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■ REVIEW ■

Clinical Applications of TSH Receptor Antibodies in Thyroid Diseases

The cloning and sequencing of thyroid-stimulating hormone (TSH) receptor (TSHR), combined with advances in molecular techniques, have facilitated the understanding of the interaction of the TSHR antibodies (TSHRAbs) with the TSHR at the molecular level and have allowed the delineation of their clinical role. TSHRAbs in vivo are functionally heterogeneous; the stimulating TSHRAbs cause hyperthyroidism and diffuse goiter in patients with Graves' disease, whereas, the blocking TSHRAbs cause hypothyroidism in some patients with autoimmune hypothyroidism and are the cause of transient neonatal hypothyroidism. Measuring TSHRAbs has potential clinical implications in differential diagnosis of Graves' disease, predicting the outcome of Graves' disease after antithyroid drug treatment, and predicting the fetal/neonatal hyperthyroidism or neonatal hypothyroidism. The existence of epitope heterogeneity in a patient, i.e., of stimulating TSHRAbs with epitopes other than on the N-terminal region of the extracellular domain, is significantly associated with favorable long-term clinical response to antithyroid drug treatment. Measuring these subtypes for thyroid-stimulating antibody (TSAb) has potential clinical implications, for example, in predicting responsiveness to treatment in untreated patients with Graves' disease.

Key Words : *Receptors, Thyrotropin; TSH Receptor antibody; Epitope heterogeneity; Graves' Disease; Hyperthyroidism; Hypothyroidism*

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INTRODUCTION

Autoimmune thyroid diseases consist of a wide spectrum of clinical presentations, with Graves' disease, Graves' ophthalmopathy, Hashimoto's thyroiditis, and atrophic thyroiditis (primary myxedema) (1, 2). Autoimmune thyroid diseases share common immunologic evidences; lymphocytic infiltration of the thyroid, circulating thyroid autoantibodies, T cell immunity, HLA association and familial aggregation. Autoantibodies against the thyroid-stimulating hormone (TSH) receptor (TSH receptor antibodies, TSHRAbs) are directly involved in the pathogenesis of Graves' disease and autoimmune hypothyroidism. The TSHRAbs compete with TSH for TSH receptor (TSHR) binding and mimic or block the TSH action, resulting in hyperthyroidism and goiter or hypothyroidism and thyroid atrophy, respectively. The heterogeneous nature of TSHRAbs is well established. Antibodies capable of interacting with multiple epitopes on the TSH receptor molecule and showing variable TSH activities, both agonistic and antagonistic, are generally present in a given patient. This variation in epitope specificity is reflected by the influence of TSHRAbs on the TSHR function, as demonstrated by the occurrence of transplacentally transmitted hyperthyroidism or hypothyroidism in the fetuses of mothers with high levels of circulating stimulating or blocking TSHRAbs (1-4).

In recent years, a number of investigators have advanced our understanding of how TSH and TSHRAbs interact with TSHR (2, 5), delineating clinical implications of TSHRAbs. The purposes of this review are, first, to summarize recent advances in TSH receptor and its autoantibodies, and second, to describe their potential clinical implications.

CHARACTERISTICS OF TSH RECEPTOR

Gene and protein structure

The TSHR is a glycoprotein expressed on the cell membrane of thyrocytes and a member of the large superfamily of G-protein-coupled seven transmembrane receptors. The TSHR gene has been cloned and sequenced (6, 7) and has been located on the long arm of chromosome 14q31 (8), spans more than 60 kb and contains 10 exons. Exons 1-9 encode for the extracellular domain (ECD) of the receptor, and exon 10 codes for the transmembrane and the cytoplasmic domain. Exons 2-8 code for the leucine-rich repeats (LRRs) (7). The open leading frame consists of 2,295 nucleotides encoding a 764-amino acid

