Prevalence of interrelated autoantibodies in thyroid diseases and autoimmune disorders

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ABSTRACT. ***Objective:*** **We determined the autoantibody profile in autoimmune thyroid diseases (AITD) and examined the distribution of thyroid-related autoantibodies in other autoimmune disorders.** ***Methods:*** **We tested sera from 234 patients with Graves' disease (GD), 130 with Hashimoto's thyroiditis (HT), 249 with other autoimmune diseases, and 50 healthy controls by enzyme-linked immunosorbent assay or radioimmunoassay.** ***Results:*** **Autoantibodies except TSH receptor antibody (Ab), anti-thyroglobulin (Tg) Ab and anti-thyroid peroxidase (TPO) Ab were not significantly prevalent in patients with AITD despite a significantly high elevation of thyroid-related Ab. Significant prevalence of autoantibodies related to AITD was observed in type 1 diabetes patients. Elevation of anti-Tg Ab was seen in patients with primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH), and anti-TPO Ab was elevated in patients with PBC. Although the prevalence of anti-acetylcholine receptor Ab and systemic lupus erythematosus (SLE)-related Ab was significant in AIH, primary Sjögren's syndrome (pSS)-related Ab were also found in both liver diseases. In *myasthenia gravis* (MG) patients, thyroid-related Ab and pSS-related Ab were detected in both MG groups, although SLE-related Ab were limited to the anti-muscle specific kinase Ab-positive MG patients. In patients with connective tissue diseases, anti-Tg Ab and anti-TPO Ab were significantly prevalent.** ***Conclusion:*** **Thyroid-related Ab were significantly elevated in all autoimmune diseases. Conversely, the elevations of Ab were not significant in the patients with AITD, suggesting a close relationship between AITD and other immune-mediated diseases. (J. Endocrinol. Invest. 31: 861-865, 2008)**

INTRODUCTION

Autoimmune disorders are triggered by both genetic and external factors (1). Autoantibody production and tissue damage are brought about by the emergence of autoreactive T-cell and subsequent B-cell expansion (2). Autoimmune disorders can be divided into two types, i.e., organ-specific and organ-non-specific disorders. Autoimmune thyroid diseases (AITD) and type 1 diabetes are thought to be organ-specific disorders. On the other hand, connective tissue diseases such as systemic *lupus erythematosus* (SLE) and primary Sjögren's syndrome (pSS) show non-specific systemic inflammation. Although these disorders have characteristic autoantibodies, it is well known that most of them are also complicated with AITD such as Graves' disease (GD) and Hashimoto's thyroiditis (HT) (3). We therefore considered that it would be of value to investigate the relationship between AITD and other autoimmune disorders from the perspective of autoantibody prevalence.

MATERIALS AND METHODS

We tested sera from 663 subjects in this study, including 234 with GD [male (M)/female (F): 56/178; age: 39.8±15.6], 130 with HT (M/F: 11/119; age: 44.7±17.5), 249 with other autoimmune diseases, and 50 age-matched healthy controls (M/F: 24/26; age: 36.2±8.1). Other autoimmune diseases included primary biliary cirrhosis (PBC) (M/F: 1/10; age: 61.0±10.3), autoimmune hepatitis (AIH) (M/F: 1/23; age: 62.9±11.6), type 1 diabetes (M/F: 13/37; age: 45.4±18.0), *myasthenia gravis* (MG) (M/F: 13/49, age: 50.2±14.4), rheumatoid arthritis (RA) (M/F: 5/24; age: 61.0±14.7), SLE (M/F: 3/41; age: 44.7±11.6) and pSS (M/F: 2/27; age: 62.3±13.1). In each autoimmune disease, the existence of other clinically diagnosed autoimmune diseases was ignored when evaluating the autoantibody profile in sera. Furthermore, no fulminant type 1 diabetes patients were included in this study, because it is suggested that fulminant type 1 diabetes is a non-autoimmune-mediated disease (4). We used newly developed autoantibody detection kits (Table 1) provided by Cosmic Corp. (Tokyo, Japan). The kits contained antibodies (Ab) for TSH receptor (TR) (TRAb 3rd generation, Cosmic) (5, 6), thyroid peroxidase (TPO) (TPOAb, Cosmic II) (7), thyroglobulin (Tg) (TgAb, Cosmic II), glutamic acid decarboxylase (GAD) (GADAb, Cosmic), IA-2 (IA-2Ab, Cosmic), and acetylcholine receptor (AChR) (AChRAb, Cosmic II). The 3rd generation TRAb assay (Cosmic Corp.), using biotin-labelled monoclonal Ab (M22) instead of biotin-labelled TSH, was determined by enzyme-linked immunosorbent assay, and others were determined by radioimmunoassay. TRAb 3rd generation, TgAb, and AChRAb fetal type are extraction kits and GADAb, IA-2Ab, and AChRAb adult type are recombinant kits. For detection of other autoantibodies against SS-A, SS-B, Scl-70, Jo-1, ribonucleoprotein (RNP), Sm, double-stranded deoxyribonucleic acid (dsDNA), and ribosomal P, a colorimetric microarray system (Cosmic Corp.) was employed, by which many kinds of Ab measurement were performed automatically. Informed consent for this study was obtained from all participants. For the statistical analysis of the prevalence of each antibody, the chi-square test was employed. Values of $p<0.05$ were considered to be statistically significant.

