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ORIGINAL ARTICLE

Associated Immunological Disorders and Cellular Immune Dysfunction in Thymoma: A Study of 87 Cases from Thailand

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Abstract Several immune disorders are often associated with thymoma. The aim of this study was to analyze the correlation between clinicopathological features of Thai patients with thymoma and concomitant immune-mediated diseases. Medical records of 87 patients diagnosed with thymoma during a 10-year period were retrospectively reviewed. Peripheral blood T cell subsets along with cytokine responses in 15 thymoma patients and 15 healthy controls were comparatively analyzed. The results demonstrated that thymoma type AB and B2 were the most common types among patients diagnosed with thymoma. The most common presentation was incidentaloma, followed by local chest symptoms and autoimmune diseases. The prevalence of autoimmune diseases, immunodeficiency states, and secondary neoplasms was 34.5, 10.3, and 10.3 %, respectively. Autoimmune diseases were most frequently found in thymoma type B2 and sometimes associated with clinical immunodeficiency, although classic Good's syndrome was rare. Patients with thymoma had significantly lower percentage $CD4^{+ve}$ T cells and interferon γ response, but higher percentage regulatory T cells than those in healthy controls. This study indicated that the aberrant immunologic disorders comprising autoimmune diseases, immunodeficiency states, and secondary neoplasms were found in almost 40 % of Thai patients with thymoma and possibly related to defectiva cytokine responses and altered T cell subsets.

Keywords Thymoma · Paraneoplastic syndromes · Autoimmunity · Immunologic deficiency syndrome · Neoplasms

Introduction

The thymus gland is one of the key organs of the immune system and serves as the nurture site for thymocyte differentiation and maturation, especially in early childhood and adolescence (Douek and Koup 2000). Although thymic function declines with age, there is evidence that the thymus maintains an active thymopoiesis throughout adult life and even in the elderly (Ferrando-Martínez et al. 2009). It also plays an important role in maintaining immunologic self-tolerance by balancing between effector and regulatory T cells (Tregs) (Itoh et al. 1999; Pennington et al. 2006).

Thymoma, the epithelial tumor of thymus, is the most common tumor of anterior mediastinum. Data indicate that the incidence of thymoma is high among Asians and Pacific Islanders (Engels 2010). The association between clinical manifestations and different types of thymoma has been documented and found to be related to many immunological disturbances (Detterbeck 2006; Kim et al. 2005). The association between thymoma and several paraneoplastic autoimmune diseases, especially myasthenia gravis (MG), has been demonstrated (Levy et al. 1998; Shelly et al. 2011; Tormoehlen and Pascuzzi 2008). There have been studies reporting the link between thymoma and other neoplasms, such as non-Hodgkin's lymphoma, soft tissue

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sarcomas, and colorectal carcinoma (Engels 2010; Engels and Pfeiffer 2003; Welsh et al. 2000). The presence of adult-onset immunodeficiency associated with thymoma has also been well-documented with the prevalence around 5–10 % in western countries (Kelleher and Misbah 2003). Nevertheless, overview data regarding the prevalence and characteristics of all immunological disorders in patients with thymoma is not much available.

The increased prevalence of autoimmune diseases, secondary malignancies, and immunodeficiency status in patients with thymoma suggests that there are cell-mediated immune defects in these patients. Several hypotheses try to explain the immunological mechanisms responsible for these findings in thymoma. The aberration of cells and cytokines responsible for cellular immunity in patients with thymoma is the one possibility. Interferon (IFN)-γ is a key T helper-1 cytokine and could be down-regulated by interleukin (IL)-10 (Moore et al. 2001; Schroder et al. 2004). The interplays between IFN-γ and IL-10 are believed to play a pivotal role in the balance of cell-mediated immune responses (Tso et al. 2005; Zhou et al. 2008). There have been a few studies discussing the alteration of T cell subset in thymoma. The alteration of T cell subsets in blood was reported in patients with thymoma (Hoffacker et al. 2000). A significantly lower CD4:CD8 ratio and lower percentages of $CD4^+$ and $CD8^+$ T lymphocytes expressing CD28 antigen was also found in thymectomized patients as compared to healthy control subjects (Krawczyk et al. 2007). The imbalance of T cell subset and cytokines may result in the failure of cell-mediated immunity in response to neoplastic changes and opportunistic infections.

