithelial cells from patients with MG and those from patients with UC thus seem to be fundamentally different in their nature.

MG-STEC increased cyclicAMP levels in mice lymphocytes but no other thymic hormonal factors increased it. Those factors increased cyclic GMP levels in them. It

Fig. 9 Effect of STEC on ConA induced suppressor activity of healthy control T cell. Suppressor activity on IgG production was inhibited by STEC from myasthenia gravis in four experiments. Two of three experiments in IgM production revealed the similar results.

Fig. 10 The effect of STEC from the patients with myasthenia gravis on *in vitro* production of IgG and anti-acetylcholine antibody (α ACh-R). No change of IgG production amount was noted on addition of STEC as shown in the left side. Measurement of the minute amount of α ACh-R by method of Dr. Kobayashi of Kitasato Institute revealed a rise of production in response to STEC as shown in the right side.

is postulated that this property is related to the maturation and differentiation of T cells.

The difference between MG-STEC and UC-STEC was further made clear by intravenous injection into mice. MG-STEC facilitated the differentiation of mouse thymocytes while UC-STEC did not.⁴⁶

The serum factors from patients with MG and UC (Thymus growth factor)

As thymic epithelial cells excrete some kinds of humoral factors, it was demonstrated that some humoral factors related to the thymus was in the serum of the patient with MG or UC. Intravenous injection of sera from patients with MG or UC into C57BL/6 mice increased the mouse thymus in weight⁴⁷ (Fig. 12). The serum factor which had this activity was revealed to be a glycoprotein with a molecular weight of 14,800. Especially the serum factors of patients with UC induced the proliferation of the thymic epithelial cells that had been demonstrated by immunohistochemical method using an anti-keratin antibody.⁴⁸ It was also demonstrated that this serum factors decreased Lyt-2-positive cell population, and increased L3T4-positive cell population by

Fig. 11 E-rosette forming activities of supernatant of thymus epithelial cell culture obtained from the patients with myasthenia gravis and ulcerative colitis. STEC indicates supernatant of thymus epithelial cell culture. The human bone marrow cells were incubated with STEC and percent E-rosette forming activity was assayed.

using FACS.⁴⁹

In vitro organ culture of normal mouse thymus was utilized to test the function of serum factors. The encapsulated fetal thymus was removed from a pregnant mouse under a stereoscopic microscope using microscalpel. Fetal thymus was then subjected to organ culture on a specific filter with addition of the patients' sera. The serum factors from patients with UC were found to increase the number of thymocytes in *in vitro* organ cultured thymus. Those from patients with MG did not have such a property.

Prospective views for autoimmune diseases

Various immunological differences were found between MG and UC. As to HLA, specific patterns were noted in both diseases, suggesting a genetic background.^{50,51} In a general outline, thymic abnormalities appear to play a major role in MG, whereas peripheral blood abnormalities were mainly responsible in UC. The abnormalities in peripheral T lymphocyte subsets are noted in UC, especially with a remarkable decrease in Leu2a positive cells. Serum factors from both diseases increased weight and cell number of mouse thymus *in vivo*, but *in vitro*, this property was found only in sera of patients with UC. In addition, various kinds of autoantibodies, such as AChR-Ab,

Fig. 12 The effect of intravenous injection of sera from patients with myasthenia gravis and from those with ulcerative colitis in C57BL/6 mice. The weight of thymus was measured after the injection. After the administration of sera from the patients with myasthenia gravis, the weight of thymus increased 2 days after and decreased 4 days after, but re-increased. When sera from the patients with ulcerative colitis was injected, the weight of thymus was increased gradually and reached the peak weight after 9 days. The effect of these injection of sera were different from each other.

anti-colon antibody, lymphocytophilic antibody and anti-thymus antibody were noted in these diseases.

MG-STEC had an activity to induce bone marrow lymphocytes into E-rosette forming cells. It also inhibited suppressor T cell activities and facilitated differentiation of lymphocytes within the thymus. No such activity was demonstrated in UC-STEC. As to the lymphocyte subsets within the thymus, no change was noted in MG, but immature cells increased in UC, possibly due to the effect of the serum factors as mentioned above. As to the anti-thymus antibody, thymic lymphocytes from patients with MG did not react with sera of patients with UC, but cells from patients with UC react with sera of patients with MG. Since anti-human thymocyte mouse antibody reacts with thymocytes of patients with MG and those with UC, some abnormalities may possibly be present in the thymocyte themselves from patients with MG, because the human serum anti-thymus antibody does not bind to thymocytes from patients with MG. Clinically, thymoma is not found in patients with UC, but in patients with MG. This fact may be related to such abnormalities of the thymocyte themselves in MG (Table 2).