Key-words: Connective tissue diseases, myasthenia gravis, primary biliary cirrhosis, thyroid disease, type 1 diabetes.

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Table 1 - *Sensitivity, specificity and cut-off value of the autoantibody detection kits in this study.*

Kits	Measurement	Sensitivity (%)	Specificity (%)	Cut-off value	Unit
TRAb	ELISA	99.6	99.6	15	%
TPOAb	RIA	76.9	100	0.3	U/ml
TgAb	RIA	92.3	83.3	0.3	U/ml
GADAb	RIA	84	90	1.5	U/ml
IA-2Ab	RIA	58	100	0.4	U/ml
AChRAb	RIA	80	100	0.1	nmol/l
Anti-SS-A Ab	Colorimetric microarray	NT	NT	<30	U
Anti-SS-B Ab	Colorimetric microarray	NT	NT	<30	U
Anti-Scl-70 Ab	Colorimetric microarray	NT	NT	<30	U
Anti-Jo-1 Ab	Colorimetric microarray	NT	NT	<30	U
Anti-RNP Ab	Colorimetric microarray	NT	NT	<30	U
Anti-Sm Ab	Colorimetric microarray	NT	NT	<30	U
Anti-dsDNA Ab	Colorimetric microarray	NT	NT	<30	IU
Anti-ribosomal P Ab	Colorimetric microarray	NT	NT	<30	U

TR: TSH receptor; Ab: antibody; TPO: thyroid peroxidase; Tg: thyroglobulin; GAD: glutamic acid decarboxylase; AChR: acetylcholine receptor; RNP: ribonucleoprotein; dsDNA: double-stranded deoxyribonucleic acid; ELISA: enzyme-linked immunosorbent assay; RIA: radioimmunoassay; NT: not tested. Sensitivity and specificity of TRAb, TPOAb/TgAb, GADAb/IA-2Ab, and AChRAb were configured against Graves' disease, Hashimoto's thyroiditis, type 1 diabetes, and *myasthenia gravis*, respectively.

RESULTS

Detection of autoantibodies in AITD

Table 2 shows the autoantibodies detected in GD and HT. TRAb, anti-Tg Ab, and anti-TPO Ab were all significantly elevated in both patients with GD and those with HT. Although the prevalence of TRAb in GD was greater than that in HT, the prevalence of anti-Tg Ab in HT was significantly greater than that in GD, reaching 96.9% in HT. The prevalence of anti-TPO Ab was equivalent in both GD and HT. Anti-GAD Ab and anti-IA-2 Ab were detected in 6.4% and 4.3% of GD patients and 4.6% and 3.8% of HT patients, respectively. The prevalence of anti-AChR Ab was 3.4% in GD and 3.8% in HT. We also examined the prevalence of anti-GAD Ab (no.=21) and anti-AChR Ab (no.=13) in the combined group of patients with GD and patients with HT (no.=364), resulting in p values of 0.08 and 0.2 compared to healthy controls as determined by the chi-square test, respectively. The antibodies related to SLE were sporadically detected in GD and HT, but this prevalence was not statistically significant. Anti-SS-A Ab and anti-Ro Ab were detected in 3.1 to 3.8% of patients with GD and HT, without significance, although no anti-SS-B Ab was detected in either group. Neither anti-Scl-70 Ab nor anti-Jo-1 Ab was detected in either group (data not shown).