There is evidence that the Tregs play an important role in the maintenance of self-tolerance and controlling autoimmunity (Leavy 2007). Neoplastic transformation in thymoma possibly contributes to the abnormal processes of positive and negative selection of thymocytes, lead to the loss of self-tolerance and release autoreactive T cells into peripheral blood (Shelly et al. 2011). The defect of Tregs in patients with thymoma has been suggested as well. The number and distribution of Tregs were extensively investigated in patients with thymoma-associated MG (Luther et al. 2005; Matsui et al. 2010; Ströbel et al. 2004; Sun et al. 2004). Although the decrease of Tregs in the thymic tissues was suggested to trigger the development of MG in patients with thymoma, data on the number of peripheral blood Tregs in patients with thymoma in general are not yet conclusive.

The purpose of this study was twofold: (1) to study the characteristics of patients diagnosed with thymoma in terms of associated immunological disorders and clinicopathological correlation, and (2) to evaluate T cell proportions and cytokine responses in thymoma patients compared to those in healthy controls.

Materials and Methods

Study Patients

Medical records of patients diagnosed with thymoma in King Chulalongkorn Memorial Hospital during a 10-year period between 2000 and 2010 were retrieved from the hospital electronic database and patients' medical history was retrospectively reviewed for clinical characteristics, histopathological diagnosis, and associated immunological disorders, such as autoimmune diseases, additional malignancies, clinical immunodeficiencies, and laboratory tests. The classifications of thymoma were categorized according to the recent World Health Organization (WHO) histological classification and the Masaoka clinical staging system (Masaoka et al. 1981; Sonobe et al. 2005).

Immunological Investigations

Fifteen thymoma patients, who underwent thymectomy and were not on corticosteroid or chemotherapeutic agents were invited by telephone to have 20 ml ACD-blood sample collected at the hospital to measure immunoglobulin levels, percentages of $CD4^+$, $CD8^+$, $CD4^+CD25^+FOXP3^+$ (Tregs, and results of IFN-γ and IL-10 enzyme-linked immunospot (ELISPOT) assays. Peripheral blood mononuclear cells (PBMCs) were isolated by lymphoprep (Robbins Scientific Corporation, Sunnyvale, CA, USA) density-gradient centrifugation. The mononuclear cell fraction was washed twice with RPMI1640 (Gibco, USA) and resuspended in R10 medium (RPMI1640 supplemented with 10,000 U/ml Penicillin G, 10,000 μg/ml Streptomycin and 10 % fetal bovine serum [Biowhittaker, Walkersville, MD, USA]). PBMC were counted and adjusted depending on the experiment.

Surface Staining of CD4, CD8, CD25 and Intracellular Staining of FoxP3

Flow cytometric measurement of surface and intracellular staining in PBMCs was done according to a manufacturer's protocol. Anti-CD4 FITC, anti-CD8 PerCP, and anti-CD25 APC were purchased from BD Bioscience (San Jose, CA, USA). Anti-FOXP3 PE and PE-conjugated Rat IgG2a isotype control were purchased from eBioscience (San Diego, CA, USA). In brief, 1×10^6 PBMCs were stained for the surface markers, anti-CD4 FITC/anti-CD8-PerCP/anti-CD25-APC, and incubated for 30 min at 4 °C in the dark and washed in a cold staining buffer. Cell permeabilization was

then performed for 45 min with fixation/permeabilization buffer (eBioscience, San Diego, CA, USA) at 4 °C in the dark. After washing twice with the permeabilization buffer, the cells were stained with PE-conjugated anti-human Foxp3 (10 μl/test) for 30 min at 4 °C. Finally, the cells were washed once with a permeabilization buffer and resuspended in 1 % paraformaldehyde before being analyzed on a FACSCalibur flow cytometer with CellQuestPro software (Becton–Dickinson, San Jose, CA, USA) for the percentages of $CD4^+$, $CD8^+$, and $CD4^+CD25^+FoxP3^+$ cells.

