Comprehensive research on thyroid diseases associated with autoimmunity: autoimmune thyroid diseases, thyroid diseases during immune-checkpoint inhibitors therapy, and immunoglobulin-G4-associated thyroid diseases

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Abstract. Various thyroid diseases are associated with autoimmunity. Major autoimmune thyroid diseases are Graves’ disease (GD) and Hashimoto’s thyroiditis (HT). Thyrotropin receptor is an autoantigen in GD, and its immunogenicity has been examined. Immune-checkpoint inhibitor (ICI) is recently widely used for treatment of malignant tumors, but cases of thyroid diseases during ICI treatment have been increasing. Thyroid diseases during ICI therapy have been investigated in immunological and clinical aspects, and their official Japanese diagnostic guidelines were established. In addition, serum and tissue immunoglobulin-G4 (IgG4) levels have been examined in association with clinicopathological characteristics in GD, HT, and Riedel’s thyroiditis. We review these diseases associated with thyroid autoimmunity and comprehensively discuss their potential application in future research and therapeutic options.

Key words: Graves’ disease, Hashimoto’s thyroiditis, Riedel’s thyroiditis, Immunoglobulin G4, Immune-checkpoint inhibitors

doi:10.1507/endocrj.EJ19-0234

Introduction

Autoimmune thyroid disease (AITD) is a thyroid-specific autoimmune disease, and Graves’ disease (GD) and Hashimoto’s thyroiditis (HT) are two major components of AITDs [1-3]. When individuals with susceptible genetic background are exposed to environmental factors (e.g. iodine, smoking, infections, stress, and trauma), AITDs may develop [1-3] (Fig. 1). Thyroid autoantigens include thyroglobulin (Tg), thyrotropin (TSH) receptor (TSHR), thyroid peroxidase (TPO), and sodium iodide symporter. TSHR is an autoantigen in GD, and anti-TSHR antibodies (TRAb) cause hyperthyroidism [1, 2, 4, 5]. Tg and/or TPO are autoantigens in HT [3]. Immunogenicity of TSHR has been examined and epitope-specific therapies for GD have been developed. Recently, immune-checkpoint inhibitors (ICIs) are widely used for treatment of malignant tumors, but cases of thyroid dysfunctions are increasing during ICI therapy. Thyroid diseases during ICIs therapy have been investigated in immunological and clinical aspects, and their official Japanese diagnostic guidelines were established. In addition, serum and tissue immunoglobulin-G4 (IgG4) levels in association with clinicopathological characteristics in GD, HT, and Riedel’s thyroiditis (RT) have been examined. We review these three thyroid diseases associated with autoimmunity: AITDs, Thyroid diseases during ICIs therapy, and IgG4-associated thyroid diseases. Moreover, we comprehensively discuss their possible application in future research and in therapeutic approaches (Fig. 2).

Autoimmune Thyroid Disease (AITD)

Autoimmunity and genetic factors in AITD

Genetic factors contribute to development of AITD [2, 3, 6, 7]. Reported factors to predisposition to GD include human leukocyte antigen (HLA) [6], polymorphisms of TSHR [6, 7], Tg [6], and Cytotoxic T-lymphocytes antigen (CTLA-4) [7]. Among these, HLA is a major genetic factor in AITD. Inheritance of HLA-DRB1*03:01 (DR3) has been reported to be susceptible gene to GD [8], and
Our common research strategy of thyroid diseases associated with autoimmunity.

Fig. 1 Relations between immunological network amongAITD, thyroid diseases during ICIs therapy, and IgG4-associated thyroid diseases are shown. APC or tumor cells present thyroid epitope or tumor-associated epitope on the surface of them to TCR. HLA-class II bound epitopes are presented to CD4⁺ T-cells, and HLA-class I bound epitopes face to CD8⁺ T-cells, respectively. HLA-binding amino acid groove (position 1-9) is shown in the window.