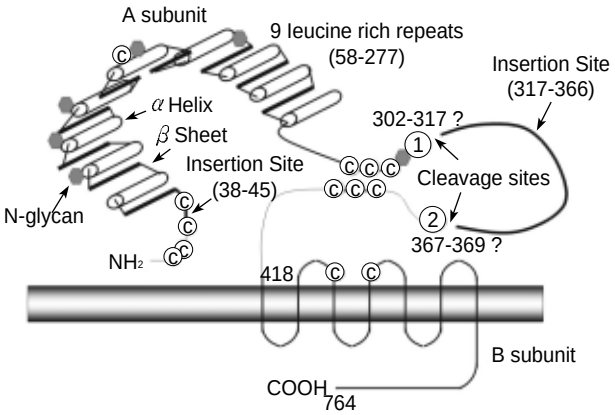


Fig. 1. Schematic representation of TSH receptor structure. Modified from ref. 2.

protein. In the ECD, TSHR has 35-45% of homology with lutenizing hormone/chorionic gonadotropin receptor (LH/CGR) and follicle stimulating hormone receptor (FSHR). The TSHR differs from LH/CGR by the presence of two unique insertions of 8 (residues 38-45) and 50 amino acids (residues 317-366) in the ECD (2, 5) (Fig. 1).

The ECD of human TSHR contains six potential asparagines-linked glycosylation sites and at least two sites are glycosylated (Fig. 1). The primary translation product of 85 kDa TSHR is glycosylated, resulting in a high-mannose form of ~100 kDa, with subsequent maturation to a 120 kDa form with complex carbohydrates (2, 4). TSH binding is increased on mature, glycosylated form of the receptor, and mutation of glycosylation sites reduces TSH binding (9).

There are 11 cysteine residues in the ECD of TSHR that could form five pairs, with one residue remaining as the orphan. Disulfide bonding is vital for the correct folding of the TSHR and the maintenance of its tertiary conformation. At present, however, there are no direct data to indicate which cysteines form the five pairs.

Two subunits model of the TSH receptor

In vitro studies suggest that the TSHR is present not only as a single chain, but also in a two-subunit form (10, 11). The glycosylated ECD, the A subunit, is cleaved from the membrane-spanning B subunit and these two subunits are linked by disulfide bonds (2). The amino-terminal cleavage site is located between residues 302 and 317 in proximity to a basic domain formed by residues 310-313 (10, 12). The carboxy-terminal cleavage site seems to be located between residues 367-369 (Fig. 1). Although there is some debate whether the cleavage site is two or not, recent studies suggest that the initial cleavage at the amino-terminal site is followed by progressive cleavage or degradation to the amino-terminal site or, alternatively, rapid degradation of the putative connecting peptide (10, 12). The physiologic significance of the cleavage and shedding phenomenon remains unresolved.

Intracellular signaling through TSH receptor

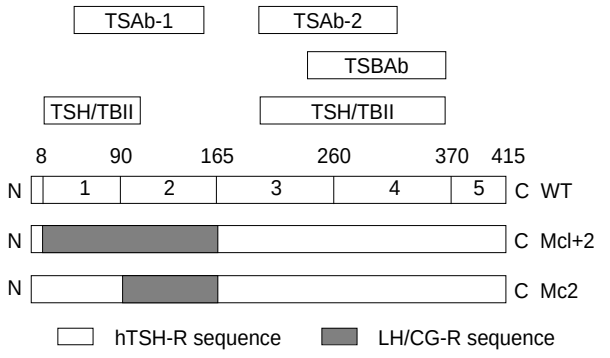
TSH binding to its receptor leads to coupling Gsα and stimulates cAMP production through activation of adenylyl cyclase (13). At higher concentrations of TSH, the TSHR couples Gq/11, resulting in activation of phospholipase C, and stimulates the inositol phosphate (IP) pathway (13). The increase in cAMP leads to phosphorylation of protein kinase A and to activation of transcription factors, such as CREB, resulting in the increase of iodide uptake, thyroid peroxidase, and thyroglobulin synthesis (13). In addition, stimulation of Gq/11 and the phospholipase C-dependent inositol phosphate /diacylglycerol pathway at the higher doses of TSH activates hydrogen peroxide generation and iodination. Graves' immunoglobulins (IgG) can enhance both cAMP and IP production in FRTL-5 cells as well as in cells transfected with human TSHR cDNA (14). The physiological significance of this dual signaling system and the clinical role of the IP pathway in Graves' disease still remain to be defined.

