Clinical Applications of TSH Receptor Antibodies in Thyroid Diseases

Bo Youn Cho
Department of Internal Medicine, Seoul National University College of Medicine and Hospital, Seoul, Korea

Received : 25 January 2002
Accepted : 26 February 2002

Address for correspondence
Bo Youn Cho, M.D.
Department of Internal Medicine, Seoul National University Hospital, 28 Yungon-dong, Chongno-gu, Seoul 110-744, Korea
Tel : +82.2-760-2242, Fax : +82.2-762-9662
Email : bycho@plaza.snu.ac.kr

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

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 TSH RAbs 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 TSH RAbs 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 TSH RAbs 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 TSH RAbs interact with TSHR (2, 5), delineating clinical implications of TSH RAbs. 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 reading frame consists of 2,295 nucleotides encoding a 764-amino acid...
Intracellular signaling through TSH receptor

TSH binding to its receptor leads to coupling Gs and stimulates cAMP production through activation of adenyl cyclase (13). At higher concentrations of TSH, the TSHR couples Go/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 Go/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 TSHR Abs 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 TSHR Abs interaction with TSHR (2), for the following reasons; they could identify only linear and non-glycosylated binding sites, but TSH and TSHR Abs 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 TSHR Abs. Several studies have shown that TSH and TSHR Abs 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, non-linear, and discontinuous.

In several reports using chimeric TSHR-LH/CGR, most patients with Graves’ disease were found to have one or more stimulating TSHR Ab epitope(s) on the N-terminal portion of the ECD of TSHR, whereas the major blocking TSHR Ab 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 TSHR Ab stimulation. The epitope for blocking TSHR Ab on the ECD of TSHR overlaps with, but is not precisely the
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 125I-TSH binding to soluble porcine TSHR or recombinant human TSHR. 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).

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 TSHR.

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

| Antibody | Function | Detection method |
|----------|----------|-----------------|
| TBII     | Inhibits 125I-TSH binding to TSHR | Radioreceptor assay: inhibition of 125I-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 cAMP production, iodine uptake, Tg synthesis compared to normal serum |

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 125I-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).
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 TSH action with a homologous receptor and the ready availability compared to FRTL-5 cells (2). The TSHR density is 5-10 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). TSBAbs 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 TBIIB activities in Graves' disease patients in many reports (1,2). This discrepancy between TBIIB and TSAb activities might be related to species specificity, as porcine receptor has been used for most TBIIB 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 TBIIB 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 TBIIB 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 TBIIB activity and a number of TBIIB-positive clones with TSH-blocking activity. Furthermore, recently, we demonstrated that 18.5% of patients with hyperthyroid Graves' disease had both TSAb and TSBAb activities (32). Thus, sera from most patients with Graves' disease contain both TSAb and TSBAb/TBIIB 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 epitope distribution (18,19). 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 hyperthyroidism (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 potential clinical implications.

CLINICAL APPLICATIONS

Diagnostic values in Graves' disease...
Graves' disease is an organ-specific autoimmune disease caused by stimulating TSH RAb that stimulate the thyroid function and growth, resulting in hyperthyroidism and diffuse goiter. Thus, theoretically, the detection of TSH RAb in a hyperthyroid patient is diagnostic of Graves' disease. However, because 80% or more of patients with Graves' disease have a diffuse goiter and heterogeneous thyroid scan, it is likely that most patients can be diagnosed by thyroid hormones measurements without TSH RAb testing. Thus, TSH RAb measurement has not been recommended as a routine investigation in recent guidelines (37, 38). However, assays for TSH RAbs 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. TSH RAbs 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, TSH RAb assay may be valuable in confirming the diagnosis of Graves' disease.

