Prevalence and Functional Significance of Thyrotropin Receptor Blocking Antibodies in Children and Adolescents with Chronic Lymphocytic Thyroiditis

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Context: TSH receptor (TSHR) blocking antibodies (Abs) inhibit TSH-induced thyroid growth and function in some adults with chronic lymphocytic thyroiditis (CLT), but their role in the pediatric age range is unknown.

Objectives: Our objectives were: 1) to determine the prevalence of TSHR blocking Abs in children and adolescents with CLT and 2) assess their functional significance both in vivo and in vitro.

Design and Setting: This was a retrospective study in a referral outpatient setting.

Patients: Sera from a total of 87 CLT patients and 33 controls were studied.

Main Outcome Measures: TSHR Abs were measured by both ELISA and bioassay.

Results: Eight of 87 children and adolescents with CLT (9.2%), including one as young as 4 yr of age, had TSHR Abs in serum as measured by ELISA. The prevalence was significantly higher in individuals whose serum TSH concentration was 20 mIU/liter or greater within 3 months of study than in less hypothyroid patients (eight of 45 vs. none of 42, \( P < 0.005 \)). Conversely, TSHR Ab-positive patients were significantly more hypothyroid at diagnosis but only when the analysis was restricted to those with severe hypothyroidism was a decreased prevalence of goiter observed. IgG purified from TSHR Ab sera retained the TSH binding-inhibitory activity and TSHR Ab-positive sera inhibited TSH-induced stimulation of cAMP significantly more than normal.

Conclusions: TSHR-blocking Abs contribute significantly to the severity of the hypothyroidism in some children with CLT, but as compared with adults, they appear to play less of a role in determining the presence or absence of a goiter. (J Clin Endocrinol Metab 94: 4742–4748, 2009)

Chronic lymphocytic thyroiditis (CLT) is the most common cause of acquired hypothyroidism in childhood and adolescence, affecting at least 1.2% of the pediatric population (1). Two clinical presentations have been distinguished: a more common goitrous form, Hashimoto’s thyroiditis, and a less common nongoitrous variety, atrophic thyroiditis (also known as primary myxedema) (2). The pathogenesis of CLT is complex and includes both apoptosis (3) and T cell- and cytokine-mediated thyroid gland destruction (4), but antibody-mediated immunological processes may also contribute by modifying the clinical features in some cases.

The role of TSH receptor (TSHR) antibodies (Abs), in particular, has received considerable attention. Unlike antithyroglobulin (Tg) and anti-thyroid peroxidase (TPO) Abs, commonly used as markers of underlying thyroid autoimmunity but without significant functional importance, TSHR Abs, can mimic or block TSH-induced stim-
ulation of thyroid growth and hormonogenesis (5). When present in a sufficiently high titer in the maternal circulation, the blocking variety can be transmitted transplacentally to the fetus and cause a syndrome of transient neonatal hypothyroidism (6), analogous to neonatal Graves’ disease. In adults, TSHR-blocking Abs, whether measured by binding assay or bioassay, have been reported to occur in a substantial proportion of patients with CLT, particularly in those with the primary myxedema variety (7–12), and disappearance of the Abs has been associated with resolution of the hypothyroidism (13), strongly suggesting a functional role.

Little is known about whether TSHR-blocking Abs occur in the pediatric age range. The known transient nature of CLT in some pediatric patients (14) and our clinical experience with a child with TSHR-blocking Abs in whom severe hypothyroidism normalized spontaneously prompted us to undertake the present study. We hypothesized that, because TSH receptor blocking Abs would impair compensatory TSH-induced thyroid stimulation, there would be a higher prevalence in patients with more severe hypothyroidism than in those with normal or mildly impaired thyroid function.

**Patients and Methods**

**Patient population**

Eighty-seven patients with CLT in whom serum was available for study were identified electronically using *International Classification of Diseases*, ninth revision codes for CLT or hypothyroidism. Patients were between 2 and 18 yr of age and seen in the Endocrine Division, Children’s Hospital Boston between April 2004 and March 2007. The diagnosis of CLT was made on the basis of clinical and laboratory findings and the presence of anti-Tg and/or anti-TPO auto-Abs in serum. Patients were stratified into those with more severe hypothyroidism (≥20 mU/liter) within 3 months of study (group 1, n = 45) and those who were euthyroid or mildly hypothyroid (group 2, n = 42). Thirty-three patients with idiopathic short stature served as the control group. Control patients had normal thyroid function tests and no anti-TPO Abs, anti-Tg Abs, thyroid function tests, and treatment status. In cases in which the initial laboratory tests were not repeated, information obtained from the laboratory used by the referring physician was used.