Lastly, thymic abnormalities and immunoregulatory therapy in MG and UC will be discussed. MG is thought to be caused by thymic abnormalities induced by infec-

Table 2 Various Immunological Differences Found between Myasthenia Gravis and Ulcerative Colitis

		Myasthenia Gravis	Ulcerative Colitis
Peripheral	HLA	A-10, B-12, (B-5)	BW52, DR2
	Lymphocyte subset	~	Leu2a ⁺ ↓ Leu3a/Leu2a ↑ reactivity with IL2 ↓
	Serum factor	<i>in vivo</i>	<i>in vivo</i>
		weight of thymus ↑	weight of thymus ↑
		cell number ↑	cell number ↑
		<i>in situ</i>	<i>in situ</i>
		~	cell number ↑
			Lyt-2 ⁺ ↓, L3T4 ⁺ ↑
	Antibodies	α ACh R lymphocytophilic α thymus α muscle	α colon lymphocytophilic α thymus
	STEC	E-rosette ↑ suppressor T ↓ mouse thymocyte proliferation ↑ differentiation ↑	E-rosette ~ mouse thymocyte proliferation ↑ differentiation ↓
Thymus	Lymphocyte subset	~	Leu2a ⁺ ↓ ↓, Leu3a ⁺ ↓
	B cell invasion	++	+
	Epithelium	proliferation +	proliferation ++
	Retrovirus	+	+
	Antigen presentation	myoid cell	?
	Reactivity with lymphocytophilic antibody		
	sera from MG	+	+
	UC	-	+

tion of retrovirus, HLA abnormalities or environmental changes. MG-STEC is secreted from thymic epithelial cells, causing lymphocyte abnormality. Thymic blood barrier may be destroyed by some unknown causes and B cells entering from the peripheral blood stream may form lymphoid follicles. AChR expressed on myoid cells may be recognized in the thymus and autoantibodies may be produced. AChR is recognized as non-self by lymphocytes and then sensitized T cells are produced. Abnormal T- and B-cells distributed in the periphery enhanced the production of AChR-Ab which damaged the neuromuscular junction. Adequate combination therapy as shown in Fig. 13, should be performed.

Fig. 13 The summary of thymic abnormalities and immunoregulatory therapy in myasthenia gravis.

Fig. 14 The summary of thymic abnormalities and immunoregulatory therapy in ulcerative colitis.

Abnormalities in UC (Fig. 14) are considered to appear at first in the periphery. The first event is inflammation of the colon due to HLA abnormalities and environmental changes in the intestinal lumen. Through such processes, thymus growth factor in the serum appears to cause thymic abnormalities. Such abnormality mainly consists the disturbed process of suppressor T cell maturation. Decreased suppressor T cell function may induce local production of anti-colon antibody, and the colon epithelium may be destroyed by an antibody-dependent cell-mediated cytotoxicity mechanism in collaboration with Killer cells. The purpose of thymectomy therefore is to cut off the circuit process of those immunological abnormalities.

The thymus is quite sensitive to steroids and markedly involutes in response to steroid administration.⁵² Three to four months after the discontinuation of administration, however, the thymus enlarges again. Effects of steroid therapy is transient for involution of the thymus. Thymectomy should be considered for such a case. An immunoregulatory therapy should be recommended from a comprehensive viewpoint of such pathophysiological process in the autoimmune diseases as mentioned above.

