

# THYROID TODAY

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## THYROID-STIMULATING ANTIBODY (TSAb) IN GRAVES' DISEASE

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The concept of an autoimmune origin for Graves' disease dates back to 1956. By that year, Adams and Purves had developed a sensitive (for those days) bioassay technique to measure thyrotropin, presumably for the purpose of testing the then-prevalent hypothesis that excess thyrotropin caused hyperthyroidism in Graves' disease. With this technique they made the pivotal observation that a peculiarity of response in the bioassay of a patient's serum was not an irrelevant, troublesome aberration (as many might have concluded) but, rather, it signified the existence of a thyroid stimulator that was not the physiological hormone. By 1960, this material had been measured in mice, and had aroused the interest of investigators who referred to it as LATS (the long-acting thyroid stimulator). By 1964, it had been identified as an immunoglobulin, class IgG.

LATS was recognized as an abnormal thyroid-stimulating substance occurring primarily (if not solely, once bioassay artifacts were recognized) in patients with Graves' disease. It was considered an obvious candidate for the prominent role of causative agent in the syndrome, and was so identified in several texts. However, by 1970, many investigators had begun to regard LATS as an epiphenomenon in the disease, or merely a marker of an underlying disorder, similar in the way antibody to thyroglobulin reflects the autoimmune pathology of Hashimoto's disease, but is not the pathogenetic agent. The primary reasons for adopting this attitude were as follows:

1. The incidence of a positive assay for LATS in unquestionable cases of hyperthyroidism in Graves' disease ranged from about 20% to, at most, 80%; the larger figures of positive assay occurred only with extreme concentrations of the patients' IgG, thus raising the question of relevance of some of the assay responses.

2. A few patients, identified as having LATS in their blood, did not have hyperthyroidism, and some even had normally suppressible thyroid function.

In contrast to these arguments against the theory of LATS-caused hyperthyroidism, some had observed that, when LATS was present, its persistence in the blood of the individual patient treated with antithyroid drugs presaged relapse on cessation of therapy.<sup>1</sup>

Only in the last decade has a general consensus evolved that the cause of hyperthyroidism in Graves' disease is indeed the presence of a thyroid-stimulating IgG. Underlying this idea has been the recognition that to measure the bioactivity of a human immunoglobulin, the most appropriate preparation is human tissue. Experience with one such assay (Table 2) allowed Zakarija et al<sup>2</sup> to postulate, with considerable supportive evidence, that LATS represents a positive assay response in the mouse to a high concentration of thyroid-stimulating antibody; in effect, it signifies cross-reaction of a human antibody, for which the natural antigen is in the human thyroid, with a similar, though foreign, antigen in the gland of the mouse. Studies of more conventional antigen-antibody interactions established the fact that higher titers of antibody are more likely to result in cross-reactions with a related antigen.

### Methods of Assay of Thyroid-Stimulating Antibody (TSAb)

Table 1 shows several procedures involving preparations of human thyroid used to assess stimulation of the gland by patients' IgG. With the exception of the assay in which colloid droplet accumulation is measured,<sup>3</sup> all have the common characteristic of reflecting stimulation of thyroid adenylate cyclase. The most effective of these procedures, in terms of identifying the thyroid-stimulating IgG,