The following clinical parameters were extracted from the patients’ medical records: age, gender, presence of concomitant autoimmune illness, Down or Turner syndrome, family history of thyroid autoimmunity, presence of a palpable goiter, anti-TPO Abs, anti-Tg Abs, thyroid function tests, and treatment status. In cases in which the initial laboratory tests were not repeated, information obtained from the laboratory used by the referring physician was used.

The study was approved by the Investigational Review Board at Children’s Hospital Boston.

**Routine thyroid function and thyroid autoantibody tests**

Thyroid function tests [TSH, T4, and T4 binding globulin index, also called the T3 resin uptake] were evaluated by electro-

**Statistical analysis**

Quantitative data were analyzed for statistical significance using the student t test or a nonparametric test (Wilcoxon rank sum test), depending on whether the data were normally distributed. Alternatively, data were logarithmically transformed before analysis and differences between groups analyzed by ANOVA, followed by the paired or unpaired student t test as appropriate. Qualitative data were evaluated using the χ² test or Fisher’s exact test. P < 0.05 was considered to be significant.

**Results**

**Clinical features of study groups**

The clinical features of the patient groups are summarized in Table 1. There was no significant difference in the gender, age, treatment, or goiter status either at the time of diagnosis or the time of the study visit. Group 1 patients, selected because of a serum TSH concentration of 20 mU/liter or greater at any time within 3 months of study, remained more hypothyroid than group 2 at the time of study [median serum TSH concentration 43.7 (0.6–400 vs. 3.9 (0.04–17.9) mU/liter P < 0.001]. Group 1 patients
were also more hypothyroid at diagnosis [median serum TSH concentration 92.4 (6.9–1145) vs. 11.9 (2–1006) mU/liter, \( P < 0.001 \)]. In patients in whom data were available, the serum T4 concentration was also significantly less in group 1 than group 2 patients (4.3 vs. 6.4 g/dl, \( P = 0.05 \)), but this information was not available in all patients. Two patients in group 2 presented initially with a transient thyrotoxic phase.

ELISA for TSHR Abs

Overall, the prevalence of TSHR Abs in unselected CLT patients was eight of 87 (9.2%). However, the prevalence was significantly higher in group 1 patients with more severe hypothyroidism than in CLT patients who were less hypothyroid (17.8 vs. 0%, \( P < 0.005 \), Fig. 1). None of the control group had detectable TSHR Abs. To quantitate the TSH binding-inhibitory activity more precisely, sera with TSHR Ab values greater than 25 U/liter (equivalent to ~75% binding inhibition) were evaluated using multiple dilutions. The concentration of TSHR Abs in Ab-positive patients varied greatly from mildly elevated (3.6–4.5 U/liter in three patients) to as high as 346 U/liter, 100 times the normal cutoff. The log TSHR Ab concentration was also significantly higher in group 1 than group 2 patients (0.22 vs. 0.05, \( P < 0.05 \), data not shown).

In seven of the TSHR Ab-positive patients, sera from subsequent clinic visits up to 33 months later were available for study (Fig. 2). In the two patients with extremely elevated values (>40 U/liter), TSHR Abs re-

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**TABLE 1. Clinical and laboratory features of study groups**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (TSH ≥ 20 mU/ml) (n = 45)</th>
<th>Group 2 (TSH &lt; 20 mU/ml) (n = 42)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/females</td>
<td>7/38</td>
<td>10/32</td>
<td>NS</td>
</tr>
<tr>
<td>Age at diagnosis (yr)</td>
<td>10.3 ± 4.6(^a)</td>
<td>11.0 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Age at study (yr)</td>
<td>11.8 ± 4.3</td>
<td>13.0 ± 3.2</td>
<td>NS</td>
</tr>
<tr>
<td>Goiter at diagnosis, n (%)</td>
<td>15 (37.5)</td>
<td>12 (37.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Goiter at visit, n (%)</td>
<td>18 (40)</td>
<td>12 (30.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Treated, n (%)</td>
<td>28 (63.6)</td>
<td>31 (73.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Diagnosis to study visit duration (yr)</td>
<td>1.5 ± 2.5</td>
<td>2.0 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>T(_4) at diagnosis (mg/dl)(^b)</td>
<td>4.3 ± 3.4</td>
<td>6.4 ± 4.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>THBR at diagnosis(^c)</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Free T(_4) Index at diagnosis(^b)</td>
<td>3.4 ± 2.9</td>
<td>4.4 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>TSH at diagnosis (mU/ml)</td>
<td>92.4 (6.9–1145)(^c)</td>
<td>11.9 (2–1007)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T(_4) at study (mg/dl)</td>
<td>6.4 ± 2.6</td>
<td>8.4 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>THBR at visit</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Free T(_4) index at study visit</td>
<td>5.9 ± 2.8</td>
<td>8.3 ± 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TSH at study visit (mU/ml)</td>
<td>43.7 (0.6–400)</td>
<td>3.89 (0.04–17.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NS, Not significant; THBR, thyroid hormone binding ratio.