Amino acids in positions 1, 4, 6, 7, and 9 bind to HLA and those in positions 2, 3, 5, and 8 are assumed to be outward facing to stimulate the TCR.

Antibodies to immune-checkpoint molecules (CTLA-4, PD-1, and PD-L1) are referred to as ICIs, and regulate those immune reactions.

Follicular helper 2 (Th2) cells as well as CD4⁺ T-cells stimulate B/plasmacytes, and thyroid-specific IgGs (TRAb, TgAb, and TPOAb) including IgG4 subtype are produced. CD4⁺ cytotoxic T-cells (CTL) as well as Treg produce TGF-β, and induce tissue fibrosis and IgG4-RD.

Epitopes include thyroid-specific antigen, tumor-associated antigen, and IgG4-RD associated antigen, as applicable. APC: antigen-presenting cell. TCR: T-cell receptor.

Also in HT [6]. When focused on GD, HLA-DQA1*05:01 was reported to predispose to GD in Caucasians [8]. In contrast, HLA-DRB1*07:01 was reported to be a protective allele for GD [9]. The second most important gene polymorphism for GD is CTLA-4. This molecule is expressed on the surface of activated T-cells, inhibiting binding of CD28 to B7 molecule on antigen-presenting cells (APC) [2]. Thus CTLA-4 suppresses T-cell mediated immunity. In addition to CTLA-4, programmed death-1 (PD-1) and programmed death ligand 1 (PD-L1) are referred to as immune-checkpoint molecules. These molecules play an important role in immune suppression [10, 11] (Fig. 1). Interaction by the complex of TSHR epitope, HLA-DR, and T-cell receptor (TCR) is modified through those suppressive signals by immune-checkpoint molecules.
**Relation of TSHR and HLA-DR in GD**

TSHR is one of a family of glycoprotein-coupled hormone receptors [12, 13]. It is crucial for TSH signal transduction and thyroid hormone production [14]. Major histocompatibility (MHC) in humans is referred to as HLA. Single polypeptide of TSHR is cleaved into extracellular A-subunit, and transmembrane and cytosolic B-subunit [15]. Extracellular A-subunit is thought to shed into circulation. The shed TSHR protein is endocytosed into APCs, and processed TSHR peptides bind to HLA-DR3 molecules for presentation on APCs to CD4+ T-cells [15] (Fig. 1). The complex of TSHR epitope on APC with HLA-DR and recognition by T-cells is the most important factor in determination of immunogenicity. Extracellular domain in TSHR (amino acids 19–417: TSHR-ECD), and part of it (A-subunit: 1–289) are thought to be immunogenic in GD [2]. In order to identify TSHR epitope in GD, we have examined binding affinity between TSHR-ECD epitopes and HLA-DR in studies *in silico*, *in vitro*, and *in vivo* [5, 16-18]. In our studies *in silico*, the computer algorithm program predicted binding affinities of TSHR-ECD peptides to epitope binding groove in various HLA-DR molecules. In studies *in vitro*, HLA-DR molecules purified by antibody-coupled affinity are used to examine binding affinity to synthesized TSHR-ECD peptides. The peptide binding groove in HLA-DR consists of nine amino acids. The positively-charged arginine in position 4 of the amino acid sequence in the binding motif of HLA-DR3 have been critical in determining binding affinity between the TSHR epitopes and HLA-DR [2]. TSHR-ECD epitopes with negatively-charged D (aspartic acid) or E (glutamic acid) in position four of the binding motif bound more strongly to HLA-DR3. Moreover, those TSHR-ECD peptides were more stimulatory to peripheral blood mononuclear cells (PBMC) of GD patients and to splenocytes from HLA-DR3 transgenic mice immunized to TSHR-ECD [16, 17]. Most importantly, TSHR-ECD peptide 78–94 (ISRIYVSIDVTLQ QLES, in which DR3 binding motif position 5 aspartic acid) or E (glutamic acid) in position four of the binding motif bound more strongly to HLA-DR3. Moreover, those TSHR-ECD peptides were more stimulatory to peripheral blood mononuclear cells (PBMC) of GD patients and to splenocytes from HLA-DR3 transgenic mice immunized to TSHR-ECD [16, 17]. Most importantly, TSHR-ECD peptide 78–94 (ISRIYVSIDVTLQ QLES, followed by TSHR-ECD peptide 132–150 (GIFNTGLKMFPDLTKVYST) were identified *in silico*, *in vitro*, and *in vivo*, and in clinical assays as important TSHR epitopes in GD (Fig. 1). These two epitopes appear important in immunogenicity to TSHR due to their favored binding to HLA-DR3, thus increasing presentation to T cells [2, 16, 17]. Pichurin, et al. reported that in HLA-DR3 transgenic mice immunized to adenovirus coding TSHR 1–289, TSHR peptide (142–161) seemed to be a TSHR epitope, which was a similar result to ours [19].