ELISPOT Assay

The responses of PBMCs after stimulation with phytohaemagglutinin (PHA) were comparatively analyzed between patients with thymoma and healthy blood donors. The numbers of IFN-γ- (or IL-5 or IL-10) releasing cells were determined using ELISPOT assay kits (Mabtech, Stockholm, Sweden). In brief, 96-well nitrocellulose membrane plates (MAIP S45; Millipore, Bedford, MA, USA) were coated for 16 h at 4 °C with 5 μg/ml of anti-IFN-γ antibody (or anti-IL-10 antibody) provided in the kit and blocked with R10 medium for 1 h at room temperature. PBMCs (2.5×10^5 cells/ml in 100 μl) were incubated for 48 h at 37 °C in 5 % CO_2 in the presence of PHA 10 μg/ml. Plates were washed six times with PBS/Tween 0.05 % and incubated for 1.5 h at 37 °C with a biotinylated anti-IFN-γ antibody (or anti-IL-10 antibody) and then extensively washed. IFN-γ (or IL-10) spot forming cells (SFCs) were developed using streptavidin–alkaline phosphatase, incubated for 1 h at 37 °C, and extensively washed before adding the substrate [5-bromo-4-chloro-3-indolylphosphate and nitro blue tetrazolium (Biorad, CA, USA)]. The numbers of IFN-γ (or IL-10) SFCs present in each well were counted using the ELISPOT reader (Carl Zeiss, Germany). The results of the IFN-γ (or IL-10) ELISPOT assay are expressed as the numbers of IFN-γ (or IL-10) SFC/10^6 PBMC cultured with PHA subtracted by the nonspecific background values obtained from PBMC cultured without PHA.

Blood samples from 15 healthy blood donors at the National Blood Center, Thai Red Cross Society were also studied as controls. The study was approved by the Faculty of Medicine Institutional Review Board and all volunteers gave written informed consent prior to blood collection.

Data Analysis

Student's t test and Chi-square test were used to analyze the quantitative data and categorical data, respectively. All statistical calculations were performed using SPSS statistics 17.0 (IBM Inc, Armonk, NY, USA). Value of $p < 0.05$ was considered statistically significant.

Results

Background Characteristics of Patients Diagnosed with Thymoma

Eighty-seven patients with confirmed histopathological diagnosis of thymoma were retrieved from the hospital electronic medical records from 2000 to 2010 (see Table 1). The average age of the first presentation was 50.2 ± 1.5 years old. The average time between symptom onset and diagnosis of thymoma was 6.9 ± 1.3 months. 29.9 % of patients diagnosed with thymoma were incidentally diagnosed during investigation for unrelated medical problems or routine check-ups, while local chest symptoms and autoimmune diseases were the clinical presentations in 27.6 and 26.4 % of patients, respectively. Thymoma type AB and B2 (23.0 % each) were the most common types, whereas thymic carcinoma was accounted for 17.2 % of thymoma patients. About half of patients with thymoma type A (42.9 %), AB (45.0 %), or B1 (53.8 %) presented as incidentaloma, while 50.0 % of thymoma type B2 presented with autoimmune diseases. The majority of patients with thymic carcinoma (80.0 %) or thymoma type B3 (58.3 %) presented with local chest symptoms or metastasis. 77.5 % of patients with thymoma types A, AB, and B1 all together were diagnosed with Masaoka stage I or II. In contrast, 63.8 % of patients with thymoma types B2, B3, and thymic carcinoma were at Masaoka stage III or IV at the time of diagnosis. The radical thymectomy was performed in 80 patients and adjuvant radiotherapy and/or chemotherapy was performed in 52 patients (data not shown).

Autoimmune Disorders in Patients with Thymoma

Autoimmune disorders were diagnosed in 34.5 % of patients with thymoma and MG was the most common thymoma-associated autoimmune disease. No difference in patient age, gender, time from first symptoms to diagnosis, Masaoka staging, and associated secondary malignancies was demonstrated in terms of autoimmune involvement. Interestingly, histopathological types are statistically different between these two groups ($p = 0.01$) with thymoma type B2 most frequently associated with autoimmune diseases (see Table 2). Clinical immunodeficiency was much higher in patients with autoimmune involvement ($p = 0.001$).