TSH RAb measurements tend to be more sensitive (85-100%) than TBII measurements (75-96%) in untreated Graves' patients (23, 39). The more sensitive and precise assays using CHO-TSHR are positive in as much as 98% of patients with untreated Graves' disease (23). Thus, TSH RAb 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 TSH RAb 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 TSH RAb 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 TSH RAb initially or developed during treatment are more responsive to antithyroid drug treatment (18, 19). Approximately 30% of patients initially have heterogeneous epitope for stimulating TSH RAbs 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 TSH RAbs 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 TSH RAb, 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 TSH RAb 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 (<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 TSH RAbs with epitopes other than on the N-terminal region of the extracellular domain, is, therefore, significant.

Guiding choice of treatment modality in Graves' disease

While the overall average rate of success is about 50%, the combination of a heterogeneous TSAb epitope and low TBII titer (<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).

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 T3+Heterogeneous epitope | 17/23 (73.9%) |
| High T3+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 T3, total T3 concentration was 350 ng/dL or less than 350 ng/dL; High T3, total T3 concentration was more than 350 ng/dL; Low TBII, <40 mL; High TBII, ≥70 mL.
significantly 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 (TBI 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 TBI-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 predictable 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 radiiodine 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, ranging from 14% (47) to 59% (28). In our study (28), the prevalences of TBI1 in goitrous and atrophic thyroiditis were 6.3% and 48% and those of TSAB1 were 10.5% and 59%, respectively.

It has been well documented that transient neonatal hypothyroidism can be induced by transplacental transfer of maternal TSAB1 since the first report of Matsuura et al. in 1980 (29). TBII and TSAB1 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 TSAB1 during the last trimester (39). Epidemiologic data suggest that this screening is indicated in those with atrophic thyroiditis (primary myxedema).
Clinical Applications of TSH Receptor Antibodies in Thyroid Diseases

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/TBII  |          | Predicting the outcome of Graves’ disease before treatment |
| Hypothyroidism         | TSBAb/TBII| Predicting neonatal hypothyroidism |
|                       |          | 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 pathogenic 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.

REFERENCES

1. Weetman AP, McGregor AM. Autoimmune thyroid disease: further developments in our understanding. Endocr Rev 1994; 15: 788-830.
2. Rapoport B, Chazenbalk GD, Jauneau JC, McLachlan SM. The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies. Endocr Rev 1998; 19: 673-716.
3. Gupta MK. Thyrotropin-receptor antibodies in thyroid diseases: advances in detection techniques and clinical applications. Clin Chim Acta 2000; 293: 1-29.
4. Kopp P. The TSH receptor and its role in thyroid disease. Cell Mol Life Sci 2001; 58: 1301-22.
5. Kohn LD, Shimura H, Shimura Y, Hitaka A, Giuliani C, Napolitano G, Ohmori M, Lagila G, Saji M. The thyrotropin receptor. Vitam Horm 1995; 50: 287-384.
6. Nagayama Y, Kaufman KD, Seto P, Rapoport B. Molecular cloning, sequence and functional expression of the cDNA for the human thyrotropin receptor. Biochem Biophys Res Commun 1989; 165: 1184-90.
7. Parmentier M, Libert F, Maenhaut C, Lefort Gerard C, Perret J, Van Sande J, Dumont JE, Vassart G. Molecular cloning of the thyrotropin receptor. Science 1989; 246: 1620-2.
8. Rousseau-Merc M, Misrahi M, Loosfelt H, Atger M, Milgrom E. Assignment of the human thyroid stimulating hormone receptor (TSHR) gene to chromosome 14q31. Genomics 1990; 8: 233-6.
9. Russo D, Chazenbalk GD, Nagayama Y, Wadsworth HL, Seto P, Rapoport B. A new structural model for the thyrotropin (TSH) receptor as determined by covalent crosslinking of TSH to the recombinant receptor in intact cells: evidence for a single polypeptide chain. Mol Endocrinol 1991; 5: 1607-12.
10. Tanaka K, Chazenbalk GD, McLachlan SM, Rapoport B. Thyrotropin receptor cleavage at site 1 does not involve a specific amino acid motif but instead depends on the presence of the unique, 50 amino acid insertion. J Biol Chem 1998; 273:1959-63.
11. Furmaniak J, Hashim FA, Buckland PR, Petersen VB, Beever K, Howells RD, Smith BR. Photoaffinity labelling of the TSH receptor on FRTL5 cells. FEBS Lett 1987; 215: 316-22.
12. Graves PN, Prittsker A, Davies TF. Post-translational processing of the natural human TSH receptor: demonstration of more than two cleavage sites. Endocrinology 1999; 84: 2177-81.
13. Dumont JE, Lamy F, Roger P, Maenhaut C. Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. Physiol Rev 1992; 72: 667-97.
14. Hidaka A, Okajima F, Ban T, Kosugi S, Kondo Y, Kohn LD. Receptor cross-talk can optimize assays for autoantibodies to the thyrotropin receptor: effect of phenylisopropyladenosine on adenosine 3’5’-monophosphate and inositol phosphate levels in rat FRTL-5 thyroid cells. J Clin Endocrinol Metab 1993; 77: 1164-9.
15. Nagayama Y, Russo D, Chazenbalk GD, Wadsworth HL, Rapoport B. Extracellular domain chimeras of the TSH and LH/CG receptors reveal the mid-region (amino acids 171-260) to play a vital role in high affinity TSH binding. Biochem Biophys Res Commun 1990; 173: 1150-6.
16. Kosugi S, Ban T, Akamizu T, Kohn LD. Site-directed mutagenesis
of a portion of the extracellular domain of the rat thyrotropin receptor important in autoimmune thyroid disease and nonhomologous with gonadotropin receptors. Relationship of functional and immunogenic domains. J Biol Chem 1991; 266: 19413-8.