On the other hand, peptides with strong binding affinities to HLA-DR molecules might induce thymic deletion of the cognate T cells, ‘negative selection in the thymus’, while those peptides exhibiting moderate binding affinities could escape the selection and enter in the circulation and participate in the development of GD [5]. Also identified as T-cell epitopes in GD were TSHR epitopes with moderate binding affinities to HLA-DR3; residues 145–163, 158–176, 207–222, 248–263, 272–291, and 343–362 [5]. Competition between low- and high-risk alleles for binding to TSHR peptides also affects the development of GD.

**Skewed balance of Th1/Th2 and AITD**

Naïve CD4+ T-cells may differentiate to T helper 1 (Th1) or T helper 2 (Th2) cells [20]. In HT, Th1 cells induce CD8+ cytotoxic T-cells, then inflammation of the thyroid gland occurs. By contrast, activated Th2 cells send signals to stimulate B-cells for production of TRAb in GD, and hyperthyroidism occurs.

**Central and peripheral tolerance in AITD**

In central tolerance, T-cells with strong affinity to autoantigen epitope are deleted in the thymus [4, 5]. In peripheral tolerance, regulatory T-cells (Tregs) suppress immunogenic T-cells [21]. Dysfunction of central or peripheral tolerance allow onset of GD. Treg dysfunction was suggested to be associated with GD [21]. We have reported pediatric and elderly GD patients with 22q11.2 deletion who exhibited dysfunction of central and peripheral tolerances [22].

**Epitope spreading during progression of GD**

Thyroid autoantigen, namely TSHR, TPO, and Tg are closely associated with ‘epitope spreading’. In the course of pathogenic amplification of immunogenic T- and B-cells, intra- or inter- ‘epitope spreading’ is often seen in GD [4, 23].

**Antigen specific treatments of GD**

Effectivity of a novel small molecular TSHR antagonist was shown in animal studies as a TSHR antigen-specific treatment for GD [24]. TSHR epitope-specific treatments using mutated TSHR peptides were reported to suppress immunogenic reaction to TSHR-ECD in HLA-DR3 transgenic mice immunized to TSHR-ECD protein [18]. With respect to TSHR peptides within HLA-DR binding groove which consists of nine amino acids, amino acids in positions 1, 4, 6, 7, and 9 bind to HLA-DR. Meanwhile, those in positions 2, 3, 5, and 8 are assumed to be outward-facing to stimulate the TCR [8]. Therefore, a mutant TSHR peptide was constructed in which the contact of peptide to TCR would be attenuated [18]. TSHR peptide 78–94: ISRIYVSIDVTLQ QLES was mutated to TSHR peptide 37m: ISRIYVSI DATLSQLES, in which DR3 binding motif position 5...
was mutated V>A, and position 8 Q>S. 37m was predicted to bind to HLA-DR3, but not to bind strongly to T-cell receptors. Both B- and T-cells responses to TSHR peptide 78–94 were significantly suppressed by peptide 37m. Other studies using mutant TSHR peptides as treatment for GD have been recently reported [25, 26]. Epitope specific treatment of GD targeted to TSHR antigen may therefore be hopeful.