Patients with Thymoma and Suspected Immunodeficiency

There were nine patients (10.3 % of thymoma patients) with clinical symptoms suggesting immunodeficiency status as shown in Table 3. Unexplained diffuse cylindrical

Table 1 Demographic data and clinical presentations of patients with the diagnosis of thymoma in King Chulalongkorn Memorial Hospital between 2000 and 2010 ($N = 87$)

Characteristics/WHO classification	A	AB	B1	B2	B3	Thymic carcinoma	Total
Gender: M/F (both)	3/4	7/13	2/11	8/12	7/5	8/7	35/52
Age at diagnosis (years)	51.1 ± 6.7	50.9 ± 2.8	50.2 ± 3.7	51.5 ± 3.3	52.6 ± 2.8	45.4 ± 3.8	50.2 ± 1.5
Time from the first symptoms to diagnosis[a] (months)	11.2 ± 6.7	9.7 ± 3.9	2.5 ± 0.9	8.5 ± 3.0	6.1 ± 2.6	3.5 ± 0.8	6.9 ± 1.3
Clinical presentations							
Local symptoms	1	4	2	2	5	10	24
Infections	1	1	0	3	0	0	5
Autoimmune symptoms	2	6	2	10	3	0	23
Incidentaloma	3	9	7	3	1	3	26
Constitutional symptoms	0	0	1	2	1	0	4
Metastatic symptoms	0	0	1	0	2	2	5
Associated diseases							
Immunodeficiencies	1	2	1	3	2	0	9
Myasthenia gravis	2	5	3	11	3	0	24
Other autoimmune diseases	1	4	0	1	2	0	8
Secondary malignancies	1	2	1	1	2	2	9
Masuoka staging							
I	4	9	4	2	0	0	19
II	1	8	5	10	3	2	29
III	1	0	2	6	5	3	17
IV	1	3	2	2	4	10	22
Total	7	20	13	20	12	15	87

[a] Incidentaloma cases were not included

bronchiectasis was demonstrated in all of these patients who had no previous history of recurrent sinopulmonary infections or underlying pulmonary diseases prior to thymoma diagnosis. Reversed CD4/CD8 ratio was detected in all five tested patients, but hypogammaglobulinemia was confirmed in only one out of seven patients tested for the immunoglobulin levels. All patients were negative for anti-HIV test and had no other identifiable causes that contributed to the infections. All but one patient also experienced autoimmune diseases as well. There was no statistical difference in terms of the histopathological types between thymoma patients with suspected immunodeficiency and without immunodeficiency ($p = 0.65$).

The Associated Malignancies in Patients with Thymoma

Nine out of 87 patients (10.3 %) in our cohort were diagnosed with secondary malignancies as shown in Table 4. Multiple primary malignant neoplasms were detected in two patients. Four patients developed synchronous neoplasms (secondary malignancies were diagnosed within 6 months before or after the diagnosis of thymoma) and the other two were also diagnosed with malignancies within 1 year prior to the detection of thymoma.

The Proportions of T Cells in Peripheral Blood and Cytokine Responses in Thymoma Patients who Underwent Thymectomy Compared to Healthy Controls

T cell subsets and cytokine responses in 15 thymoma patients who underwent thymectomy were comparatively analyzed to 15 healthy blood donors. Their average age at thymoma diagnosis was 46.4 ± 2.7 years old and the average time after thymectomy was 3.6 ± 2.0 years. The incidental findings, local chest symptoms, MG, pure red cell aplasia, and esophageal candidiasis were the first presentations in 6, 4, 3, 1, and 1 patient, respectively. None of them had associated secondary neoplasms.

The percentages of $CD4^{+ve}$ T cells and the ratio of $CD4/CD8^{+ve}$ T cells in patients with thymoma were lower than those in healthy controls ($p = 0.029$ and $p = 0.038$, respectively). In contrast, the percentages of $CD4^{+}CD25^{+}$ $FoxP3^{+}$ Tregs in thymoma patients were significantly higher than those in healthy controls (4.06 ± 0.52 vs. 1.44 ± 0.28, $p = 0.000$).

After stimulation of PBMCs with PHA, IFN-γ responses evaluated by ELISPOT were significantly lower in the patient group compared to healthy controls ($p = 0.000$). IL-10 responses between both groups were comparable,

Table 2 The comparative clinical characteristics between thymoma patients with and without autoimmune disorders

Characteristics	Autoimmunity present[a] ($N = 30$)	Autoimmunity absent ($N = 57$)	p value
Gender (M/F)	13/17	22/35	0.44
Age (years)	48.4 ± 2.6	51.5 ± 1.7	0.34
First symptoms to diagnosis (months)	9.2 ± 3.3	5.9 ± 1.3	0.26
WHO classification			0.01*
A	3	4	
AB	8	12	
B1	3	10	
B2	12	8	
B3	4	8	
C (thymic carcinoma)	0	15	
Staging			0.61
I	7	12	
II	11	17	
III	7	10	
IV	6	17	
Secondary malignancies	1 (3.3 %)	8 (14.0 %)	0.16
Clinical immunodeficiency	8 (26.7 %)	1 (1.8 %)	0.001*