TSH and autoantibody binding to the TSH receptor

Several approaches have been used to map the sites on TSHR responsible for TSH or TSHRAbs binding, all having serious problems. These include the use of synthetic peptide sequences and anti-peptide antibodies as well as site-directed mutagenesis. Although studies using synthetic peptides have provided useful information on functionally important sites on TSHR, they failed to provide conclusive evidence for TSHRAbs interaction with TSHR (2), for the following reasons; they could identify only linear and non-glycosylated binding sites, but TSH and TSHRAbs were expected to interact with nonlinear conformational epitopes.

Site-directed mutagenesis has been used to facilitate the search for TSH-binding sites and for epitopes of TSHRAbs. Several studies have shown that TSH and TSHRAbs interact with TSHR at different sites. From chimeric TSHR-LH/CGR data, important TSH contact points are present in the mid-region (residues 171-260) and carboxyl-terminal segments (residues 261-418) of the TSHR ectodomain (15). Homologous or nonhomologous substitutions in the TSHR suggest that amino acids between 201-211, 222-230, 295-302, and 387-395 are the binding sites for TSH (15, 16). Thus, the binding sites of TSH on the ECD of TSHR are multiple, nonlinear, and discontinuous.

In several reports using chimeric TSHR-LH/CGR, most patients with Graves' disease were found to have one or more stimulating TSHAbs epitope(s) on the N-terminal portion of the ECD of TSHR, whereas the major blocking TSHRAb epitope in patients with hypothyroidism and idiopathic myxedema was located in the C-terminus (17-19). The N-terminal third of the TSHR (amino acids 8-165) is necessary for TSHRAb stimulation. The epitope for blocking TSHRAb on the ECD of TSHR overlaps with, but is not precisely the



TSHR type	cAMP response			TBII
	TSH	TSAb-1	TSAb-2	
WT	+++	+++	+++	+++
Mc1+2	+++	-	+	-
Mc2	+++	-	+	++

Fig. 2. Schematic structure of the wild type TSH receptor and two TSH and LH/CG receptor chimeras designated as Mc1+2 and Mc2, illustrating binding sites for TSH, TBII, TSBAb and major and minor binding epitopes for TSAb subtypes. The amino acid numbers indicate the restriction sites used for chimeras. TSH, TSAb, and TBII responses in these cell lines are depicted as graded positive or negative in the table (bottom). The TSAb-2 epitope was considered to be minor because of its low level of reactivity in Mc1+2 or Mc2 cell lines and is boxed in dotted lines. Modified from ref. 17 and 18.

same as, the TSH-binding site and the stimulating TSHRAb epitope. Overlap between these three ligands is greatest in the C-terminal region of the ECD of TSHR (Fig. 2).

TSHRAb ASSAYS

TSHRAbs can be detected by two techniques. 1) TSHRAbs compete with TSH for receptor binding, and are detected by radioreceptor assay based on the competitive inhibition of ^{125}I-TSH binding on TSHR. The TSHRAb detected by this assay is designated TSH binding inhibitor immunoglobulin (TBII). 2) TSHRAbs stimulate the adenylyl cyclase system that leads to the production of cAMP, or blocks TSH-stimulated cAMP production in an in vitro thyroid cell bioassay. The TSHRAb detected by this bioassay is designated thyroid-stimulating antibody (TSAb, stimulating TSHRAb) or thyroid stimulation blocking antibody (TSBAb, blocking TSHRAb), respectively. Each of these assays has different diagnostic effectiveness, limitations, and clinical utility. The classification of TSHAbs based on their effect on thyroid cell function and their detection methods are listed in Table 1.