**Table 1**  
**Assays for Thyroid-Stimulating Antibody (TSAb)**

Name given the antibody	Acronym	Method
<b>Stimulating assays</b>		
Long-acting thyroid stimulator <sup>1</sup>	LATS	Discharge <i>in vivo</i> of radioiodine-labelled thyroid components measured as an increase in blood radioactivity
Human thyroid stimulator <sup>3</sup>	HTS	Colloid droplet formation or increase in cyclic AMP concentration in human thyroid slices incubated for 10 min
Human thyroid adenyl cyclase stimulator <sup>4</sup>	H-TACS	Stimulation of adenyl cyclase in human thyroid membranes; 30 min incubation
Thyroid-stimulating immunoglobulin <sup>5</sup>	TSIg	Increase in cyclic AMP concentration in human thyroid slices incubated for 20 min
Thyroid-stimulating antibody <sup>6</sup>	TSAb	Stimulation of adenyl cyclase in human thyroid membranes; 60 min incubation
Thyroid-stimulating antibody <sup>7</sup>	TSAb	Increase in cyclic AMP concentration in human thyroid slices incubated for 2h
<b>Receptor-modulation assays</b>		
Long-acting thyroid stimulator-protector <sup>8</sup>	LATS-P	Binding to human thyroid preventing ("protecting") subsequent binding of LATS
Thyroid-stimulating immunoglobulins <sup>9</sup>	TSI	Inhibition of <sup>125</sup> I-thyrotropin binding to human thyroid membranes
Thyroid-stimulating antibody <sup>10</sup>	TSAb	
Thyrotropin displacement activity <sup>11</sup>	TDA	
TSH-binding inhibitor immunoglobulin <sup>12</sup>	TBII	
TSH-binding inhibitor activity <sup>13</sup>	TBIA	

appear to be the direct measure of adenylate cyclase in membranes, as developed by Bech and her colleagues<sup>6</sup> and the thyroid slice technique used in this laboratory.<sup>7</sup> Considering that IgG stimulates the thyroid by activation of adenylate cyclase, it is not surprising that the various

assay procedures differ only in sensitivity and complexity. This is not the case, however, when they are compared with the second group of procedures, classified in Table 1 as the "receptor-modulation" type of assay.

This type of assay depends upon the ability of thyroid-stimulating antibody (TSAb) to interfere with the binding of a measurable marker, such as a ligand, to the human thyroid gland. Reported procedures use an impure fraction of thyroid follicular cell membrane as receptor and, in all but one instance, radioiodinated bovine thyrotropin as the ligand. The exception is the first method of this type developed, i.e., the LATS-protector assay,<sup>8</sup> in which the ligand is a standard, highly potent preparation of LATS-IgG measured in the routine bioassay for LATS. The factors influencing the binding of LATS-IgG to the human thyroid gland have not been analyzed in as much detail as have those affecting binding of thyrotropin. Consequently, although some of the apparent discrepancies observed in attempts to measure the thyroid-stimulating IgG by its influence on the binding of thyrotropin (see below) can be rationalized fairly readily, it is only an assumption that similar arguments may apply to results obtained with the LATS-protector procedure.

#### Specificity of Assays for TSAb

a) **Thyroid-Stimulation Type Assays.** One of the earliest procedures developed to measure thyroid-stimulating antibody by its effect on the human thyroid was based on an increase in intracellular colloid droplets as its end-point.<sup>3</sup> Since then, only adenylate cyclase in membranes,<sup>4,5,6</sup> or an increase in the concentration of cyclic AMP in thyroid slices,<sup>7</sup> has been used. The procedure developed in this laboratory is outlined in Table 2. Not surprisingly, the sensitivity of these assays appears to vary, although there has been no direct comparison reported. The techniques having the greatest percentages of positive results are those of Bech et al,<sup>6</sup> in which 83% of 57 patients were found to have IgG that stimulated thyroid adenylate cyclase, and those of Zakarija et al,<sup>7</sup> in which 93% of 102 patients were positive. With the thyroid slice technique, we have found only one apparent false positive result in the assay for TSAb in 25 non-Graves' disease subjects (and this was with IgG from a patient with Hashimoto's disease)<sup>7</sup>; we found no positive assay response in testing blood from euthyroid relatives of patients with active Graves' disease.<sup>14</sup>

b) **Receptor-Modulation Assays for TSAb.** The procedure dependent upon the inhibition of thyrotropin-binding to thyroid membranes has been used most widely. The frequency of positive results in Graves' disease was initially reported as almost 100%,<sup>15</sup> but greater experience showed that about 70% is a more accurate figure.<sup>16,17</sup> With the LATS-protector assay, 90% positive results in hyperthyroid patients have been reported.<sup>18</sup> For the LATS-protector assay, little has appeared regarding "false positives," but for the thyrotropin-binding inhibition assay,

positive results have been described with IgG or serum from significant proportions of patients with Hashimoto's disease, thyroid cancer,<sup>16</sup> and subacute thyroiditis.<sup>19</sup> It is difficult, especially in the last two situations, to see that all of these instances reflect underlying or accompanying Graves' disease, and it seems much more likely that the procedure may be picking up other non-stimulating antibodies directed against the thyroid cell membrane.