Table 2 - *Autoantibodies detected in Graves' disease and Hashimoto's thyroiditis.*

Autoantibodies	Graves' disease (no.=234)	Hashimoto's thyroiditis (no.=130)	Healthy control (no.=50)
TRAb	209 (89.3%)[b]	10 (7.7%)[a,c]	0 (0.0%)
Anti-Tg Ab	189 (80.8%)[b]	126 (96.9%)[b,c]	2 (4.0%)
Anti-TPO Ab	191 (81.6%)[b]	113 (81.6%)[b]	1 (2.0%)
Anti-GAD Ab	15 (6.4%)	6 (4.6%)	0 (0.0%)
Anti-IA-2 Ab	10 (4.3%)	5 (3.8%)	0 (0.0%)
Anti-AChR Ab	8 (3.4%)	5 (3.8%)	0 (0.0%)
Anti-dsDNA Ab	1 (0.4%)	3 (2.3%)	0 (0.0%)
Anti-Sm Ab	1 (0.4%)	3 (2.3%)	0 (0.0%)
Anti-RNP Ab	1 (0.4%)	1 (0.8%)	0 (0.0%)
Anti-ribosomal P Ab	0 (0.0%)	0 (0.0%)	0 (0.0%)
Anti-SS-A Ab	9 (3.8%)	4 (3.1%)	0 (0.0%)
Anti-Ro52 Ab	9 (3.8%)	4 (3.1%)	0 (0.0%)
Anti-SS-B Ab	0 (0.0%)	0 (0.0%)	0 (0.0%)

TR: TSH receptor; Ab: antibody; Tg: thyroglobulin; TPO: thyroid peroxidase; GAD: glutamic acid decarboxylase; AChR: acetylcholine receptor; dsDNA: double-stranded deoxyribonucleic acid; RNP: ribonucleoprotein. Diagnosis of Graves' disease was confirmed by the presence of clinical signs including thyrotoxicosis; diffuse thyroid goiter or exophthalmos with increased free T_3 or T_4, reduced TSH, or positive anti-TSH receptor antibody. Diagnosis of Hashimoto's thyroiditis was done by the presence of diffuse goiter with positive microzome (anti-TPO) antibody, anti-Tg antibody, or lymphocytic infiltration by cytology. The prevalence of autoantibodies was calculated by the chi-square test. $p<0.05$ was statistically significant. Graves' disease/Hashimoto's thyroiditis *vs* healthy control; [a]$p<0.05$, [b]$p<0.01$, Hashimoto's thyroiditis *vs* Graves' disease; [c]$p<0.01$.

Thyroid-related autoantibodies in autoimmune diseases

In the 50 patients with type 1 diabetes, the prevalence of anti-GAD Ab and anti-IA-2 Ab was 35/50 (70.0%) and 15/50 (30.0%), respectively (Table 3). The prevalence of TRAb, anti-Tg Ab, and anti-TPO Ab was significantly elevated in type 1 diabetes. Regarding anti-AChR Ab or connective tissue disease-related autoantibodies in type 1 diabetes, there was no significant elevation (Table 3). In autoimmune liver diseases, the prevalence of anti-Tg Ab was significantly elevated in both PBC and AIH compared to that in healthy controls, although neither anti-GAD Ab nor anti-IA-2 Ab were found (Table 4). The prevalence of anti-TPO Ab was significantly increased in patients with PBC, and the prevalence of this autoantibody in patients with AIH was significantly smaller than that in patients with PBC. Although anti-AChR Ab was not detected in the sera of PBC patients, this Ab was found in the sera of 3/24 (12.5%) of AIH patients (Table 4). With regard to autoantibodies related to connective tissue disorders, the prevalence of SLE-related autoantibodies in AIH was greater than that in PBC. Anti-SS-A Ab, anti-Ro52 Ab, and

