Interference with thyrotropin receptor antibody determination by a spuriously occurring anti-bovine TSH antibody

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Abstract. Abnormally negative values of thyrotropin binding inhibitor immunoglobulin (TBI) were found in the sera from a patient with Graves' disease. This was due to the presence of potent bovine TSH (bTSH) binding activity in the sera. This activity was demonstrated to be in immunoglobulin G (IgG) with a light chain isotype, which was shown to have an affinity for bTSH with a $K_{d}$ value of $3.5 \times 10^{10} \text{M}^{-1}$ and a maximum binding capacity of $1.1 \times 10^{-14} \text{mg} \text{IgG.}$. F(ab')$_2$ fragments obtained through pepsin digestion from the patient's IgG retained bTSH binding activity. [125I] bTSH binding to this IgG was inhibited by the TSH receptor. The inhibition was not completely competitive, suggesting the presence of different binding sites for this IgG and the TSH receptor on the TSH molecule. This IgG, however, could not bind labelled human TSH (hTSH). Since neither TSH nor other pituitary derivatives had ever been given to the patient, this bTSH binding activity was considered to be due to a spuriously occurring anti-bTSH antibody.

Graves' disease is now considered as the autoimmune disease characterized by the presence of autoantibodies to thyroid antigens such as thyroglobulin, the thyroid microsomal fraction, and the TSH receptor. Some cases of patients with Graves' disease and their infants were reported to have heterophilic antibodies to rabbit immunoglobulins (Igs), which led to falsely raised TSH values (Hedenborg et al. 1979; Czernichow et al. 1981). Lazarus et al. (1983) also reported the presence of heterophilic antibodies to rabbit IgG that also bind hTSH in a healthy mother and her infant. Antibodies to hTSH (Chaussin et al. 1972) or bTSH (Greenspan et al. 1974) were reported; the latter, however, turned out to be iatrogenically produced antibodies by the previous immunization with bTSH.

We found a patient with Graves' disease, whose sera had abnormally negative TBII indices. This was due to the presence of a spuriously occurring anti-bTSH antibody in the sera. We report here the characterization of this rare antibody.

Case Report

A 32 year-old woman was referred to our hospital for the treatment of Graves' disease in 1979. The patient had mild exophtalmos, and her serum total T$_3$, T$_4$, and hTSH values were 9.7 nmol/l, 290 nmol/l, and 0.6 mU/l, respectively. LATS was not detected in her serum. The patient was initially treated with methimazole followed by a partial thyroidectomy in 1979. Hyperthyroidism, however, recurred in 1982. At that time, her serum total T$_3$, T$_4$, and hTSH values were 11.5 nmol/l, over 260 nmol/l, and 1.3 mU/l, respectively. The titer of anti-thyroglobulin antibody and antithyroid microsomal antibody were negative and 1:1600, respectively. She was then treated with Na$^{131}$I. After radiiodine therapy, the titer of anti-thyroid microsomal antibody temporarily rose to 1:25600, and then gradually decreased to 1:1600. Anti-thyroglobulin antibody remained negative. Since the administration of Na$^{131}$I, her thyroid function remained within the normal range. The value of hTSH remained within the normal range except for a transient rise after radio-
iodine therapy. Abnormally negative TBII index of \(-82\) was first detected in the serum obtained in 1982. Subsequent TBII measurements revealed that all the patient’s serum samples had abnormally negative TBII values.

Methods

Preparation of IgG and F(ab')\(_2\)

IgG was purified from the patient’s serum obtained in 1984 by Protein A Sepharose affinity chromatography (Pharmacia) and was suspended in 10 mM Tris-HCl buffer, pH 7.4, containing 50 mM NaCl and 0.1% bovine serum albumin (TNB buffer). F(ab')\(_2\) fragments of IgG were prepared by pepsin digestion (Stanworth & Turner 1978) and were separated by gel filtration on Sephadex G-100 in TNB buffer. Contamination with undigested IgG and Fc fragments were removed by Protein A Sepharose. The concentration of IgG and F(ab')\(_2\) fragments were adjusted to 10 g/l and 5 g/l, respectively.

Detection of TSH binding activity

Highly purified bTSH (generous gift from Dr. John G. Pierce) and hTSH (donated by National Pituitary Agency) were iodinated by lactoperoxidase and further purified using a receptor adsorption method (Smith et al. 1977). Fifty µl of either serum or IgG were incubated with either \[^{125}\text{I}[^b\text{TSH} or h\text{TSH}] (~1 \times 10^4 \text{CPM})\] in 100 µl of TNB buffer for 60 min at 37°C. The mixture was then made up to 500 µl with TNB buffer, and 500 µl polyethylene glycol in 1 M NaCl was added. After centrifugation at 1500 \(\times\) g for 30 min, radioactivity in the pellet was counted. Immunoprecipitation analysis was also performed on the mixture of 20 µl of serum and \[^{125}\text{I}[^b\text{TSH}, in which bound and free radioactivity was separated by antibodies to human IgGs specific for \(\gamma\), \(\mu\), \(\alpha\), \(\kappa\), and \(\lambda\) chains (DAKO). Non-specific binding was estimated from the radioactivity precipitated in the presence of the same amount of either normal serum or IgG and was subtracted.