References

1. Tsuchiya, M.: Clinical prospect of thymology. *Keio J. Med.* 21: 127-145, 1972
2. Tsuchiya, M.: Immunological abnormalities involving the thymus in ulcerative colitis and therapeutic effects of thymectomy. *Gastroenterol. Jpn.* 19: 232-246, 1984
3. Blalock, A., Mason, M. F. and Morgan, H. J.: Myasthenia gravis and tumors of the thymic lesion. *Ann. Surg.* 110: 544-561, 1939
4. Tsuchiya, M.: Pneumomediastinography. *Medicina* 5: 921-924, 1968 (in Japanese)
5. Yoshimatsu, H., Ikeda, T., Tsuchiya, M. and Shimabukuro, K.: Mediastinoscopy and its application: Thymectomy via the suprasternal notch. *Keio J. Med.* 20: 29-43, 1971
6. Yoshimatsu, H., Tsuchiya, M., Kamegaya, K. and Oda, M.: Mediastinoscopy and thymectomy via the suprasternal notch. In: *Mediastinoscopy: Proceedings of an International Symposium*, Odense University, 1970, edited by Jepsen, O. and Sorensen, H. R., Odense Univ. Press, 1971, 94-96
7. Cesnik, H.: Thymectomie bei colitis ulcerosa. *Vlaufinger Bericht uber einen Behandlungsversuch bei 7 Patienten.* *Langenbecks Arch. Klin. Chir.* 321: 86-98, 1968
8. Nokita, H., Suenaga, H., Isomoto, H., Satoh, T., Yoshiwara, T., Morishige, F., Yamauchi, T. and Wakisaka, J.: Experiences of thymectomy for ulcerative colitis. *Jpn. J. Gastroenterol. Surg.* 6: 169-170, 1973 (in Japanese)
9. Tsuchiya, M., Shimabukuro, K., Asakura, H., Mizunio, Y., Oda, Y., Ono, A., Morizane, T., Yamanaka, I. and Tsuji, K.: Abnormality of thymus-T-cell system and HL-A in ulcerative colitis. *Jpn. J. Gastroenterol.* 71: 989-997, 1974 (in Japanese)
10. Reinherz, E. L. and Schlossman, S. F.: The differentiation and function of human T lymphocytes. *Cell* 19: 821-827, 1980
11. Reinherz, E. L., Kung, P. C., Goldstein, G., Levey, R. H. and Schlossman, S. F.: Discrete stages of human intrathymic differentiation: Analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. *Proc. Natl. Acad. Sci. USA* 77: 1588-1592, 1980
12. Bhan, A. K., Reinherz, E. L., Poppema, S., McCluskey, R. T. and Schlossman, S. F.: Location of T cell and major histocompatibility complex antigens in the human thymus. *J. Exp. Med.* 152: 771-782, 1980