TBII assay

In the TBII assay system, TSHR preparation and ^{125}I-labeled TSH are essential components. Porcine thyroid receptor protein has been established as an adequate alternative to human TSH receptors because of its ready availability and stability (20). Purified bovine TSH has higher biological activity than human TSH and is a more suitable ^{125}I-labeled ligand for this assay. This test's simplicity, precision, and cost-effectiveness, along with its commercial availability, have made it the most widely used test in clinical laboratories. However, there are a number of limitations to this assay. The TBII assay does not differentiate between the TSH-binding inhibitory antibodies that stimulate (TSAbs) and those that block the TSH activity (TSBAbs). In addition, about 10% or more of clinically hyperthyroid Graves' patients test negative with the TBII assay (21), a finding that may be caused by the use of TSHR and TSH from heterologous species (porcine TSHR and bovine TSH) that may influence the detection of autoantibodies to the human TSHR (22).

Table 1. Classification of TSHRAbs based on functional activities and detection methods

Antibody	Function	Detection method
TBII	Inhibits ^{125}I-TSH binding to TSHR	Radioreceptor assay: inhibition of ^{125}I-TSH binding to soluble porcine TSHR or recombinant human TSHR
TSAb	Stimulates cAMP production, iodine uptake, Tg synthesis	Bioassay: % increase in cAMP over normal serum in cultured thyrocytes (FRTL-5, hTSHR expressed CHO cells)
TSBAb	Inhibits TSH-induced cAMP production, iodine uptake, Tg synthesis	Bioassay: % inhibition of TSH-induced cAMP production compared to normal serum

TBII, TSH binding inhibitor immunoglobulin; TSAb, thyroid-stimulating antibody; TSBAb, thyroid stimulation blocking antibody; Tg, thyroglobulin.

A recently developed second-generation assay for TBII uses recombinant human TSHR expressed in eukaryotic cells, grown in suspension at a high density (23). The hTSHR was then captured in the test tubes coated with a monoclonal antibody to native TSHR. This assay showed better diagnostic sensitivity (99%) and specificity (99%) than the conventional TBII assay.

Bioassays for TSHRAbs measurement

Thyroid-stimulating antibody assay

After a sensitive cAMP response to TSH or TSHRAbs in cultured human thyrocytes using a medium free of sodium chloride for incubation was demonstrated (24), direct measurement of cAMP in the medium (instead of within the cells) simplified this assay and significantly enhanced its sensitivity. Later the assay was further simplified and made practical by using rat thyroid cell line called FRTL-5 instead of human

thyroid cells. Nevertheless, the major limitation of FRTL-5 cells has been the meticulous culture conditions they require, including expensive 6-hormone (including TSH) medium and growth for 3-5 days in TSH-deprived medium (3). Furthermore, in our experience (18), the FRTL-5 bioassay fails to detect stimulating activity in roughly 10 to 20% of patients with Graves' disease. The reported diagnostic sensitivity generally ranges between 75 and 90%.

The stable expression of the human TSHR in CHO (CHO-TSHR) cells has combined the advantage of detecting TSAb activity with a homologous receptor and the ready availability compared to FRTL-5 cells (2). The TSHR density is 5- to 10-times higher in CHO-TSHR cells than in FRTL-5 cells or human thyroid cells. This enhances analytical sensitivity, thus allowing detection of some borderline positive patients (2, 3). Thus, the use of CHO-TSHR cells reduces the frequency of false negative results seen with the FRTL-5 cells (18). The reported diagnostic sensitivity ranges between 90 and 95% (18). Further advantages are that CHO-TSHR cells are easier to maintain in culture, they do not require lengthy pre-culture time for assay, and they are superior to FRTL-5 cells in accuracy, simplicity, and cost (3).

Thyroid stimulation blocking antibody assay

Some patients with atrophic hypothyroidism have antibodies that block TSH binding and action (25). These blocking antibodies (TSBAbs) compete with TSH for receptor binding and block the biological effects of TSH on thyroid cells, including cAMP production, iodine uptake, and thyroid cell growth (26-28). They induce transient neonatal hypothyroidism when transferred across the placenta (29, 30). TSBAb assay quantitates inhibition by autoantibodies of the increase in cAMP production or the iodide uptake in response to a standard concentration of TSH in the FRTL-5 cell (26-28) or CHO-TSHR cell (31, 32).