Thyrotropin binding inhibitory immunoglobulin (TBII) assay

TBII in serum or IgG was measured using the Thyrotropin Receptor Antibody Kit (Japan Travenol) according to Sheiring & Smith (1982).

Gel filtration analysis

Either 100 µl of the patient’s serum (obtained in 1979 and 1984) or IgG was incubated with \[^{125}\text{I}[^b\text{TSH} (2–4 \times 10^4 \text{CPM})\] for 60 min at 37°C. The mixture was then applied to a column (1 \(\times\) 50 cm) of Sephacryl S-300 equilibrated with 10 mM Tris-HCl buffer, pH 7.4, containing 50 mM NaCl. The column was calibrated with Molecular Weight Calibration Kit (Pharmacia). Binding of \[^{125}\text{I}[^b\text{TSH to either the patient’s IgG or F(ab')\(_2\) fragments was also examined on a Sephadex G-100 column (1 \(\times\) 50 cm) in TNB buffer.

Radioimmunoelectrophoresis

Radioimmunoelectrophoresis was performed according to Yagi et al. (1963). After the immunoelectrophoresis of the serum (obtained in 1984) from the patient and a control using anti-human whole serum antibody (DAKO), unprecipitated protein was washed away with saline. The agar plate was incubated with \[^{125}\text{I}[^b\text{TSH} (1 \times 10^5 \text{CPM/l})\] overnight at room temperature. The agar plate was then extensively washed with saline, and dried on the filter paper. The plate was then exposed on Sakura RX film for 2 weeks.

Kinetic studies of TSH binding

A Scatchard plot was obtained from the displacement of \[^{125}\text{I}[^b\text{TSH by unlabelled bTSH (Sigma) on the patient’s IgG or serum samples. The labelled bTSH was assumed to have a potency of 40 µU/mg and a molecular weight of 28 000. In order to examine the effect of the TSH receptor on \[^{125}\text{I}[^b\text{TSH binding to the patient’s serum (obtained in 1980), \[^{125}\text{I}[^b\text{TSH was incubated with the patient’s normal serum for 60 min at 37°C in the presence or absence of 10 µl of solubilized porcine TSH receptor from the Thyrotropin Receptor Antibody Kit. Bound and free \[^{125}\text{I}[^b\text{TSH was separated by rabbit anti-human IgG antibody (DAKO). Non-specific binding was subtracted, and a double reciprocal analysis was performed.

Assay of hormones and autoantibodies

Serum total T\(_3\), T\(_4\), and bTSH were measured by RIA. Anti-thyroid microsomal antibody and anti-thyroglobulin antibody were measured by the microscope test and thyroid test, respectively (Fujizoki). LATS was measured by mouse bioassay (McKenzie 1967).

Results

TSH binding to the patient’s serum and IgG

All the patient’s serum which could be examined had bTSH binding activity. Specific binding of \[^{125}\text{I}[^b\text{TSH to 50 µl of the serum samples obtained in 1979, 1980, 1982, and 1984 were 58%, 62%, 42%, and 41% of the total radioactivity, respectively. The binding was so potent that these samples gave abnormally negative TBII indices. The patient’s serum, however, did not show any specific binding to hTSH (data not shown). Other serum samples from 105 patients with Graves’ disease or Hashimoto’s disease, and 32 normal subjects did not have bTSH binding activity.
Fig. 1.

Gel filtration analysis of $[^{125}\text{I}]b$TSH binding to the patient's sera on Sephacryl S-300. ($\Delta$) serum obtained in 1979, (●) serum or IgG obtained in 1984. Both peaks were displaced in the presence of large amount of unlabelled bTSH (○).

Fig. 2.

Elution pattern of the patient's IgG (●) or F(ab')$_2$ fragments (○) and $[^{125}\text{I}]b$TSH mixture on Sephadex G-100. The second peak of the elution curve corresponds to unbound $[^{125}\text{I}]b$TSH.
Radioimmunoelectrophoresis of the patient’s and a normal serum. Among the various proteins precipitated by anti-human whole serum antibody, only IgG fraction of the patient’s serum had bTSH binding activity.

**Gel filtration analysis**

The binding of $[^{125}]$bTSH to various serum samples of the patient was analyzed chromatographically (Fig. 1). $[^{125}]$bTSH added to the serum obtained in 1979 resulted in a macromolecule with the approximate molecular weight of 589K. $[^{125}]$bTSH added to the serum obtained in 1984, however, resulted in a complex having an approximate molecular weight of 182K. In both of these cases, binding was inhibited in the presence of large amount of unlabelled bTSH. The elution pattern of the mixture of the patient’s IgG and $[^{125}]$bTSH was identical with that of the mixture of $[^{125}]$bTSH and the serum obtained in 1984. F(ab’)$\_2$ fragments from the patient’s IgG were bound to $[^{125}]$bTSH, and the complex was eluted after IgG and $[^{125}]$bTSH complex on a Sephadex G-100 column (Fig. 2).