13. Janossy, G., Tidman, N., Papageorgiou, E. S., Kung, P. C. and Goldstein, G.: Distribution of T lymphocyte subsets in the human bone marrow and thymus: An analysis with monoclonal antibodies. *J. Immunol.* 126: 1608–1613, 1981
14. Hammar, J. A.: The new views as to the morphology of the thymus gland and their bearing on the problem of the function of the thymus. *Endocrinology* 5: 543–573, 1921
15. Boyd, E.: The weight of the thymus gland in health and in disease. *Am. J. Dis. Child.* 43: 1162–1214, 1932
16. Tsuchiya, M.: Pneumomediastinography. *Saishin-Igaku* 28: 1355–1361, 1973 (in Japanese)
17. Mizuno, Y.: Correlation of pneumomediastinography and thymic change in various autoimmune diseases. *J. Keio Med. Soc.* 53: 63–79, 1976 (in Japanese)
18. Mizuno, Y., Shimabukuro, K., Kurita, K. and Tsuchiya, M.: Thymus abnormalities in ulcerative colitis—Comparative study with other autoimmune diseases. *Gastroenterol. Jpn.* 11: 208–214, 1976
19. Tsuchiya, M. and Mizuno, Y.: Myasthenia gravis. *J. Clin. Exp. Med.* 90: 707–712, 1974 (in Japanese)
20. Adner, M. M., Sherman, J. D., Ise, C., Schwab, R. S. and Dameshek, W.: An immunologic survey of forty-eight patients with myasthenia gravis. *N. Engl. J. Med.* 271: 1327–1333, 1964
21. Kornfeld, P., Siegal, S., Weiner, L. B. and Osseman, K. E.: Studies in myasthenia gravis; Immunologic response in thymectomized and nonthymectomized patients. *Ann. Int. Med.* 63: 416–428, 1965
22. Oda, Y., Morizane, T., Mizuno, Y., Asakura, H. and Tsuchiya, M.: Influence of thymectomy on leukocyte migration inhibition test in myasthenia gravis and ulcerative colitis. *Keio J. Med.* 24: 289–296, 1975
23. Tanaka, Y.: A study of the cell-mediated immunity in chronic inflammatory bowel disease: Role of the enterobacterial common antigen. *J. Gastroenterol.* 77: 722–731, 1980
24. Geld, H. W. van der and Struss, A. J.: Myasthenia gravis. Immunological relationship between striated muscle and thymus. *Lancet* 1: 57–60, 1966
25. Strauss, A. J. L., Geld, H. W. van der, Kemp, P. G., Jr., Exum, E. D. and Goodman, H. C.: Immunological concomitants of myasthenia gravis. *Ann. NY Acad. Sci.* 124: 744–766, 1965
26. Tsuchiya, M., Shimabukuro, K., Mizuno, Y., Oda, Y., Kurita, T., Yoshida, T. and Ono, A.: Serum antibody to thymus in myasthenia gravis. *J. Keio Med. Soc.* 51: 237–245, 1974 (in Japanese)
27. Aiso, S., Yoshida, H., Hibi, T., Watanabe, M., Ishikawa, M., Kobayashi, K., Asakura, H., Tsuchiya, M., Shimabukuro, K. and Tsuru, S.: Lymphocytophilic antibody and hyperplastic thymus in patients with ulcerative colitis. *Dig. Organ Immunol.* 7: 205–207, 1981 (in Japanese)
28. Pirskanen, R., Leftvert, A. K., Matell, G., Mizuno, Y., Muraoka, M., Ono, A., Osterman, P.-O., Satoyoshi, E., Tsuchimoto, K. and Tsuchiya, M.: HL-A, receptor antibodies (AChR-Ab), and clinical state in Japanese, Finnish, and Swedish myasthenia gravis (MG). In: *Myasthenia Gravis: Pathogenesis and Treatment*, edited by Japan Medical Research Foundation, University of Tokyo Press, 1981, 233–248
29. Hibi, T., Aiso, S., Yoshida, T., Watanabe, M., Asakura, H., Tsuru, S. and Tsuchiya, M.: Anticolon antibody and lymphocytophilic antibody in ulcerative colitis. *Clin. Exp. Immunol.* 49: 75–80, 1982
30. Tsuchiya, M.: Educational lecture on recent advances in the etiological and therapeutic studies in ulcerative colitis. *Jpn. J. Int. Med.* 76: 1372–1377, 1987 (in Japanese)
31. Aiso, S., Yoshida, T., Watanabe, M., Hibi, T., Asakura, H., Tsuchiya, M. and Tsuru, S.: Characterization of thymus cells in hyperplastic thymuses in patients with myasthenia gravis and ulcerative colitis with monoclonal antibodies. *J. Clin. Lab. Immunol.* 13: 137–139, 1984