**Conclusion of research on AITD**

Genetic and environmental factors contribute to the development of AITD. HLA has been reported as a major genetic factor that influences autoimmunity in the thyroid gland. Thyroid autoantigens presented to T-cells with certain HLA/MHC molecules play an important role in the initiation of AITD. Identification of thyroid epitopes and epitope spreading, cytokine balance, and immunological tolerance, therefore seem to be important for future application to antigen-specific treatments of AITD.

**Thyroid Diseases during Immune-Checkpoint Inhibitors (ICIs) Therapy**

**Immune-checkpoints and their inhibitors (ICIs)**

As described above, immune-checkpoints play an indispensable role not only in autoimmunity, but also in anti-tumor immunity [10, 11] (Fig. 1). The CTLA-4 pathway predominantly acts in lymph nodes, and the PD-1 pathway is involved with tumor microenvironment. APC or tumor cells present cancer-related antigen with HLA-class II to CD4+ T-cell (Fig. 1). APC presents cancer-related antigen with HLA-class II to CD4+ T-cell (Fig. 1). CD4+ T-cell and CD8+ T-cell cooperate in anti-tumor immunity. Monoclonal antibodies to immune-checkpoints are referred to as immune-checkpoint inhibitors (ICIs), they promote T-cell-mediated cytotoxicity directed to cancer cell antigens, and 20–30% of patients with advanced cancer were found to be responders of ICIs [27].

**ICI and immune-related adverse events (irAEs)**

During ICI therapy, various immune-related adverse events (irAEs) can occur [27]. In the endocrine system, hypopituitarism, thyroid dysfunction, adrenalitis, and type 1 diabetes mellitus (T1DM) have been reported [28-32]. Of these, thyroid irAE is most frequently seen [28, 30-32]. Briefly, the thyroid gland is preferentially affected by anti-PD-1 antibody rather than anti-CTLA-4 antibody. Most cases of thyroid irAE manifest as destructive thyroiditis. The prevalence of thyrotoxicosis and hypothyroidism is similar [32]. Patients who have anti-TPO-antibody (TPOAb) or anti-Tg-antibody (TgAb) are reported to be prone to development of thyroiditis related to irAE during nivolumab therapy [33]. Treatment of thyroid irAE was also described by Arima, et al. [32].

We have experienced a case of thyroid irAE and isolated ACTH deficiency induced by ICIs [28]. A 63-year-old woman with advanced malignant melanoma had received an anti-PD-1 antibody, nivolumab for eight cycles. On day 168, nivolumab was switched to an anti-CTLA-4 antibody: ipilimumab. Twenty-eight days later, she was diagnosed with thyrotoxicosis due to painless thyroiditis (day 196). In the case, eosinophilia, thrombocytopenia, ESR/CRP/LDH elevation, and liver dysfunction might be important for early detection of thyrotoxicosis. Yamauchi, et al. reported that patients with thyroid uptake of FDG-PET before nivolumab therapy showed high incidences of overt thyroid irAE [34]. Routine check-up of those predictive factors for thyroid irAE as well as serum FT3, FT4 and TSH levels are recommended.