HETEROGENEITY OF TSHRAbs

The heterogeneous nature of TSHRAbs in terms of function and epitopes is well recognized. A single patient has functionally different kinds of TSHRAbs. In addition, since the molecular cloning of the TSHR, it has become possible to discern epitopic differences even within a population of autoantibodies with similar function.

Functional heterogeneity

It has been well known that there are two primary types of functional TSHRAbs: 1) TSHRAbs that stimulate the adenylyl cyclase-cAMP cascade through TSHR and cause hyperthyroidism and goiter (TSAb); and 2) TSHRAbs that block TSH binding and action, thereby causing atrophic hypothyroidism (TSBAb). In addition, recently neutral TSHRAbs, which neither stimulate the TSHR nor block TSH action, have been also demonstrated (33).

It is well established that not all TSHRAbs that inhibit TSH binding to the TSHR are stimulatory. In fact, there has been no good correlation between TSAb and TBII activities in Graves' disease patients in many reports (1, 2). This discrepancy between TBII and TSAb activities might be related to species specificity, as porcine receptor has been used for most TBII studies and rat thyroid cells for most TSAb studies. However, the correlation between these two activities was also weak when using recombinant human TSHR for TBII and human thyroid cells for TSAb (34). Thus, the cause of the disparity is more likely to be the TSBAbs that are detected in the TBII assay but, in fact, mask TSAb activity in the bioassay (32).

The functional heterogeneity of TSHRAbs was confirmed by Kohn et al. (35), who found that the clones generated from lymphocytes from a hypothyroid woman included TSAb-producing clones with weak TBII activity and a number of TBII-positive clones with TSH-blocking activity. Furthermore, recently, we demonstrated that 18.5% of patients with hyperthyroid Graves' disease had both TSBAb and TSAb activities (32). Thus, sera from most patients with Graves' disease contain both TSAb and TSBAb/TBII activities, and the clinical effect may depend on the relative concentration and affinity of the predominating antibody.

Epitope heterogeneity

Heterogeneity within the stimulating TSHRAbs with respect to epitope recognition on TSHR has been demonstrated using chimeras of the human TSHR and the LH/CGR (18, 19, 36). Using such a chimeric cell line, Kim et al. recently reported that patients with Graves' disease have been divided into two subgroups according to the TSHRAbs epitopes distribution (18). Of 66 patients studied, 45 patients (68%) had stimulating TSHRAbs with homogeneous epitope distribution that recognize only the N terminus (residues 90-165) of the TSHR ectodomain. Heterogeneous epitope distribution, with epitopes other than on the N-terminus of the TSHR, occurred in 21 patients (32%). Moreover, the functional stimulating TSHRAb epitope changed from residues 90-165 to residues outside this region in half of the patients with Graves' disease during treatment of hyperthyrodism (19). Patients with heterogeneous epitope for stimulating TSHRAb initially or developed during treatment are more responsive to antithyroid drug treatment (18, 19). Thus, this epitope heterogeneity in patients with Graves' disease has clinical implications.

CLINICAL APPLICATIONS

Diagnostic values in Graves' disease

Graves' disease is an organ-specific autoimmune disease caused by stimulating TSHRAb that stimulate the thyroid function and growth, resulting in hyperthyroidism and diffuse goiter. Thus, theoretically, the detection of TSHRAb in a hyperthyroid patient is diagnostic of Graves' disease. However, because 80% or more of patients with Graves' disease have a diffuse goiter and homogeneous thyroid scan, it is likely that most patients can be diagnosed by thyroid hormones measurements without TSHRAb testing. Thus, TSHRAb measurement has not been recommended as a routine investigation in recent guidelines (37, 38). However, assays for TSHRAbs is helpful in diagnosing patients with mild hyperthyroidism and small or no goiter and may also be useful in differentiating Graves' disease from other forms of thyrotoxicosis, such as toxic nodular goiter, painless (autoimmune) thyroiditis, or factitious thyrotoxicosis. TSHRAbs measurements may also be helpful for pregnant women or patients with recent iodine load where radioiodine uptake or thyroid scan is contraindicated. In a patient who presents with Graves' ophthalmopathy but is biochemically euthyroid, TSHRAb assay may be valuable in confirming the diagnosis of Graves' disease.