**Table 2**  
**Method of Zakarija et al<sup>7</sup> for Assay of TSAb**

Basis:	increase in cyclic AMP in human thyroid <i>in vitro</i>
Tissue:	slices of fresh "normal" thyroid* from O.R.
Test material:	IgG from patients' sera; (NH <sub>2</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate or purified by diethylaminoethyl cellulose chromatography
Control:	normal human IgG
Incubation:	2 hr at 37°C in Krebs-Ringer-bicarbonate-glucose-albumin with theophylline
End-point:	cyclic AMP, extracted from the tissue, and measured by radioimmunoassay

\*Usually paranodular tissue obtained at lobectomy for a solitary nodule or cyst.

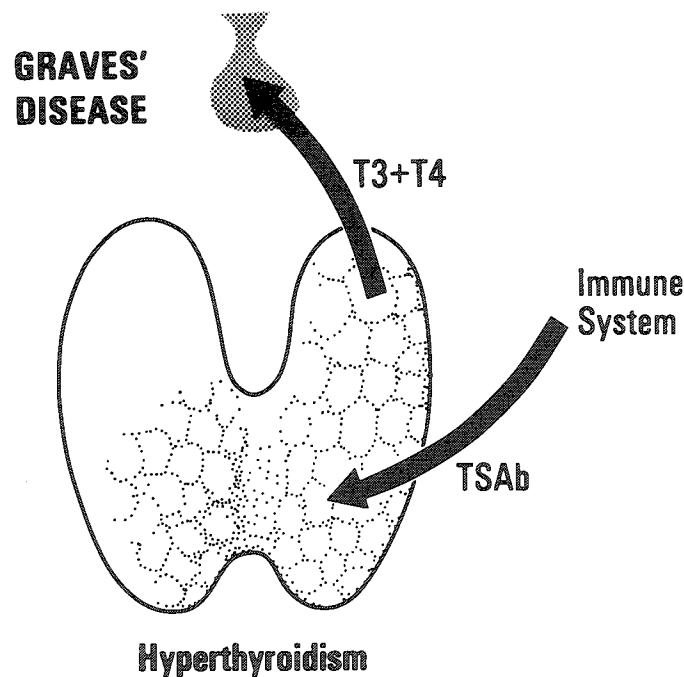
#### Clinical Usefulness of Assays for TSAb

At present, the clinical situations in which assay of TSAb would be desirable are limited. Certainly a positive assay may be seen as confirmation of a diagnosis of Graves' disease, but resulting hyperthyroidism is probably dependent upon several other variables, including most importantly the responsiveness of the thyroid gland. In euthyroid Graves' disease—i.e., where there is ophthalmopathy with apparently normal thyroid function—our limited experience suggests that testing for TSAb has diagnostic usefulness similar to carrying out an *in vivo* assessment of responsiveness to thyrotropin-releasing hormone (TRH); of 9 such patients we have investigated, 5 had both no thyrotropin response to TRH and had TSAb in the blood, whereas 4 showed normal responsiveness to TRH and were negative for TSAb.<sup>7</sup> Thus, TSAb may be viewed in this situation as a useful (and clinically comforting) index of the existence of Graves' disease in euthyroid ophthalmopathy, although there is no reason to see it as pathogenetically involved.

There are two situations wherein TSAb measurement may be of specific advantage in clinical management of Graves' disease patients. One is in forecasting the likelihood of neonatal hyperthyroidism occurring in the offspring of a woman, who has either clinically active Graves' disease, or a history of the condition. It appears that with a certain minimum level of TSAb in the maternal blood at term (>500% increase in cyclic AMP by the assay developed in this laboratory<sup>20</sup>), the expectation of neonatal hyperthyroidism is very high. Two important points should be

emphasized. First, we have encountered this circumstance in patients who previously had apparently been treated successfully for hyperthyroidism with <sup>131</sup>I, and with no remaining physical stigmata of the syndrome. Secondly, the concentration of maternal TSAb may decline significantly throughout the period of gestation. We have seen a value in the first trimester (>2000%) that suggested an inevitable problem for the neonate; however, a subsequent third trimester assay of 230% was associated with no hyperthyroidism in the child.