**Possible mechanisms in endocrine irAEs**

Activation of pre-existing autoimmunity to thyroid glands is possibly involved in the pathogenesis of thyroid irAE. Moreover, multiple factors may influence the development of thyroid irAE. In animal models, anti-CTLA-4-antibodies were reported to induce thyroiditis [35]. Expression of PD-L1 and PD-L2 on the thyroid gland may be associated with thyroid irAE [36]. In addition, genetic mutation of immune-checkpoint molecules may contribute to the development of thyroid irAE. In AITD, genetic mutations of CLTA-4 in GD [7] and HT [37] were reported, and association of a single nucleotide polymorphism in PD-L1 with GD has been shown [38]. Regarding molecular mimicry of tumor antigen and autoantigen in thyroid glands, a tumor-associated antigen, NY-ESO-1, possesses common amino acid sequences with thyroid autoantigens (TSHR, Tg, and TPO), and type 1 diabetes mellitus (T1DM) have been reported [28-32]. Of these, thyroid irAE is most frequently seen [28, 30-32]. Briefly, the thyroid gland is preferentially affected by anti-PD-1 antibody rather than anti-CTLA-4 antibody. Most cases of thyroid irAE manifest as destructive thyroiditis. The prevalence of thyrotoxicosis and hypothyroidism is similar [32]. Patients who have anti-TPO-antibody (TPOAb) or anti-Tg-antibody (TgAb) are reported to be prone to development of thyroiditis related to irAE during nivolumab therapy [33]. Treatment of thyroid irAE was also described by Arima, et al. [32].

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HLA molecules may be related to thyroid irAE (Fig. 1).

Conclusions of thyroid irAE

If anti-tumor therapy is effective, continuation of ICI treatment is warranted with proper management of thyroid irAE [32]. In addition, development of thyroid irAE was reported to have positive correlation with feasible outcome of lung cancer [34]. In the future, identification of individuals who are susceptible to thyroid irAEs is necessary (age, gender, genetic predispositions such as HLA and immune-checkpoint molecules).

Immunoglobulin-G4-Associated Thyroid Diseases

Introduction

Immunoglobulin G4-related disease (IgG4-RD) is a systemic disease characterized by elevated serum IgG4 levels and infiltration of IgG4-bearing lymphoplasmacytes into multiple organs [40]. It preferentially affects lacrimal glands, salivary glands, and the pancreas. Estimated prevalence of IgG4-RD is 6 out of 100,000, and male predominance has been shown [41]. IgG4 accounts for only 5% of total IgG in healthy subjects. IgG4 has neither antibody-dependent cellular cytotoxicity (ADCC) nor complement dependent cytotoxicity (CDC) [42]. Th2 cells and Tregs have been thought to contribute to the pathogenesis of IgG4-RD, but recently Mattoo et al. showed that CD4+ cytotoxic T-cells (CD4+ CTLs), follicular helper 2 cells (Th2) are mainly associated with the progression of IgG4-RD [43] (Fig. 1). IL-4 secreted from Th2 cells and possibly from Th2 cells promotes IgG class switching to IgG4, and tumor growth factor (TGF)-β produced by immune-mediated cells promotes tissue fibrosis [43]. In the immune reaction, logically, APC should present antigens to T-cells, and thus they may play an important role in the development of IgG4-RD. Recently, Shiokawa et al. reported that Laminin 511 might be a target antigen in autoimmune pancreatitis [44], however, other specific antigens or epitopes in IgG4-RD remains unknown. Histological diagnostic criteria of IgG4-RD are follows: 1) dense lymphoplasmacytic infiltrates, 2) storiform fibrosis, 3) obliterative phlebitis, and 4) eosinophilic infiltration [40-42]. In addition, Umehara et al. proposed comprehensive criteria of IgG4-RD as follows: 1) serum IgG4 concentration >135 mg/dL, and 2) IgG4+ plasmacytes >10/high power field (HPF), and IgG4+ plasmacytes/IgG+ plasmacytes ratio >40% in the same tissue [45].

IgG4-RD and thyroid diseases

Komatsu, et al. found significant increases in the prevalence of TgAb and hypothyroidism in patients with autoimmune pancreatitis compared to those with chronic pancreatitis (34.1 vs. 7.3%, and 26.8 vs. 0%, respectively) [46]. In addition, Watanabe et al. reported that 19% of patients with IgG4-RD had hypothyroidism [47]. Close relation of IgG4-RD and AITD was therefore suggested. In line with these findings, we have investigated thyroidal manifestation of IgG4-RD in subsets of GD, HT (including its fibrotic variant), and RT, in association with clinicopathological characteristics.