* WHO classification and clinical immunodeficiency status were statistically different between thymoma patients with and without autoimmune disorders

[a] Twenty-four patients was diagnosed with MG, three patients with pure red cell aplasia, two patients with lichen planus, one patient with aplastic anemia, one patient with sclerosing cholangitis, and one patient with paraneoplastic pemphigus. Two patients had multiple autoimmune disorders

resulting in a lower IFN-γ/IL-10 ratio in thymoma patients ($p = 0.022$) as shown in Table 5. The levels of IgG, IgM and IgA in these patients were within normal ranges (data not shown).

Discussion

More than 40 % of thymoma patients in our cohort had associated immunological abnormalities in which autoimmune diseases were the most common manifestation, followed by secondary malignant neoplasms and clinical immunodeficiency. Autoimmunity, particularly MG, was highly associated with thymoma type B2 but not found in thymic carcinoma, which agreed with a previous study (Okumura et al. 2008). The incidentaloma was the first presentation in almost half of patients with benign pathologies, whereas the initial presentation of local chest symptoms or metastasis was prevalent in patients with thymoma type B3 or thymic carcinoma.

The clinical features of thymoma with immunodeficiency in our study were similar to those reported in other literature. Bronchiectasis was very common in this patient group and alerted clinical immunologists to investigate for abnormal antibody response. Although very low B cell percentages were detected in some patients, a classic Good's syndrome (an adult-onset immunodeficiency associated with thymoma and hypogammaglobulinemia) was rare. The prevalence of Good's syndrome in our study (1.1 % of all patients with thymoma) was much lower than that in western countries, but only slightly higher than the prevalence in Japan (0.2–0.3 %) (Kikuchi et al. 2011). The clinical constellation of combined humoral and cellular immunodeficiency in these patients was similar to that seen in patients with classic Good's syndrome or common variable immunodeficiency. In our opinion, these abnormalities could be defined as "incomplete Good's syndrome" since the clinical characteristics resemble classic Good's syndrome although immunoglobulin levels remained in normal range. The disorders of cell mediated immunity were also evident in these patients (increased opportunistic intracellular infections and concurrent autoimmune diseases), supported by abnormal immunological tests (negative delayed type hypersensitivity skin test ± reversed CD4/CD8 ratio). In fact, the concomitant autoimmune diseases in patients with Good's syndrome were well-recognized (Khan et al. 2009; Seneschal et al. 2008; van der Marel et al. 2007). The combined feature of autoimmunity and immunodeficiency, and the existence of clinical immunodeficiency in the absence of hypogammaglobulinemia in patients with thymoma were previously observed (Federico et al. 2010; Holbro et al. 2012). It is worth noting that the outcome of patients with associated immunodeficiency and/or autoimmune diseases (excluding MG) was poor. According to our cohort study, only 2 out of 11 thymoma patients in this category recovered from their associated immunological disorders after thymectomy and five of the remaining patients succumbed to infections.

The prevalence of second malignancies in patients with thymoma in this study was comparable to the Taiwanese