TSAb measurements tend to be more sensitive (85-100%) than TBII measurements (75-96%) in untreated Graves' patients (23, 39). The new more sensitive and precise assays using CHO-TSHR are positive in as much as 98% of patients with untreated Graves' disease (23). Thus, TSAb by CHO-TSHR is a better biologic marker than TBII and should be preferred for diagnostic purposes.

Guiding choice of treatment modality in Graves' disease

The major problem with antithyroid drug is the high relapse rate (approximately 50% or more) after 1-2 yr of treatment. Thus, it is clinically important to identify patients who are likely to achieve remission with antithyroid drug treatment. In general, however, most reported studies suggest that initial TSHRAb activity has not had satisfactory positive and negative predictive values for remission or relapse after antithyroid drug treatment (40, 41). Nevertheless, taking into account the initial titer of TSHRAb and other clinical parameters such as, age, gender, goiter size, the severity of hyperthyroidism, and the presence of ophthalmopathy, subgroups of patients can be identified as harboring a high or low risk of relapse. In a study by Vitti et al. (42), the combination of a small goiter (<40 mL) and low TBII level (<30 U/L) conferred a 45% chance of remission during the 5 yr after completion of 12-24 months course of antithyroid drug treatment. In contrast, patients with a large goiter (>70 mL) and a higher TBII level had less than a 10% chance to remain in remission.

As discussed above, it has been reported that patients with heterogeneous epitope for stimulating TSHRAb initially or developed during treatment are more responsive to antithyroid drug treatment (18, 19). Approximately 30% of patients initially have heterogeneous epitope for stimulating TSHRAbs whose activity depends on sites other than the N-terminal locus. In patients with heterogeneous epitope, 94% became euthyroid within 3 months, whereas this was true of only 70% of patients with homogeneous epitope. Furthermore, there was a decrease in the duration of antithyroid drug therapy necessary to achieve a euthyroid state in the heterogeneous epitope group. Thus, the authors (18) demonstrated for the first time that patients with a heterogeneous population of stimulating TSHRAbs involving receptor determinants other than the N-terminus have a better clinical response to antithyroid therapy. Recently we expanded this observation in 159 patients with Graves' disease for 4 to 7 yr (43). In 52 patients (32.7% of 159) with heterogeneous epitope of stimulating TSHRAb, 34 patients maintained remission for more than 12 months, with 65.4% of success rate of antithyroid drug therapy. However, 46 out of 107 patients, whose IgGs had activities of stimulating TSHRAb with homogeneous epitope, attained remission after antithyroid drug therapy with 43.0% of rate. Thus, the success rate of antithyroid drug therapy in the heterogeneous epitope group, was significantly higher than that in the homogeneous epitope group (p=0.011). While the overall average rate of success is about 50%, the combination of a heterogeneous TSAb epitope and low TBII titer ($\leq$40%) conferred a 82% chance of remission, and a patient with a homogeneous TSAb epitope and high TBII titer (>40%) has a 32% chance of remission. Likewise, combination of heterogeneity of TSAb with size of goiter and initial T3 concentration had different probability of success ranging from 32 to 74% (Table 2).