HT and IgG4

Li et al. described a novel subtype of IgG4 thyroiditis on the basis of clinical and histopathological features, and found a close relationship between the fibrous variant of HT (FVHT) and IgG4-RD [48]. Deshpande et al. also described eight cases of FVHT, they found storiform fibrosis in all eight cases and one obliterative phlebitis in FVHT [49]. Histologically Li et al. defined IgG4 thyroiditis as follows: IgG4+ plasmacytes >20 in HPF and IgG4/IgG ratio >30% in thyroid gland [48]. They categorized HT into IgG4 thyroiditis and non-IgG4 thyroiditis, and 27% of surgical cases of HT were found to be IgG4 thyroiditis. IgG4 thyroiditis showed infiltration of IgG4-bearing lymphoplasmacytes, dense fibrosis, and thyroid follicular cell degeneration, suggesting that IgG4 thyroiditis might be related to IgG4-RD, based on the similar histopathological features of IgG4-RD. They concluded that IgG4 thyroiditis shows 1) young and male predominance, 2) rapid progression and often subclinical hypothyroidism, 3) higher levels of serum thyroid autoantibodies, and 4) diffuse low echogenicity at ultrasound. They also demonstrated that the thyroid gland is the source of serum IgG4 in patients with IgG4 thyroiditis, based on the reduction in serum IgG4 levels after thyroidectomy. Although a relationship between IgG4 thyroiditis and IgG4-RD had been suggested, the significance of serum IgG4 in HT has been uncertain. We have therefore prospectively evaluated serum IgG4 levels and clinical features of 149 patients with HT [50]. According to the comprehensive diagnostic criteria of IgG4-RD [45], the cutoff level of serum IgG4 was set to 135 mg/dL. HT patients were divided into two groups: elevated IgG4 (>135 mg/dL) and non-elevated IgG4 (≤135 mg/dL). Six patients (4.0%) categorized in elevated IgG4 group were older, and exhibited enlarged hypoechogenic areas in the thyroid gland. Two of the six HT patients with serum IgG4 elevation had extra-thyroid organ manifestations as often seen in IgG4-RD (one patient had swelling of bilateral eyelids and lacrimal glands, and another patient had swelling of the pituitary gland). These two patients imply that the extrathyroidal organ as well as thyroid glands might be the source of serum IgG4. Taken together, HT patients with serum IgG4 elevation might
share clinical features with both IgG4 thyroiditis and IgG4-RD. Kawashima et al. also reported similar frequency of HT with elevated IgG4 in Japan (5/94, 5.3%) [51]. As Zhang et al. suggested, elevated levels of TPOAb-IgG4 and TgAb-IgG4 rather than serum total IgG4 might be specific to IgG4 thyroiditis [52]. The discrepancy of prevalence of IgG4 thyroiditis or prevalence of HT with serum IgG4 elevation might be derived from surgical or non-surgical patients, or methods ofrequiring and screening patients. We reported a case of HT with IgG4-RD, and proposed a close relationship between a subtype of HT and IgG4-RD [53]. A 73-year-old man with HT was diagnosed with IgG4-RD. Broader areas of low echogenicity in ultrasound in thyroid gland, TgAb elevation, and hypothyroidism were observed. TgAb levels declined and hypothyroidism was partially improved in response to glucocorticoid therapy, the case might therefore be HT closely associated with IgG4-RD [53]. As known, HT shows Th1 predominance [54], but in this case, skewed cytokine balance to Th2 (elevation of IL4 and IL6) and Treg (elevation of TGF-β) was seen, as reported in IgG4-RD [55]. Interestingly, the ratio of Th1/Th2 cells in PBMCs was increased, similar to those of IgG4-RD [56] or severe HT [54]. We speculate that in this case, preferential Th1 balance in HT appeared to be followed by IgG4-RD which is characterized with Th2 and Treg polarization. We also found that the measurement of cytokines and chemokines appeared to be beneficial to predict and diagnose IgG4-RD. In the case, antigen exposure in HT might be involved with cross presentation on the surface of APCs, and subsequently, epitope spreading of autoantigens and alloantigens might occur. Similarly, Watanabe et al. reported that hypothyroidism in patients with IgG4-RD normalized after prednisolone treatment [47]. The histology of their case revealed IgG4-bearing plasma cells and loss of thyroid follicles, they therefore proposed that their cases could be referred to as IgG4-related thyroiditis [47]. To note, we have reported a case of IgG4-RD with HT mimicking thyroid associated orbitopathy (TAO) [56]. Thus, similar etiology may exist among HT, TAO and IgG4-RD.