Table 3 Characteristics of thymoma patients with suspected immunodeficiency

Gender/age (years)	Clinical presentations	Thymoma classification and staging	Infectious complications	Associated immune-mediated diseases	Underlying diseases	Immunoglobulin levels	Cell-mediated immunity investigation
Female/53	Chronic cough, dyspnea with weight loss	B2II most likely[a]	Diffuse cylindrical bronchiectasis, oral Candidiasis, *Pseudomonas pneumonia*	Lichen planus	Multinodular thyroid goiter	IgG = 1,740 mg/dL, IgM = 301.1 mg/dL, IgA = 267.7 mg/dL, IgE = 11.6 mg/dL	Negative DTH for tetanus, candida, and tuberculin CD4 = 925 cells/μL (31 %), D19 = 90 cells/μL (3 %)
Male/42	Chronic cough and weight loss	AI	Recurrent *Streptococcal pneumonia*, chronic bronchiectasis, recurrent herpes zoster	Sclerosing cholangitis	None	IgG = 445 mg/dL, IgM < 21.4 mg/dL, IgA = 28.5 mg/dL	CD4 = 168 cells/μL (18 %), CD8 = 505 cells/μL (56 %), CD19 = 1 %
Female/38	Dysphagia	B2III	Esophageal candidiasis, diffuse cylindrical bronchiectasis	None	None	IgG = 2,180 mg/dL, IgM = 206 mg/dL, IgA = 239 mg/dL, (IgG1 = 1,550 mg/ml, IgG2 = 463 mg/ml, IgG3 = 73.5 mg/ml, IgG4 = 113 mg/ml)	Impaired PHA stimulation test
Male/68	MG, weight loss	B2II	Diffuse cylindrical bronchiectasis, recurrent *Klebsiella pneumonia*	MG	Hypertension, benign prostate hyperplasia	IgG = 1,840 mg/dL, IgM = 153 mg/dL, IgA = 236 mg/dL, IgE = 83.9 mg/ml, (IgG1 = 1,120 mg/ml, IgG2 = 636 mg/ml, IgG3 = 25.2 mg/ml, IgG4 = 54.2 mg/ml)	CD4 = 759 cells/μL (30 %), CD8 = 1,012 cells/μL (40 %), CD19 = 1 %, CD56 = 12 %
Female/44	MG	B3IV	Diffuse cylindrical bronchiectasis, Pseudomonas p*neumonia*, Candida septicemia	MG, paraneoplastic pemphigus		IgG = 1,070 mg/dL,IgM = 71.3 mg/dL,IgA = 409 mg/dL	CD3 = 1320 cells/μL (57 %), CD4 = 440/cells/μL (30 %), CD8 = 857 cells/μL (37 %), CD56 = 261 cells/μL (11.3 %)
Female/52	Chest pain	B1III	Diffuse cylindrical bronchiectasis, pulmonary aspergilloma	MG	None	NA	NA
Female/61	Chronic cough	ABII	Diffuse cylindrical bronchiectasis	Pure red cell aplasia	None	IgG = 906 mg/dL, IgM = 97.1 mg/dL, IgA = 121 mg/dL	NA

Table 3 continued

Gender/age (years)	Clinical presentations	Thymoma classification and staging	Infectious complications	Associated immune-mediated diseases	Underlying diseases	Immunoglobulin levels	Cell-mediated immunity investigation
Male/54	Anemic symptoms	B3IV	Diffuse cylindrical bronchiectasis, pulmonary mycobacterial infection, invasive aspergillosis, Candida septicemia	Aplastic anemia	None	IgG = 1,790 mg/dL, IgM = 110 mg/dL, IgA = 173 mg/dL (IgG1 = 1,330 mg/dL, IgG2 = 274 mg/dL, IgG3 = 38 mg/dL, IgG4 = 20.4 mg/dL)	NA
Male/38	MG	ABII	Bronchiectasis, *Klebsiella pneumonia*, Staph septicemia	MG, lichen planus, nephrotic syndrome (minimal change)	None	NA	CD4 = 666 cells/μL (34 %), CD8 = 921 cells/μL (47 %), Negative DTH for tetanus, candida, and tuberculin

NA not available, *DTH* delayed type hypersensitivity skin testing, *MG* myasthenia gravis

[a] Histopathological diagnosis by mediastinoscopic biopsy

study, and types of malignancies were similar to those previously reported (Engels 2010; Pan et al. 2001; Welsh et al. 2000). The occurrence of synchronous neoplasms and multiple primary malignant neoplasms in our study reflected the defects of cancer immunosurveillance in thymoma patients. As most of associated malignancies were diagnosed simultaneously or prior to the development of thymoma, it was unlikely that these neoplasms were the consequences of chemoradiation or immunosupressive drugs from thymoma management.

Our study confirms an earlier report that the ratio of CD4/CD8 in patients who underwent thymectomy were lower than those in healthy controls (Krawczyk et al. 2007). The impaired IFN-γ response compared to IL-10 in patients with thymoma possibly explains the defective cellular immunity frequently observed. The increased percentages of peripheral blood Tregs in patients with thymoma may contribute to the altered cytokine responses and T cell dysfunction as well. These immunological aberrations may be responsible for increased opportunistic infections and secondary malignancies frequently found in thymoma patients. It was not clear whether this phenomenon is an intrinsic abnormality of patients with thymoma or the redistribution of Tregs in thymectomized patients. The dual or aberrant function of Tregs in patients with thymoma or the breakdown of peripheral tolerance is also possible since the development of immunodeficiency and autoimmunity in the same patients could occur (Verbsky and Chatila 2011).