\(^a\) Mean ± SD; \(^b\) Information not available in all patients; \(^c\) Median (range).
mained elevated without much change. In two patients with intermediate values, TSHR Abs decreased progressively for the duration of follow-up, whereas in the three remaining patients with the lowest serum TSHR Ab concentrations, considerable fluctuation was observed.

Clinical and laboratory features of TSHR Ab-positive vs. TSHR Ab-negative patients

The clinical features of TSHR Ab-positive and TSHR Ab-negative patients were compared (Table 2). TSHR Ab-positive patients had a significantly higher serum TSH level at diagnosis [268.6 (9.2–883) vs. 31.6 (2–1145) mU/liter, \( P < 0.05 \)], and in patients in whom it was measured, the free \( T_4 \) index was also significantly lower (0.56 vs. 4 ± 2.7, \( P < 0.01 \)). Seven of the eight TSHR Ab-positive patients were being treated with \( L \)-thyroxine at the time of study. Although the serum TSH concentration was higher in group 1 than group 2 patients at this time [29.8 (4.9–261) vs. 9.4 (0.04–400) mU/liter] and the free \( T_4 \) index was lower, these differences were no longer significant. A significantly reduced prevalence of goiter in TSHR blocking Ab-positive patients was observed only when the analysis was restricted to those with severe hypothyroidism (31.7 vs. 14.3%, \( P < 0.001 \)) but not when the entire cohort of CLT patients was considered. An unexpected finding was the high prevalence of TSHR Abs in patients with Down syndrome as compared with the remaining CLT patients [two of four (50%) vs. two of 79 (7.2%), \( P < 0.05 \)].

Three of the TSHR Ab-positive patients were prepubertal [mean age 6.3 (range 4–10) yr] at diagnosis and presented with extremely severe hypothyroidism [TSH 657 (409–884) \( \mu \)U/ml]. Overall, however, although TSHR Ab-positive patients were slightly younger (Table 2) and tended to have a higher incidence of concomitant autoimmune disease and a somewhat higher family history (data not shown), these differences were not significant, and there was no significant difference in any of the other clinical parameters examined.

TSHR Ab activity of purified IgG

To verify that the inhibitory activity observed was due to an IgG, IgG was purified from six TSHR Ab-positive sera using NAB spin protein G purification kits (Pierce, Athens OH) and retested in the ELISA (Fig. 3). In all cases, the TSH binding-inhibitory activity was retained in the purified IgG fraction. In contrast, no TSH binding-inhibitory activity was found in the non-IgG-containing eluate.

![FIG. 3. Retention of TSH binding-inhibitory activity by IgG. Values given are log percent binding inhibition (TBI) (mean ± SEM). *, \( P = 0.04 \); **, \( P = 0.02 \).](image-url)
To further explore whether serum TSH might have accounted, in part, for the high TSH binding-inhibitory activity observed, human TSH (hTSH; Genzyme Corp., Cambridge MA) was evaluated in the TSHR Ab assay. No effect was observed up to a concentration of 5,000 mU/liter; only at a concentration of 10,000 mU/liter was slight (18%) binding inhibition observed. In support of these observations, two patients with serum TSH concentrations greater than 400 mU/liter were TSHR Ab negative. The mean serum TSH concentration of the eight TSHR Ab-positive patients at the time of study was 29.8 mU/liter.

**Biological activity of TSHR Abs**

Sufficient sample was available from seven TSHR Ab-positive sera to assess biological activity in vitro directly. Once again, inhibition of TSH-induced cAMP in TSHR Ab-positive sera was significantly greater than that in four normal controls evaluated in the same assay (percent inhibition 25.7 ± 2.1 vs. −1.3 ± 3.8, P < 0.001) (Fig. 4). Preliminary studies using recombinant hTSH suggested that 100 μU/ml hTSH significantly increased cAMP activity in this assay system.

**Discussion**

Although numerous studies have evaluated the prevalence of TSHR-blocking Abs in adults with CLT, there are very few data published in children and adolescents. The present study establishes unequivocally that these Abs occur in the pediatric population as well, including children as young as 4 yr of age, and that, as in the adults, they play an important role in the observed hypothyroidism in these individuals. Overall, significant TSH binding-inhibitory activity was observed in eight of 87 children and adolescents with CLT (9.2%) and only in patients whose serum TSH was 20 mU/liter or greater within 3 months of study. Furthermore, TSHR Ab-positive patients were significantly more hypothyroid at diagnosis than TSHR Ab-negative individuals strongly supporting an etiological role. These findings are consistent with similar studies in adults in which these Abs were found almost exclusively in hypothyroid patients (11) and probably explain our failure to find TSHR Abs previously in euthyroid children with CLT (17). In view of their relatively low prevalence, TSHR blocking Abs are but one of a number of immunopathogenic mechanisms contributing to thyroid gland failure in this patient population.