**RT and IgG4**

RT is a rare chronic fibrosing disorder characterized by a hard, infiltrative lesion in the thyroid gland [57], and estimated incidence is 1.06 per 100,000 [58]. Compressive symptoms such as dyspnea, hoarseness and dysphagia are often seen in RT, and its typical ultrasonographic feature is a diffuse, hypechoic, hypovascular appearance due to the extensive fibrosis [59]. Histologically, RT has fibroinflammatory cell infiltrates and extra-thyroidal extension into surrounding tissues, inflammatory destruction of the thyroid follicles, and obliteratorive phlebitis, and can thus be distinguished from HT. RT was firstly described as a thyroidal manifestation of IgG4-RD by Dahlgren, et al. [60] with a case in which immunohistochemistry positive staining for IgG4 was seen. In order to evaluate the clinicopathological features of RT and its relationship with IgG4-RD, we performed a search of Japanese literature using the keywords “Riedel” and “Riedel’s thyroiditis.” [61]. We have also used the web-based databases Medline and Igaku Chuo Zasshi. The diagnosis of RT was based on the presence of a microscopically or macroscopically confirmed fibroinflammatory process with extension into surrounding tissues [62]. Ten patients (3 Males and 7 Females) in Japan fulfilled the diagnostic criteria during the 25-year period between 1988 and 2012. Further, we have conducted immunohistochemical analyses concerning two selected cases. A patient showed 43 IgG4+ plasmacytes/HPF, and 20% of IgG4/IgG ratio. Another patient had 13 IgG4+ plasma cells/HPF, and the IgG4/IgG ratio was <5%. Although the presence of IgG4+ plasma cells was observed in both patients, the comprehensive diagnostic criteria for IgG4-RD was partially met in each case [45]. In both patient tissues, the total number of IgG4+ plasma cells was >10/HPF, but the IgG4/IgG ratio was <30%. Similarly, a case of RT reported by Pusztaszeri partially fulfilled the criteria (70 of IgG4+ plasma cells/HPF, and the IgG4/IgG ratio was 35%) [63]. Dahlgren et al. reported three cases of RT: 1) a patient with 53 IgG4+ plasmacytes/HPF, and 80% of IgG4/IgG ratio, 2) a patient with 8 IgG4+ plasma cells/HPF, and 50% of IgG4/IgG ratio, and 3) a patient with 10 IgG4+ plasma cells/HPF, and 20% of IgG4/IgG ratio [60]. Fatourechi et al. confirmed the presence of IgG4+ plasma cells in two patients with RT [62]. Stan, et al. more recently reported six cases of RT, and three patients showed IgG4+ plasma cells >10/HPF [64]. Of those 12 patients including our two cases, only one patient met the comprehensive criteria [45]. Conversely, relevantly increased number of IgG4+ plasma cells and dense fibrous tissue were observed in thyroid gland in most of the cases, which would respond to glucocorticoid therapy. Therefore, RT seems to have similar characteristics to IgG4-RD [59, 60]. In our study, female predominance (7/10) and higher frequency of thyroid autoantibodies positivity (3/8) were observed as were seen in report by Fatourechi et al. [62]. Out of 10 cases in our study, two received glucocorticoids, one of whom experienced marked shrinkage of the thyroid lesion. One patient had extrathyroidal involvement manifesting as retroperitoneal fibrosis. These clinicopathological features of some cases with RT suggested association with IgG4-RD.
GD and IgG4