32. Aiso, S., Watanabe, M., Hibi, T., Yoshida, T., Tsuchiya, M. and Tsuru, S.: Characterization of immunoregulatory T cells and lymphocytophilic antibodies in ulcerative colitis: Analysis with monoclonal antibodies. *J. Clin. Lab. Immunol.* 9: 109-112, 1982
33. Watanabe, M.: Analysis of anticolon antibody and lymphocytophilic antibody in sera from patients with ulcerative colitis. *Jpn. J. Gastroenterol.* 82: 413-423, 1985 (in Japanese)
34. Tsuchiya, M., Saito, H., Fujisawa, T., Watanabe, M., Mizuno, Y., Ono, A., Hibi, T., Yoshimatsu, H. and Ando, K.: Autoimmune diseases and thymus abnormalities. *Jpn. J. Chest Dis.* 46: 709-715, 1987 (in Japanese)
35. Tsuchiya, M., Watanabe, M., Ono, A., Mizuno, Y. and Yoshimatsu, H.: Mechanisms of immunosuppressive therapy "thymectomy". *Saishin Igaku* 41: 2525-2530, 1986 (in Japanese)
36. Ono, A., Higashinakagawa, T., Kondo, S., Nakamura, T., Saito, H., Suzuki, H., Yoshimatsu, H., Murakami, M. and Tsuchiya, M.: RNA tumor virus-like particles in thymus cells of patients with myasthenia gravis. *Keio J. Med.* 32: 49-58, 1983
37. Ono, A., Saito, H., Kondo, S., Yoshimatsu, H. and Tsuchiya, M.: RNA tumor virus in human thymomas and thymus hyperplasias. *Semin. Surg. Oncol.* 1: 39-152, 1985
38. Mizuno, Y., Muraoka, M., Munakata, Y., Tsuchiya, M., Shimabukuro, K., Itoh, M. and Notake, Y.: *In vitro* culture of thymus epithelial cells from various autoimmune diseases. *J. Clin. Exp. Med.* 103: 631-634, 1977 (in Japanese)
39. Mizuno, Y., Tsuchiya, M., Muraoka, M., Ito, M., Shimabukuro, K., Ono, A. and Yoshimatsu, H.: Humoral factor produced by thymic epithelium in myasthenia gravis. *Keio J. Med.* 28: 131-138, 1979
40. Goldstein, A. L., Slater, F. D. and White, A.: Preparation, assay, and partial purification of a thymic lymphocytopoietic factor (thymosin). *Proc. Natl. Acad. Sci. USA* 56: 1010-1017, 1966
41. Goldstein, G.: Isolation of bovine thymim: A polypeptide hormone of the thymus. *Nature* 247: 11-14, 1974
42. Trainin, N., Small, M. and Globerson, A.: Immunocompetence of spleen cells from neonatally thymectomized mice conferred *in vitro* by syngeneic thymus extract. *J. Exp. Med.* 130: 765-775, 1969
43. Bach, J-F., Dardenne, M., Pleau, J-M., Rosa, J.: Biochemical characterization of a serum thymic factor. *Nature* 266: 55-57, 1977
44. Mizuno, Y., Muraoka, M. and Tsuchiya, M.: Biological activities of supernatant of human thymic epithelial culture. *J. Clin. Exp. Med.* 130: 647-652, 1979 (in Japanese)
45. Tsuchiya, M.: Thymomas and autoimmune diseases: Myasthenia gravis. In: *Thymomas and Their Related Tumors*, edited by Suemasu, K. and Shimosato, Y., Kyowa Kikaku Tsuushin 1987, 83-98 (in Japanese)
46. Watanabe, N., Watanabe, M., Iwao, Y., Yoshida, T., Hayashi, T., Asakura, H., Tsuchiya, M., Aiso, S., Hibi, T., Muraoka, M., Mizuno, Y. and Tsuru, S.: Effect of "thymus growth factor" derived from patients with ulcerative colitis on mouse thymus and spleen. *Dig. Organ Immunol.* 17: 88-91, 1986 (in Japanese)
47. Watanabe, M., Yoshida, T., Watanabe, N., Hibi, T., Asakura, H., Tsuchiya, M., Aiso, S., Tsuru, S. and Jinnaka, H.: Analysis of thymus growth factor derived from patients with ulcerative colitis. *Dig. Organ Immunol.* 15: 136-141, 1985 (in Japanese)
48. Ogata, H., Watanabe, M., Watanabe, N., Iwao, Y., Niwa, H., Ohhara, S., Fujisawa, T., Aiso, S., Hibi, T., Asakura, T., Tsuru, S. and Tsuchiya, M.: Immunohistochemical analysis of thymus cells by "thymus growth factor" derived from sera of patients with ulcerative colitis. *Dig. Organ Immunol.* 19: 95-99, 1987 (in Japanese)
49. Watanabe, M., Aiso, S., Hibi, T., Watanabe, N., Iwao, Y., Yoshida, T., Asakura, H., Tsuru, S. and Tsuchiya, M.: Alteration of T cell maturation and proliferation in the mouse thymus induced by serum factors from patients with ulcerative colitis. *Clin.*

- Exp. Immunol. 68: 596–604, 1987
50. Yoshida, T., Tsuchiya, M., Ono, A., Yoshimatsu, H., Satoyoshi, E. and Tsuji, K.: HLA antigens and myasthenia gravis in Japan. *J. Neurol. Sci.* 32: 195–201, 1977
 51. Asakura, H., Tsuchiya, M., Aiso, S., Watanabe, M., Kobayashi, K., Hibi, T., Ando, K., Tanaka, H. and Sekiguchi, S.: Association of the human lymphocyte-DR2 antigen with Japanese ulcerative colitis. *Gastroenterology* 82: 413–418, 1982
 52. Pascuzzi, R. M., Coslett, H. B. and Johns, T. R.: Long-term corticosteroid treatment of myasthenia gravis: Report of 116 patients. *Ann. Neurol.* 15: 291–298, 1984