Table 3 - *Autoantibodies detected in type 1 diabetes.*

Autoantibodies	Type 1 diabetes (no.=50)	Healthy control (no.=50)
Anti-GAD Ab	35 (70.0%)[a]	0 (0.0%)
Anti-IA-2 Ab	15 (30.0%)[a]	0 (0.0%)
TRAb	10 (20.0%)[a]	0 (0.0%)
Anti-Tg Ab	22 (44.0%)[a]	2 (4.0%)
Anti-TPO Ab	25 (50.0%)[a]	1 (2.0%)
Anti-AChR Ab	0 (0.0%)	0 (0.0%)
Anti-dsDNA Ab	0 (0.0%)	0 (0.0%)
Anti-Sm Ab	0 (0.0%)	0 (0.0%)
Anti-RNP Ab	0 (0.0%)	0 (0.0%)
Anti-ribosomal P Ab	0 (0.0%)	0 (0.0%)
Anti-SS-A Ab	1 (1.8%)	0 (0.0%)
Anti-SS-B Ab	0 (0.0%)	0 (0.0%)

GAD: glutamic acid decarboxylase; Ab: antibody; TR: TSH receptor; Tg: thyroglobulin; TPO: thyroid peroxidase; AChR: acetylcholine receptor; ds-DNA: double-stranded deoxyribonucleic acid; RNP: ribonucleoprotein. The diagnosis of acute-onset type 1 diabetes was made based on the criteria of the American Diabetes Association for type 1 diabetes (8). The prevalence of autoantibodies was calculated by the chi-square test. $p<0.05$ was statistically significant. type 1 diabetes *vs* healthy control; [a]$p<0.01$.

anti-SS-B Ab were significantly detected in both PBC and AIH, although neither anti-Scl-70 Ab nor anti-Jo-1 Ab was found in either group (data not shown). In MG patients, the prevalence of anti-Tg Ab and anti-TPO Ab was greatly increased in both MG patient groups (Table 5). In contrast, anti-GAD Ab was significantly detected in the anti-AChR Ab-positive MG groups, although no anti-IA-2 Ab was detected in either group. Interestingly, autoantibodies related to SLE were detected in sera from anti-muscle-specific tyrosine kinase (MuSK) Ab-positive MG patients only, although there was no significant difference in either of the groups (Table 5). Just as in the case of AIH or PBC, pSS-related autoantibodies were detected in both anti-AChR Ab-positive and anti-MuSK Ab-positive MG groups. Neither anti-Scl-70 Ab nor anti-Jo-1 Ab was found in the sera of MG patients (data not shown).

As concerns autoantibodies detected in connective tissue diseases, no significant elevation of TRAb was observed among the patients with SLE, pSS, and RA (Table 6). Anti-Tg Ab and anti-TPO Ab were significantly detected in these groups, although the prevalence of anti-165 TPO Ab in pSS was smaller than that in the other groups. Anti-GAD Ab was not significantly detected in these diseases, although anti-IA-2 Ab and anti-AChR Ab were significantly detected in RA only.

DISCUSSION

Thyroid-related Ab were detected in patients with AITD; in particular, anti-Tg Ab and anti-TPO Ab were significantly elevated in HT patients. The etiological causes of HT are thought to include Ab-dependent cellular cytotoxicity or activation of complements, while the Fas/Fas ligand system has also been reported to be related to the pathogenesis of GD (9). In this study, the prevalence of anti-Tg Ab and anti-TPO Ab in HT was different, suggesting that this might be related to their different action mechanisms. Although autoantibodies other than anti-Tg Ab and anti-TPO Ab were not significantly elevated in AITD, Ohta et al. (10) previously reported detectable levels of anti-GAD Ab in 3.53% of their patients with AITD, which is in accordance with our results. A low