**Radioimmunoelectrophoresis and immunoprecipitation**

Radioimmunoelectrophoresis clearly demonstrated that the bTSH binding activity was in the IgG fraction (Fig. 3). Immunoprecipitation showed that the bTSH binding activity was in IgG with a λ light chain isotype (Fig. 4).

**Kinetic studies of bTSH binding**

$[^{125}]$bTSH binding to the patient’s IgG was inhibited dose dependently by unlabelled bTSH (Fig. 5). The inset of Fig. 5 shows a linear Scatchard plot with a $K_a$ value of $3.5 \times 10^{10} \text{M}^{-1}$ and a maximum binding capacity of $1.1 \times 10^{-14} \text{mg IgG}$.

The TSH receptor interfered with the binding of $[^{125}]$bTSH to the patient’s serum. A double reciprocal plot showed that the binding inhibition by the TSH receptor was not competitive (Fig. 6). It suggests that the patient’s IgG and the TSH receptor did not bind to exactly the same sites on the TSH molecule.

**Discussion**

Abnormally negative TBII indices led us to find out potent bTSH binding activity in the sera from a patient with Graves’ disease. TSH binding activity in the patient’s sera was due to an IgG with a λ light chain isotype. The serum obtained in 1979 and bTSH formed a macromolecule, indicating that IgG in that serum, although it must be aggregated in long-term storage, retained bTSH binding activity. F(ab’)$\_2$ fragments of the patient’s IgG also retained bTSH binding activity. This IgG had extremely high affinity for bTSH with a $K_a$ value of $3.5 \times 10^{10} \text{M}^{-1}$. As the patient had never received bTSH injections, this IgG was considered to be a spuriously occurring anti-bTSH antibody.
Immunoprecipitation of sera and \(^{125}\text{I}\)bTSH mixture by antibodies to human Iggs specific for \(\gamma, \mu, \alpha, \beta,\) and \(\lambda\) chain.

Solid column: the patient's serum, open column: normal serum.

Thyroid stimulating immunoglobulin (TSI), which is now considered to be a TSH receptor antibody (Petersen et al. 1977), plays an important role in the pathogenesis of Graves' disease. Several studies have documented that anti-idiotypic antibodies (\(\alpha\)-Ids) raised against anti-hormone antibodies or against antibodies to hormone agonist or antagonist could bind to the corresponding hormone receptors. For instance, Farid et al. (1982) showed that \(\alpha\)-Ids to anti-hTSH antibodies...
raised in rabbits mimicked the action of TSH or TSI, supporting the hypothesis that TSH receptor antibodies might be α-Ids to anti-TSH antibodies (Beall & Kruger 1983).

For α-Ids against anti-hormone antibodies to recognize hormone receptors, one would expect that the antigen binding site of anti-hormone antibodies must be specifically directed to the hormone’s receptor binding site. In the present case, however, the bTSH antibody and the TSH receptor did not bind to exactly the same sites on the TSH molecule, suggesting that α-Ids to the bTSH antibody, if any, might not be able to stimulate the TSH receptor as much as did TSH or TSI. Recently, it has been shown that α-Ids can neutralize the activity of the original antibodies (Abdou et al. 1981; Cohen & Eisenberg 1982), and that α-Ids themselves can be neutralized by anti-αIds antibodies (Couraud et al. 1983). Since the bTSH antibody was continuously present in the patient regardless of the disease activity and similar antibodies were also found in patients with Hashimoto’s disease (Eto et al. 1984), it seems unlikely that the bTSH antibody had any relation to the pathogenesis of Graves’ disease.

An alternate explanation for the presence of TSH binding Igs in patients with Graves’ disease, which has recently demonstrated by Baker et al. (1984), is that these Igs might be α-Ids to TSH receptor antibodies. This hypothesis also seems unlikely in our case, since TSI activity was not neutralized and the patient relapsed even after subtotal thyroidectomy.

Previously, the hTSH-anti-hTSH antibody complex was reported to produce a LATS type response in a mouse bioassay (Meek 1970). Since the bTSH antibody in our case did not bind hTSH, it might not be able to modify the biological response of hTSH to thyroid gland to induce LATS-like activity.

Recently, similar bTSH binding Igs were found in patients with Graves’ disease (Kajita et al. 1983; Akamizu et al. 1984). These Igs also gave abnormal TBII value because of their potent bTSH binding activity. The incidence of these Igs were low, and these Igs did not seem to have any relation to both the thyroid function and the TSI activity, suggesting that they may not play an important role in the production of TSI. It still remains unclear whether these potent bTSH binding Igs exist exclusively in patients with autoimmune thyroid diseases, and have any specific relation to thyroid diseases.

It is very difficult to explain the presence of

Double reciprocal analysis of [125I]bTSH binding to the patient’s serum in the presence (O) or absence (●) of the TSH receptor. The slope was determined by linear regression analysis.
bTSH antibodies in patients who have never received a TSH injection. Interestingly, human lymphocytes stimulated by T cell mitogen were reported to produce an immunoreactive TSH (Smith et al. 1983), which might act as an antigen for the production of TSH binding Igs. Further studies are necessary to elucidate their immunological implication.

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References


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