To elucidate the relationship between GD and IgG4-RD, we prospectively evaluated clinical significance and implication of IgG4 elevation in GD [65]. Serum IgG4 levels were measured in 109 patients with GD, and seven patients (6.4%) were found to have elevated serum IgG4 levels. The prevalence of GD with IgG4 elevation in the Japanese population is similar to later studies by Torimoto et al. (5/72, 6.9%) [66], and by Hiratsuka et al. (2/28, 7.1%) [67]. We have found that GD patients with serum IgG4 elevation were 1) older age at diagnosis, 2) having increased hypoechogenic areas in the thyroid, and 3) responding well to antithyroid drug (ATD) or prone to be hypothyroid after ATD treatment. Thus, the study suggested the presence of a novel subtype of GD, and measuring serum IgG4 levels might help to distinguish the new entity and provide potential therapeutic options for GD. Considering that serum IgG4 levels declined after thyroidectomy of a similar patient with GD with IgG4 elevation [68], thyroids can be a source of IgG4 in patients with GD with elevated IgG4 levels. We also reported a significant correlation between serum TSAb levels and IgG4 levels [65], and this observation is consistent with a report indicating correlation of serum IgG4 levels with TRAb levels [69]. Hiratsuka, et al. reported that although statistically not significant, serum IgG4 levels in patients with GD who had serum IgG4 elevation decreased after ATD treatment (IgG4 levels before and after the achievement of euthyroid were 66.2 ± 74.0 mg/dL vs. 50.5 ± 47.3 mg/dL, respectively [67]). Regarding Graves’ orbitopathy (GO), Bozkirli et al. reported that cases of GD with GO tend to have IgG4 elevation [70]. It is interesting that long-standing GD may switch subclass of TRAb from IgG1 to IgG4 [71]. Notably, McLachlan et al. showed that in patients with GO and elevated TRAb, an IgG4 shift toward TgAb was observed [72]. The main limitation is that histological confirmation cannot be obtained in most cases of GD.

Conclusion of IgG4-associated thyroid diseases

Evidence for IgG4-RD involvement in the spectrum of thyroid diseases currently seem to be limited, but certain subtypes of HT, RT, and GD may be related to IgG4-RD. Increase of IgG4 may be considered as a result of immune-mediated inflammation or antigen-antibody reaction [42]. Future studies are required to clarify the significance of IgG4 in serum and tissue with increased number of patients including histological analysis.

Summary of the Review

In summary, common immunological mechanisms were found among three diseases associated with autoimmunity: AITDs, Thyroid diseases during ICIs therapy, and IgG4-associated thyroid diseases. Thyroid autoantigens presented to T-cells with certain HLA/MHC molecules play an important role in initiation of each disease. Based on these characteristics, our common research strategy was established (Fig. 2). In addition to the identification of thyroid epitopes molecules including binding interaction to HLA molecules and presentation to T-cells, in vivo effects of the epitopes have been extensively investigated in silico, in vitro, in vivo, and in clinical settings. Dysfunction of central and peripheral tolerance could contribute to development of each thyroid disease. Further investigations are warranted to elucidate more precise immunological mechanisms to establish antigen-specific treatments for each entity.

Disclosure Statement

Authors declare nothing to disclose. All authors were involved in the preparation and writing of the manuscript. This work was partially supported by Grants-in-Aid for Scientific Research 26461385, and Takeda Science Foundation.

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Thyroid diseases related to autoimmunity


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Endocrine Journal Advance Publication