Table 4 - *Autoantibodies detected in autoimmune liver diseases.*

Autoantibodies	PBC (no.=11)	AIH (no.=24)	Healthy control (no.=50)
TRAb	1 (9.1%)[a]	1 (4.2%)	0 (0.0%)
Anti-Tg Ab	6 (54.5%)[b]	13 (54.2%)[b]	2 (4.0%)
Anti-TPO Ab	5 (45.5%)[b]	2 (8.3%)[c]	1 (2.0%)
Anti-GAD Ab	0 (0.0%)	0 (0.0%)	0 (0.0%)
Anti-IA-2 Ab	0 (0.0%)	0 (0.0%)	0 (0.0%)
Anti-AChR Ab	0 (0.0%)	3 (12.5%)[a]	0 (0.0%)
Anti-dsDNA Ab	0 (0.0%)	2 (8.3%)[a]	0 (0.0%)
Anti-Sm Ab	0 (0.0%)	2 (8.3%)[a]	0 (0.0%)
Anti-RNP Ab	0 (0.0%)	1 (4.2%)	0 (0.0%)
Anti-ribosomal P Ab	0 (0.0%)	1 (4.2%)	0 (0.0%)
Anti-SS-A Ab	3 (27.3%)[b]	8 (33.3%)[b]	0 (0.0%)
Anti-Ro52 Ab	3 (27.3%)[b]	7 (29.2%)[b]	0 (0.0%)
Anti-SS-B Ab	2 (18.2%)[b]	4 (16.7%)[b]	0 (0.0%)

TR: TSH receptor; Ab: antibody; Tg: thyroglobulin; TPO: thyroid peroxidase; GAD: glutamic acid decarboxylase; AChR: acetylcholine receptor; dsDNA: double-stranded deoxyribonucleic acid; RNP: ribonucleoprotein; PBC: primary biliary cirrhosis; AIH: autoimmune hepatitis. Diagnosis of PBC was done by the presence of chronic non-suppurative destructive cholangitis (CNSDC), positive antimitochondrial antibody without CNSDC, or positive anti-mitochondrial antibody with clinical manifestations. Autoimmune hepatitis is characterized by the presence of autoantibodies, elevation of γ-globulin/IgG, continuous elevation of transaminase, negative hepatitis viral markers and histological findings of chronic hepatitis or liver cirrhosis with interface hepatitis. The prevalence of autoantibodies was calculated by the chi-square test. $p<0.05$ was statistically significant. PBC/AIH *vs* healthy control; [a]$p<0.05$, [b]$p<0.01$, AIH *vs* PBC; [c]$p<0.05$.

Table 5 - *Autoantibodies detected in myasthenia gravis (MG).*

Autoantibodies	Anti-AChR Ab positive MG (no.=23)	Anti-MuSK Ab positive MG (no.=39)	Healthy control (no.=50)
Anti-AChR Ab	23 (100.0%)[b]	1 (2.6%)[c]	0 (0.0%)
Anti-MuSK Ab	0 (0.0%)	39 (100.0%)[b,c]	0 (0.0%)
TRAb	1 (4.3%)	0 (0.0%)	0 (0.0%)
Anti-Tg Ab	6 (26.1%)[b]	7 (17.9%)[a]	2 (4.0%)
Anti-TPO Ab	6 (26.1%)[b]	7 (17.9%)[b]	1 (2.0%)
Anti-GAD Ab	3 (13.0%)[b]	1 (2.6%)	0 (0.0%)
Anti-IA-2 Ab	0 (0.0%)	0 (0.0%)	0 (0.0%)
Anti-dsDNA Ab	0 (0.0%)	2 (5.1%)	0 (0.0%)
Anti-Sm Ab	0 (0.0%)	2 (5.1%)	0 (0.0%)
Anti-RNP Ab	0 (0.0%)	1 (2.6%)	0 (0.0%)
Anti-ribosomal P Ab	0 (0.0%)	1 (2.6%)	0 (0.0%)
Anti-SS-A Ab	3 (13.0%)[b]	8 (20.5%)[b]	0 (0.0%)
Anti-Ro52 Ab	3 (13.0%)[b]	7 (17.9%)[b]	0 (0.0%)
Anti-SS-B Ab	2 (8.7%)[a]	4 (10.2%)[a]	0 (0.0%)