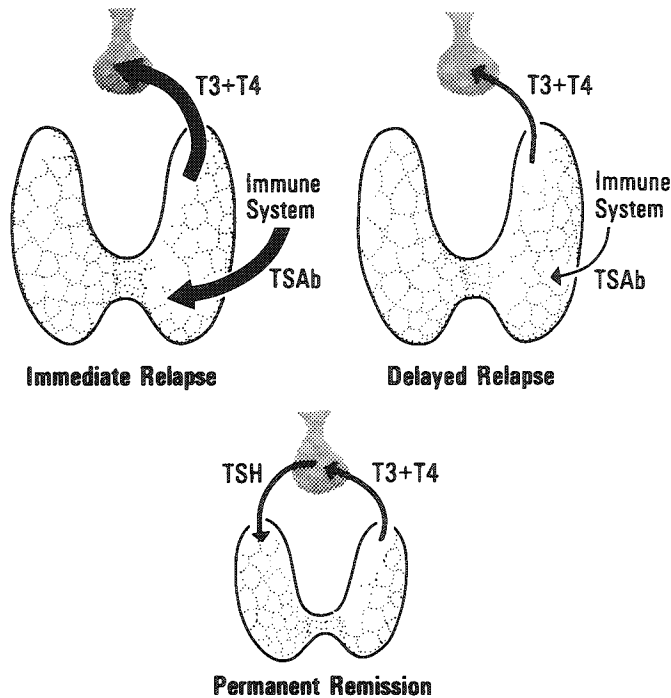
The other instance in which it appears that an assay of TSAb would be clinically desirable is in predicting clinical remission or relapse of hyperthyroidism following a course of treatment with antithyroid drugs. Although experience in this laboratory is still limited,<sup>7</sup> it appears that a positive assay for TSAb in blood taken at cessation of therapy indicates with 100% efficiency that relapse will occur, usually within 3 months. This concept is illustrated in Fig. 1. On the other hand, a negative assay at that juncture is associated with a strong probability that a prolonged



**Figure 1**  
a) The modification of the thyroid-pituitary axis found in active hyperthyroidism of Graves' disease.

clinical remission will ensue; indeed, subsequent hyperthyroidism perhaps ought to be seen as a recurrence, i.e., the development of a fresh episode of the disease, rather than a simple relapse.

These somewhat dogmatic statements are made on the strength of experience with our current method of assay of TSAb<sup>7</sup> and of even longer experience with the fore-



b) Possible outcomes of the medical (antithyroid drug) therapy of hyperthyroidism of Graves' disease. Emphasis is given to the concept that relapse signifies persistence of a greater or lesser concentration of TSAb in the blood, and that permanent remission is associated with TSAb being no longer measurable. It is also assumed that the delayed response relapse reflects a lower level of TSAb.

runner, the assay of LATS.<sup>1</sup> Measurement of TSAb by thyrotropin-binding inhibition has similar implications but, as already indicated, there appears to be poorer specificity. For instance, no relapse was described despite persistence of TSH-binding inhibitory activity (TBIA),<sup>10,21</sup> and neonatal hyperthyroidism was not observed in a child of a mother with Graves' disease who had an extreme level of TBIA.<sup>22</sup> The present situation thus appears to be that the more readily available assay for TSAb (inhibition of binding

of thyrotropin) is too non-specific to be thoroughly relied upon as a clinical tool. On the other hand, the specific assays dependent upon direct measurement of thyroid stimulation are, technically and logistically, too demanding to be widely available. However, experience gained so far indicates that when a specific, technically simple assay is designed, its application to the routine assay of TSAb will be a significant adjunct to the management of patients with Graves' disease.

**Before prescribing any drug mentioned in this article, the readers should consult full prescribing information.**

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