17. Tahara K, Ban T, Minegishi T, Kohn LD. Immunoglobulins from Graves’ disease patients interact with different sites on TSH receptor/LH-CG receptor chimeras than either TSH or immunoglobulins from idiopathic myxedema patients. Biochem Biophys Res Commun 1991; 179: 70-7.

18. Kim WB, Cho BY, Park HY, Lee HK, Kohn LD, Tahara K, Koh C-S. Epitopes for thyroid stimulating antibodies in Graves’ sera: a possible link of heterogeneity to differences in response to antithyroid drug treatment. J Clin Endocrinol Metab 1996; 81: 1758-67.

19. Kim WB, Chung HK, Lee HK, Kohn LD, Tahara K, Cho BY. Changes in epitopes for thyroid-stimulating antibodies in Graves’ disease sera during treatment of hyperthyroidism: therapeutic implications. J Clin Endocrinol Metab 1997; 82: 1953-9.

20. Shewring GA, Rees Smith B. An improved radioresceptor assay for TSH receptor antibodies. Clin Endocrinol (Oxf) 1982; 17: 409-17.

21. Ilicki A, Gansniedt A, Karlsson FA. Hyperthyroid Graves’ disease without detectable thyrotropin receptor antibodies. J Clin Endocrinol Metab 1992; 74: 499-503.

22. Vitti P, Elisei R, Tonacchera M, Chiovato L, Mancusi F, Rago T, Mammoli C, Ludgate M, Vassart G, Pinchera A. Detection of thyroid-stimulating antibody using Chinese hamster ovary cells transfected with cloned human thyrotropin receptor. J Clin Endocrinol Metab 1993; 76: 499-503.

23. Costagliola S, Morgenthaler NG, Hoermann R, Badenhoop K, Struck I, Ilicki A, Gamstedt A, Karlsson FA. Hyperthyroid Graves’ disease sera: a new in vitro assay for human thyroid stimulator using cultured thyroid cells. J Clin Endocrinol Metab 1999; 84: 90-7.

24. Kasagi K, Konishi J, Iida Y, Ikukubo K, Mori T, Kuma K, Torizuka K. Second generation assay for thyrotropin receptor antibodies has superior diagnostic sensitivity for Graves’ disease. J Clin Endocrinol Metab 1999; 84: 90-7.