The existence of epitope heterogeneity in a patient, i.e., of stimulating TSHRAbs with epitopes other than on the N-terminal region of the extracellular domain, is, therefore, sig-

Table 2. Success rate after antithyroid drug treatment according to combination of heterogeneity of epitope for TSAb with other prognostic parameters

Combination of parameters	Success rate
Smaller goiter+Heterogeneous epitope	20/28 (71.4%)
Larger goiter+Homogeneous epitope	19/60 (31.7%)
Low T_3+Heterogeneous epitope	17/23 (73.9%)
High T_3+Homogeneous epitope	15/46 (32.6%)
Low TBII+Heterogeneous epitope	22/27 (81.5%)
High TBII+Homogeneous epitope	15/47 (31.9%)

Smaller goiter, none/small on clinical examination; Larger goiter, medium/large on clinical examination; Heterogeneous epitope, group who had IgG with residual TSAb activity in the chimeras; Homogeneous epitope, group with epitope only in the N-terminal portion of the extracellular domain; Low T_3, total T_3 concentration was 350 ng/dL or less than 350 ng/dL; High T_3, total T_3 concentration was more than 350 ng/dL; Low TBII, titer of TBII was 40% or less than 40%; High TBII, titer of TBII was more than 40%.

nificantly associated with favorable long-term clinical response to antithyroid drug treatment. We suggest, therefore, that the measurement of difference in epitopes for TSHRAbs in patients with untreated Graves' hyperthyroidism may be useful to predict the long-term outcome after antithyroid drug treatment.

Monitoring the response to therapy in Graves' disease

In general, most studies demonstrate that the titers of TSHRAb (TBII and/or TSAb) are decreased during treatment and their falling titers indicate a good response (44). One question merits consideration: could the determination of TSHRAb during antithyroid drug treatment contribute to the adaptation of the treatment plan to each case or be benefit for the patient to monitor the level of TSHRAb during treatment? Previously Cho et al. (41) reported that in a 174 patients with an overall remission rate of 52%, the proportion of remitters was much higher among patients treated for 24 months who had become TBII-negative with normal basal TSH after 6 (94% remission rate) or 12 (75% remission rate) months of treatment than in patients whose TSHRAb had remained detectable for 18 (63% remission rate) or 24 months (52% remission rate). Thus, the rate of fall of TSHRAb during antithyroid drug treatment is predictive of subsequent outcome.

The duration of antithyroid drug treatment can be adapted to the TSHRAb status. Cho et al. (41) previously reported that the remission rate of patients, whose treatment was discontinued on the normalization of their TSHRAbs and TSH levels, was not significantly different from that of patients, who were treated for 24 months irrespective of their TSH levels and TSHRAbs activities (52% vs 63%, $p<0.05$). The mean treatment of duration of the former group was 10 months, 14 months shorter than the latter group. These findings suggest that the sequential monitoring of TSHRAb during the treatment of Graves' disease gives valuable information concerning the optimum time for withdrawal of medication.

Because the TSHRAb stimulates the thyroid function and results in hyperthyroidism in Graves' disease, theoretically, the disappearance of TSHRAbs at the end of the treatment reflects remission. However, in a recent report of meta-analysis that included 18 studies comparing the relapse rate in TSHRAbs-positive and -negative patients, only 10 of 18 studies showed a statistically significant difference (44). The authors concluded that the absence of TSHRAb was significantly protective against relapse, but that it was indicative of either relapse or remission after antithyroid drug treatment in individual patients. As recently discussed by Davies et al. (37), variations in the effectiveness of the assays, along with the known heterogeneity of TSHRAbs and the fact that blocking antibodies are detected differently by TBI or TSAb assays, may have an impact on the final analysis. Furthermore, as discussed above, our data suggest that both TSAb and TBAbs are heterogeneous (18, 19, 31, 32) and that a subtype of TSAb detected by chimeric TSHR is associated with increased responsiveness to antithyroid drug therapy (18, 43).