AChR: acetylcholine receptor; Ab: antibody; MuSK: muscle-specific tyrosine kinase; TR: TSH receptor; Tg: thyroglobulin; TPO: thyroid peroxidase; GAD: glutamic acid decarboxylase; dsDNA: double-stranded deoxyribonucleic acid; RNP: ribonucleoprotein; Diagnosis of MG was done by easy fatigability, waning detected by myoelectrogram, positive anti-acetylcholine receptor antibody and effectiveness of anti-cholinesterase agents. The prevalence of autoantibodies was calculated by the chi-square test. The $p<0.05$ was statistically significant. MG *vs* healthy control; [a]$p<0.05$, [b]$p<0.01$, anti-MuSK Ab positive MG *vs* anti-AChR positive MG;[c]$p<0.01$.

Table 6 - *Autoantibodies detected in connective tissue diseases.*

Autoantibodies	SLE (no.=44)	pSS (no.=29)	RA (no.=29)	Healthy control (no.=50)
TRAb	3 (6.8%)	0 (0.0%)	2 (6.9%)	0 (0.0%)
Anti-Tg Ab	15 (34.1%)[b]	10 (30.0%)[b]	9 (31.0%)[b]	2 (4.0%)
Anti-TPO Ab	12 (27.3%)[b]	4 (13.8%)[a]	9 (31.0%)[b]	1 (2.0%)
Anti-GAD Ab	1 (2.3%)	1 (3.4%)	1 (3.4%)	0 (0.0%)
Anti-IA-2 Ab	0 (0.0%)	2 (6.9%)	3 (10.3%)[a,c]	0 (0.0%)
Anti-AChR Ab	3 (6.8%)	1 (3.4%)	3 (10.3%)[a]	0 (0.0%)

TR: TSH receptor; Ab: antibody; Tg: thyroglobulin; TPO: thyroid peroxidase; GAD: glutamic acid decarboxylase; AChR: acetylcholine receptor; SLE: systemic lupus erythematosus; pSS: primary Sjögren's syndrome; RA: rheumatoid arthritis. Diagnosis of SLE was confirmed by the existence at least 4 items out of malar erythema, discoid erythema, photosensitivity, oral ulcer, arthritis, serositis, renal involvement, neurological involvement, hematological involvement, immunological involvement, and positive anti-nuclear antibody. Diagnosis of pSS was done by at least 4 items out of dry eye, dry mouth, reduction of lacrimal fluid or salivary flow, positive anti-SS-A/SS-B antibodies or positive finding from salivary gland biopsy. Diagnosis of RA was confirmed by the existence at least 4 items out of morning stiffness, swelling of at least 3 joints, swelling of wrist, metacarpophalangeal joints or proximal interphalangeal joints, symmetrical joint swelling, abnormality of hand or finger X-ray, rheumatoid nodule, and rheumatoid factor. The prevalence of autoantibodies was calculated by the chi-square test. $p<0.05$ was statistically significant. SLE/pSS/RA *vs* healthy control; [a]$p<0.05$, [b]$p<0.01$, [c]*p*SS or RA *vs* SLE; [c]$p<0.05$.

prevalence of anti-IA-2 Ab has also been observed in patients with GD (11). Finally, low titers of GAD Ab have also been reported in the sera of AITD patients, although those in AITD were greater than those in healthy subjects (12). Type 1 diabetes is characterized by insulitis and destruction of β cells in functional islets (13, 14) followed by production of specific autoantibodies (15-17). Although GAD consists of two isoforms, the main isoform detected in humans is GAD65 (18, 19). In the present study, the prevalence of anti-GAD Ab in type 1 diabetes was greater than that of anti-IA-2 Ab, suggesting that the Ab against GAD65 is a better diagnostic indicator of type 1 diabetes. As for GAD Ab in type 1 diabetes, type 1 diabetes patients with AITD show high levels of GAD Ab (20). Furthermore, Goswami et al. (21) previously reported that the prevalence of anti-TPO Ab in 100 patients with type 1 diabetes was 42.1%. The prevalence of anti-TPO Ab in this study was similar to that observed in their study, suggesting the availability of these Ab for diagnosis of HT in type 1 diabetes. However, the prevalence of these Ab reported in the Czech Republic is much less than that suggested by our data (22), indicating that there may be some racial variation.