25. Endo K, Kasagi K, Konishi J, Ikukubo K, Okuno T, Takeda Y, Mori T, Torizuka K. Detection and properties of TSH binding inhibitor immunoglobulins in patients with Graves’ disease and Hashimoto’s thyroiditis. J Clin Endocrinol Metab 1982; 54: 108-14.

26. Konishi J, Iida Y, Endo K, Misaki T, Nohara Y, Matsuura N, Mori T, Torizuka K. Inhibition of thyrotropin-induced adenylate cyclase activation and growth of rat thyroid cells, FTL-5, by immunoglobulin G from patients with primary myxedema: comparison with activities of thyrotropin-binding inhibitor immunoglobulins. J Clin Endocrinol Metab 1983; 57: 544-9.

27. Cho BY, Shong YK, Lee HK, Koh C-S, Min HK. Inhibition of thyrotropin stimulated adenylate cyclase activation and growth of rat thyroid cells, FTL-5, by immunoglobulin G from patients with primary myxedema: comparison with activities of thyrotropin-binding inhibitor immunoglobulins. Acta Endocrinol (Copenh) 1989; 120: 99-106.

28. Cho BY, Kim WB, Chung JH, Yi K-H, Shong YK, Lee HK, Koh C-S. High prevalence and little change in TSH receptor blocking antibody titres with thyroxine and antithyroid drug therapy in patients with non-goitrous autoimmune thyroiditis. Clin Endocrinol (Oxf) 1995; 43: 465-71.

29. Matsuura N, Yamada Y, Nohara Y, Konishi J, Endo K, Kojima H, Wataya K. Familial neonatal transient hypothyroidism due to maternal TSH-binding inhibitor immunoglobulins. N Engl J Med 1980; 303: 738-41.

30. Cho BY, Shong YK, Lee HK, Koh C-S, Min HK, Lee M. Transient neonatal hypothyroidism due to transplacental transfer of maternal immunoglobulins that inhibit TSH binding, TSH-induced cAMP increase and cell growth. Endocrinol Jpn 1988; 35: 819-26.

31. Chung H-K, Kim WB, Park DJ, Kohn LD, Tahara K, Cho BY. Two Graves’ disease patients who spontaneously developed hypothyroidism after antithyroid drug treatment: characteristics of epitopes for thyrotropin receptor antibodies. Thyroid 1999; 9: 393-9.

32. Kim WB, Chung HK, Park YJ, Park DJ, Tahara K, Kohn LD, Cho BY. The prevalence and clinical significance of blocking thyrotropin receptor antibodies in untreated hyperthyroid Graves’ disease. Thyroid 2000; 10: 579-86.

33. Tonacchera M, Costagliola S, Cetani F, Ducobu J, Stordeur O, Vassart G, Ludgate M. Patient with monoclonal gammopathy, thyrotoxicosis, pretibial myxedema and thyroid-associated ophthalmopathy; demonstration of direct binding of autoantibodies to the thyrotropin receptor. Eur J Endocrinol 1996; 134: 97-103.

34. Filetti S, Foti D, Costante G, Rapoport B. Recombinant human TSH receptor in a radioresceptor assay for the measurement of TSH receptor autoantibodies. J Clin Endocrinol Metab 1991; 72: 1096-101.

35. Kohn LD, Suzuki K, Hoffman WH, Tombaccini D, Marconcì C, Shimoji N, Watanabe Y, Amino N, Cho BY, Kohno Y, Hirai A, Tahara K. Characterization of monoclonal thyroid-stimulating and thyrotropin binding-inhibiting autoantibodies from a Hashimoto’s patient whose children had intrauterine and neonatal thyroid disease. J Clin Endocrinol Metab 1997; 82: 3998-4009.