Our study suggests that the prevalence of classic Good's syndrome in Asian populations is quite rare although the other immune-related characteristics are very similar to reports from other geographic regions. We speculate that the clinical spectrum of immunodeficiency in thymoma could possibly range from completely asymptomatic to fully immunocompromised with combined T and B cell deficiency and hypogammaglobulinemia. The aberrant cytokine production and increased peripheral blood Treg cells observed in this study could partially explain impaired cell-mediated immunity in patients with thymoma.

There were certain limitations due to the retrospective feature of this study. Some immunological tests were incomplete or missing from the hospital electronic medical records. Whether the immunological alterations observed in thymoma patients in this study were the underlying abnormalities predisposing to the development of thymoma, the results of thymoma transformation, or the consequences of thymectomy and other therapeutic modalities, needs further investigation (Fattorossi et al. 2005; Huang et al. 2004). The quantitative and qualitative analysis of peripheral blood T cells in patients with thymoma before and after thymectomy, compared to baseline

Table 4 Characteristics of thymoma patients with associated malignancies

Gender/age (years)	Thymoma classification and staging	Types of associated malignancies	Onset of malignancies	Associated immune-mediated diseases
Female/34	CIV	Invasive squamous cell cervical carcinoma	9 months prior to thymoma	None
Female/67	XIV	Renal cell carcinoma	Concurrent with thymoma	Pure red cell aplasia
Female/42	B3III	Poorly differentiated non-Hodgkin's lymphoma	5 years prior to thymoma	None
Male/76	B2III	Poorly differentiated, non-keratinized squamous cell carcinoma of skin	1 year prior to thymoma	None
Male/69	ABI	Non-small cell lung cancer	Concurrent with thymoma	None
Male/67	ABIV	Carcinoma of descending colon, rectum, and squamous cell carcinoma of scalp	Colon, rectum, and skin carcinomas were diagnosed 3, 6, and 7 years after thymoma, respectively	None
Female/68	B1II	Diffuse large cell lymphoma	7 years after thymoma	None
Female/69	AII	Breast cancer (mammary ductal carcinoma), non-small cell lung cancer	Breast cancer was diagnosed 7 months after thymoma, lung cancer was diagnosed 4 months prior to thymoma	None
Male/31	CIV	Epithelioid sarcoma, proximal type	Concurrent with thymoma	None

Table 5 The CD4/CD8 ratio, regulatory ($CD4^+CD25^+FoxP3^+$) T cells, and IFN-γ and IL-10 responses to PHA stimulation in 15 thymoma patients underwent thymectomy compared to 15 healthy individuals

Parameters	Age (years) gender	% CD4	% CD8	CD4/CD8	% Regulatory $CD4^+$ T cells ($CD25^+FoxP3^+$ $CD4^+$ cells)	PHA stimulation (SFC/106 PBMCs)		
						IFN-γ SFU/10^5 cells	IL-10 SFU/10^5 cells	IFN-γ/IL-10 ratio
Thymoma patients ($N = 15$)	46.33 ± 3.39 M:F = 8:7	28.73 ± 2.27	34.30 ± 3.24	0.94 ± 0.12	4.06 ± 0.52	1,845.87 ± 223.28	2,239.67 ± 237.77	1.01 ± 0.21
Healthy controls ($N = 15$)	42.40 ± 2.62 M:F = 6:9	36.65 ± 2.58 $p = 0.029$	26.49 ± 2.00 $p = 0.049$	1.58 ± 0.26 $p = 0.038$	1.44 ± 0.28 $p = 0.000$	3,586.40 ± 254.44 $p = 0.000$	2,243.60 ± 309.54 $p = 0.992$	1.93 ± 0.33 $p = 0.022$

T cell characteristics in the same patients prior to thymoma development would be very helpful to study the cause–effect relationship of immunological changes associated with thymoma.

In conclusion, several immunological disorders are often associated with thymoma in the Thai population. The prevalence of thymoma with clinical immunodeficiency was not uncommon, although classic Good's syndrome was rare. The immunodysregulation in these patients was possibly related to the imbalance of cytokine responses and altered T cell subsets. The temporal relationship between clinical and immunological laboratory findings at various stages of thymoma development should be further explored.

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Conflict of interest The authors have no conflicts of interest.

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