The significantly high prevalence of anti-Tg Ab and anti-SS-A/SS-B Ab in both PBC and AIH has suggested that these autoimmune liver diseases are closely related to pSS (23). We should also pay attention to the difference in the prevalence of anti-TPO Ab, which might be a clue to segregating PBC from AIH from a serological perspective because of the low prevalence of anti-TPO Ab in AIH. Sporadically detected autoantibodies related to SLE and MG might explain the pathological characteristics of AIH (24) due to the remarkable infiltration of plasma cells in the portal area, which is closely associated with autoantibody production. In regard to the target antigen of anti-AChR Ab, both fetal-type and adult-type antigens have been identified. MacLennan et al. (25) reported that fetal-type negative MG included the adult-type positive population. In the present study, we used a kit that was composed of both types of AChR. In contrast, MuSK Ab-positive MG (26) is characterized by the presence of IgG4 subclass autoantibody with a low prevalence of complement deposition in motor end-210 plates. Although it is known that MG commonly complicates GD (27), we noticed that TRAb levels were not elevated in any of the MG patients in our study. However, both anti-Tg Ab and anti-TPO Ab were equally detected in both MG groups, implying a co-existence of HT. As concerns the prevalence of thyroid-related autoantibodies in MG, Kiessling et al. (28) have previously reported a much lower prevalence of anti-Tg Ab than that observed in our patients with anti-AChR Ab-positive MG, which might have been caused by differences in the measurement kits or racial variations between the studies. In addition, pSS-related Ab in both MG groups were suggested to be of benefit for examining complications of subclinical pSS. Various autoantibodies are found in connective tissue diseases. With regard to the prevalence of thyroid-related autoantibodies, Pyne et al. (29) reported that 42 of their 300 patients with SLE (14%) were positive for thyroid-related antibodies. Prior to their observation, others reported the prevalence of thyroid-related Ab to be 21-45% in SLE patients (30, 31), which was close to our results. It is well known that pSS is also associated with a variety of autoantibodies (32), and our data showed the prevalence of anti-Tg and anti-TPO antibodies in pSS to be elevated with low prevalence of TRAb. These findings imply that connective tissue diseases are easily complicated with AITD, but less readily with type 1 diabetes and MG. For the statistical analysis with regard to the prevalence of autoantibodies, we should note that the statistical significance differs according to the number of patients in each disease. Because we had a sufficient number of GD and HT patients, the prevalence of autoantibodies in these diseases was considered to be reliable. Nonetheless, the number of patients with PBC or AIH was relatively small; inclusion of a larger number of patients in these disease groups might have yielded a higher seropositivity toward each autoantibody or a higher level of statistical significance. In summary, we found that thyroid-related autoantibodies, especially anti-Tg Ab, can be detected in all autoimmune diseases. Regarding the elevated prevalence of both anti-Tg Ab and anti-TPO Ab, high prevalence of the former was particularly indicative of the clinical and subclinical existence of HT. In type 1 diabetes patients, the prevalence of TRAb and anti-Tg Ab was elevated, which is a distinct finding compared to the profiles in other autoimmune diseases. However, as reported in SLE patients (29), there would be a difference between hypothyroidism and hyperthy-

roidism in autoimmune diseases. Since a varying degree of B-cell activation followed by plasma cell expansion in these autoimmune diseases results in various clinical and serological characteristics, each disease might have a preference with regard to thyroid-related autoantibodies. Therefore, increased thyroid-related autoantibody profile is considered to be useful for investigating the mechanism of immune-mediated diseases.

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