36. Grasso YZ, Kim MR, Faiman C, Kohn LD, Tahara K, Gupta MK. Epitope heterogeneity of thyrotropin receptor-blocking antibodies in Graves’ patients as detected with wild-type versus chimeric thyrotropin receptors. Thyroid 1999; 9: 531-7.

37. Davies TF, Roti E, Braverman LE, Degroot LJ. Therapeutic controversy: thyroid-stimulating antibodies. J Clin Endocrinol Metab 1998; 83: 3777-85.

38. Saravanan P, Dayan CM. Thyroid autoantibodies. Endocrinol Metab Clin North Am 2001; 30: 315-37.

39. Orgiazzi J. Anti-TSH receptor antibodies in clinical practice. Endocrinol Metab Clin North Am 2000; 29: 339-55.

40. Schleusener H, Schwander J, Fisher C, Holle R, Holl G, Badenhoop K, Hensen J, Finke R, Bogner U, Mayr WR, Schernthaner G, Schatz H, Pickardt CR, Kotulla P. Prospective multicenter study on the prediction of relapse after antithyroid drug treatment in patients with Graves’ disease. Acta Endocrinol (Copenh) 1989; 120: 689-701.

41. Cho BY, Shong MH, Yi K-H, Lee HK, Koh C-S, Min HK. Evaluation of serum basal thyrotropin levels and thyrotropin receptor antibody activities as prognostic markers for discontinuation of antithyroid drug treatment in patients with Graves’ disease. Clin Endocrinol (Oxf) 1992; 36: 585-90.
42. Vitti P, Rago T, Chiovato L, Pallini S, Santini F, Flore E, Rocchi R, Martino E, Pinchera A. Clinical features of patients with Graves’ disease undergoing remission after antithyroid drug treatment. Thyroid 1997; 7: 369-75.

43. Kim TY, Park YI, Kim WB, Park DI, Kohn LD, Cho BY. Prediction of outcome in Graves’ disease after antithyroid drug treatment according to heterogeneity in epitopes of thyroid-stimulating antibodies. J Clin Endocrinol Metab (submitted).

44. Feldt-Rasmussen U, Schleusenter H, Carayon P. Meta-analysis evaluation of the impact of thyrotropin receptor antibodies on long term remission after medical therapy of Graves’ disease. J Clin Endocrinol Metab 1994; 78: 98-102.

45. Zakarija M, McKenzie JM. Pregnancy associated changes in thyroid stimulating antibody of Graves’ disease and the relationship to neonatal hyperthyroidism. J Clin Endocrinol Metab 1983; 57: 1036-40.

46. Laurberg P, Nygaard B, Glinoer D, Grussendorf M, Orgiazi J. Guideline for TSH-receptor antibody measurements in pregnancy: results of an evidence-based symposium organized by the European Thyroid Association. Eur J Endocrinol 1998; 139: 584-6.

47. Tamaki H, Amino N, Kimura M, Hidaka Y, Takeoka K, Miyai K. Low prevalence of thyrotropin receptor antibody in primary hypothyroidism in Japan. J Clin Endocrinol Metab 1990; 71: 1382-6.

48. Wood LC, Ingbar SH. Hypothyroidism as late sequela in patients with Graves’ disease treated with antithyroid drugs. J Clin Invest 1979; 64: 1429-36.

49. Tamai H, Kasagi K, Takaichi Y, Takamatsu J, Komaki G, Matsu-bayashi S, Konishi J, Kuma K, Kumagai LF, Nagataki S. Development of spontaneous hypothyroidism in patients with Graves’ disease treated with antithyroid drugs: clinical, immunological and histological findings in 26 patients. J Clin Endocrinol Metab 1989; 69: 49-53.

50. Cho BY, Shong YK, Lee HK, Koh C-S, Min HK. Graves’ hyperthyroidism following primary hypothyroidism: sequential changes in various activities of thyrotropin receptor antibodies. Acta Endocrinol (Copenh) 1989; 120: 447-50.