In the initial studies in adults, TSHR-blocking Abs appeared to be more strikingly common in patients with primary myxedema than in those with goitrous CLT (7–9), although it should be noted that one of the initial TSHR Ab-positive patients reported by Endo and colleagues (18) had severe goitrous hypothyroidism. In the present study, there was no difference in the prevalence of goiter between TSHR Ab-negative vs. TSHR Ab-positive patients when the entire cohort of CLT patients was considered. However, when our analysis was restricted to patients with more severe hypothyroidism (group 1 patients), nongoitrous patients had a significantly increased prevalence of TSHR Abs. It is possible that the lack of a difference overall was affected by the retrospective nature of our study as well as to the fact that many patients were being treated. However, our findings are remarkably similar to the only other previous study in children and adolescents in which potent TSHR Abs were observed in three of 32 goitrous CLT patients but in none of 18 nongoitrous ones (2). Therefore, it is likely that TSHR-blocking Abs play a significant role in blocking TSH-induced thyroid growth in children as in adults, but this effect appears to be less pronounced perhaps because the TSH concentration in children is higher (19) or because other non-TSH-dependent determinants of thyroid size, including lymphocytic infiltration, apoptosis, and both T-and cytokine-mediated thyroid destruction, mask this influence.

The prevalence of TSHR Abs we observed (9.2%) is somewhat less than most similar reports in adults (10). Whether this difference is apparent or real cannot be determined from the present study because many of our patients were being treated and TSHR Abs have been reported to disappear in some patients. Indeed, of the seven patients in whom sequential samples for up to 33 months were available, TSHR Abs did decrease in some of the patients during the period of follow-up. However, in the two patients with the most potent activity (≥40 U/liter), TSHR Abs remained extremely elevated without much change over the duration of follow-up. Although longer-term data are not available, patients such as these may be
at risk of having children with transient congenital hypothyroidism in the future, so continued follow-up will be important.

Several lines of evidence confirmed that the TSH binding-inhibitory activity observed in serum was due to an IgG. Potent TSH binding-inhibitory activity was retained in the purified IgG fraction but not in the non-IgG containing eluate. In contrast, recombinant hTSH did not inhibit binding of bTSH in concentrations 5000 mU/liter or greater, and sera obtained from two hypothyroid patients whose TSH concentration was greater than 400 mU/liter were TSHR Ab negative. These results are consistent with other reports in the literature that suggest that a serum TSH concentration less than 500 mU/liter does not interfere in the TSH binding-inhibition assay, at least under the conditions used in this study (porcine thyroid membranes and bTSH) (20). TSHR Ab-positive sera also inhibited TSH-induced stimulation of cAMP significantly greater than normal, indicating that the TSHR Abs blocked TSH action as well as binding.

Two patients with CLT presented with an initial transient thyrotoxic phase but neither had Graves’ disease, a precursor of primary myxedema in some adult patients (6), and in both patients, TSHR Abs were negative. An unexpected finding was the increased prevalence of TSHR-blocking Abs in patients with Down syndrome. Patients with Down syndrome are known to have an increased risk of both CLT and Graves’ disease (21), but an increased prevalence of TSHR-blocking Abs has not been reported previously. In view of the small sample size, this intriguing result needs to be confirmed in a larger study.

As noted previously, a limitation of this retrospective study was that many of our patients were being treated. Therefore, we arbitrarily stratified patients according to whether their serum TSH concentration had been 20 mU/liter or greater within 3 months of serum sample, reasoning that treatment during this time period should permit a normalization in the serum TSH concentration in newly diagnosed hypothyroid patients but have less influence on IgG, the half-life of which is much longer (4–6 wk). This approach allowed us to separate patients into two groups with significantly different degrees of hypothyroidism at diagnosis and probably accounted for the lack of a difference in serum TSH concentration at the time of study between TSHR Ab-positive and -negative patients.

We conclude that TSHR-blocking Abs occur in at least 9.2% of children and adolescents with CLT patients including in children as young as 4 yr of age, that they contribute significantly to the severity of the hypothyroidism in these patients and that they are unlikely to be found in those individuals whose serum TSH concentration is less than 20 mU/liter. Although more data are needed, our preliminary data suggest that TSHR-blocking Abs may remain elevated indefinitely in individuals with extremely elevated concentrations in serum, and there appears to be an increased prevalence of TSHR-blocking Abs in patients with Down syndrome. Unlike the typical picture reported in adults, TSHR-blocking Abs may be found in both goitrous and nongoitrous patients in the pediatric age range.

Acknowledgments

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