Guidelines for the management of Graves' disease in pregnancy

Although maternal TSHRAbs values normally decline during the third trimester, an elevated TSAb level at 28 to 30 weeks of gestation is associated with an increased frequency of Graves' hyperthyroidism in the neonate (45). The recommendations of European Thyroid Association for measuring TSHRAbs in pregnancy can be summarized as follows (46):

1. Euthyroid women without medication and previously treated with antithyroid drugs alone: no TSHRAbs testing is required.
2. Women previously treated with radioiodine or surgery for Graves' disease: measure TSHRAbs early in pregnancy. If the level is high, the fetus should be monitored closely for signs of hyperthyroidism and antithyroid drugs treatment for mother should be considered. Antibody titers should be repeated in the last trimester.
3. Women currently treated with antithyroid drugs: adjustment of treatment is needed to achieve a high-normal level of free T4 in the mother. TSHRAbs should be checked in the last trimester.

TSHRAb and autoimmune hypothyroidism

Indeed, the pathogenetic role of blocking TSHRAb in autoimmune hypothyroidism is suggested not only by their prevalence but also by their capacity to induce neonatal transient hypothyroidism. The reported prevalence of these antibodies in atrophic autoimmune thyroiditis varies greatly, raging from 14% (47) to 59% (28). In our study (28), the prevalences of TBII in goitrous and atrophic thyroiditis were 6.3% and 48% and those of TSBAb were 10.5% and 59%, respectively.

It has been well documented that transient neonatal hypothyroidism can be induced by transplacental transfer of maternal TSBAb since the first report of Matsuura et al. in 1980 (29). TBII and TSBAb were detected in 5% and 4%, respectively, among the mothers of hypothyroid newborns. On the whole, antibody-related transient neonatal hypothyroidism accounts for 1% of all causes of congenital hypothyroidism. Thus, transient transplacental neonatal hypothyroidism is as predictable as feto-neonatal hyperthyroidism by the assay of TBII and, if positive, by an assay of TSBAb during the last trimester (39). Epidemiologic data suggest that this screening is indicated in those with atrophic thyroiditis (primary myxedema).

Table 3. Current clinical indications of TSHRAbs assays

Clinical presentation	Antibody	Indications
Hyperthyroidism	TSAb/TBII	Differential diagnosis of Graves' disease
		Predicting the outcome of Graves' disease after antithyroid drug treatment
		Predicting the fetal and neonatal hyperthyroidism
	Epitope of TSAb	Predicting the outcome of Graves' disease before treatment
Hypothyroidism	TSBAb/TBII	Predicting neonatal hypothyroidsm
		Evaluation of fluctuating thyroid function during treatment of Graves' disease
Euthyroid Graves'	TSAb/TBII	Diagnosis of subclinical hyperthyroidism disease

TBII, TSH binding inhibitor immunoglobulin; TSAb, thyroid-stimulating antibody; TSBAb, thyroid stimulation blocking antibody.

Monitoring fluctuating thyroid function

Spontaneous evolution of hyperthyroid Graves' disease to hypothyroidism has been well characterized (48). Although thyroid destruction caused by concomitant autoimmune thyroiditis has been proposed as the major pathogenetic mechanisms of this phenomenon, the presence of blocking TSHRAbs is considered to be another possible cause (31, 49). TSBAbs may result from a conversion of the bioactivity of TSAb or may coexist with TSAb at the time of hypothyroidism. In addition, several patients with fluctuating thyroid function from hypothyroidism to hyperthyroidism by changing the activity of TSBAb to TSAb have been reported (50). Thus, the measurement of bioactivities of TSHRAbs during the course of Graves' disease is valuable in the case, especially, of fluctuating thyroid function.

CONCLUSION

The cloning and sequencing of TSHR, combined with advances in molecular techniques, have facilitated the understanding of the interaction of the TSHRAbs with the TSHR at the molecular level and have allowed the delineation of their clinical role. Recently developed new techniques confirm that TSHRAbs in vivo are functionally or epitopically heterogeneous, and indicate that the thyroid function may depend on the balance of the functional activities of TSHRAbs. In addition, the existence of epitope heterogeneity in a patient is significantly associated with apparently long-term clinical response to antithyroid drug treatment. Measuring these subtypes for TSAb has potential clinical implications, for example, in predicting responsiveness to treatment in Graves' disease. Table 3 summarizes the author's view on the current clinical indications for the use of TSHRAbs measurements.

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