Detection of TSH-Binding Inhibitor Immunoglobulins by Using the Triton-Solubilized Receptor from Human Thyroid Membranes

YASUHIRO IIDA, JUNJI KONISHI, KANJI KASAGI,
KANJI KUMA* AND KANJI TORIZUKA

*Department of Nuclear Medicine, Kyoto University School of
Medicine, Kyoto 606 and *Kuma Hospital, Kobe 650, Japan*

Abstract

A radioreceptor assay of TSH using Triton-solubilized human thyroid receptors was applied to the detection of TSH-binding inhibitor immunoglobulins (TBII). In 26 untreated patients with Graves' disease, 20 (76.9%) were found positive in this assay, while 18 (69.2%) cases were found positive by the conventional assay using human thyroid particulate receptors. In 25 patients with goitrous Hashimoto's thyroiditis, 2 (8.0%) were found positive by the assay using soluble receptor, while 3 (12.0%) were found positive by the conventional one. A significant correlation was found between TBII activities in the patients with untreated Graves' disease detected by these two assay systems ($r=0.69$; $n=26$; $p<0.001$). These data support the concept that TBII are antibodies against the TSH receptor or its closely related structures.

By using the radioreceptor assay of TSH, immunoglobulins which inhibit TSH binding to human thyroid membranes have been detected in the sera of patients with Graves' disease (Manley *et al.*, 1974; Smith and Hall, 1974; Mehdi and Nussey, 1975; Bryson *et al.*, 1976; Petersen *et al.*, 1977; Endo *et al.*, 1978; O'Donnel *et al.*, 1978).

These immunoglobulins, originally called thyroid stimulating immunoglobulins by Smith and Hall (1974), are more appropriately termed TSH-binding inhibitor immunoglobulins (TBII) (Endo *et al.*, 1978), and are now considered to be antibodies to portion of the thyroid plasma membranes including the TSH receptor (Volpé, 1978; Editorial, 1979). In these studies, the particulate fraction of the human thyroid homogenate has been used as receptor (Smith and

Hall, 1974; Mehdi and Nussey, 1975; Bryson *et al.*, 1976; Endo *et al.*, 1978; O'Donnel *et al.*, 1978).

It was reported that the TSH receptor could be solubilized with non-ionic detergents and the solubilized receptor maintain its binding activity (Tate *et al.*, 1975a, b; Petersen *et al.*, 1977; Czarnocka *et al.*, 1979; Iida *et al.*, 1981). However, no detailed study on the detection of TBII using soluble receptor has yet been reported.

In the present study, a radioreceptor assay for TBII activity by using Triton-solubilized human thyroid receptors was developed and the assay results were compared with those obtained by using particulate receptors.

Materials and methods

Patients

Sera were obtained from 26 patients with untreated Graves' disease, 25 patients with goitrous

Hashimoto's thyroiditis and 16 laboratory personnel who served as normal control subjects. The clinical diagnoses of patients were based on clinical findings, measurement of serum T₃, T₄, anti-thyroglobulin antibody, anti-microsome antibody, scintigraphic examination and histology of needle biopsy specimen of the thyroid.

Preparation of immunoglobulin G (IgG)

IgG were prepared from the sera by column chromatography through DEAE-Sephadex (Baumstark *et al.*, 1964). The protein concentration was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Radioiodination of TSH

Highly purified bovine TSH (40 IU/mg) was a generous gift from Dr. John G. Pierce (Los Angeles, CA). It was iodinated by the lactoperoxidase method (Miyachi *et al.*, 1972) and purified further by receptor absorption (Manley *et al.*, 1974).

Radioreceptor assay using particulate receptors

Radioreceptor assay using particulate receptor was performed according to Endo *et al.* (1978). The thyroid gland was obtained from a single patient with Graves' disease at the time of operation. 100 μ l 10,000 $\times$ g fraction of human thyroid homogenate (about 500 μ g protein, 100 mg wet tissue equivalent), 50 μ l [¹²⁵I]TSH and 150 μ l 100 mM Tris, 50 mM NaCl with 1 mg IgG were incubated for 60 min. at 37°C. Subsequently 500 μ l 0.32% human γ -globulin and 800 μ l 20% polyethylene glycol (PEG), 2M NaCl were added. After vortexing and leaving for 10 min. at 4°C, the mixture was centrifuged at 3,000 $\times$ g for 20 min. and the radioactivity in the pellet was measured in a γ -counter.

The result was expressed as a percentage of the mean value of the TSH specifically bound in the presence of 16 normal IgGs. Specific binding was obtained by subtracting [¹²⁵I]TSH bound to the receptor in the presence of 100 mU/ml unlabeled bTSH (Thyropar®, Armor Pharm. Co.). The TBII activities of patients were measured in a single assay.

Radioreceptor assay using Triton-solubilized receptor

Solubilization of TSH receptors was performed from human thyroid obtained from patients with Graves' disease at the time of operation by using 0.5% Triton X-100 by the method of Petersen *et al.* (1977) with minor modification (Iida *et al.*, 1981).

Radioreceptor assay using Triton-solubilized receptor was performed as follows: 100 μ l Triton-solubilized receptor (200 μ g protein, 200 mg wet tissue equivalent), 50 μ l [¹²⁵I]TSH and 50 μ l 10 mM Tris, 50 mM NaCl with 1 mg IgG were incubated for 60 min. at 25°C. Subsequently, 500 μ l 0.32% human γ -globulin and 700 μ l 30% PEG, 1M NaCl were added.

After centrifugation, the radioactivity in the pellet was measured as in the assay using particulate receptor.

Results

Detection of TBII by using particulate receptors

When the particulate receptors were used, about 15% of [¹²⁵I]TSH were specifically bound. TBII activities ranged from 83.6–116.7% ($100 \pm 9.6\%$; mean $\pm$ SD) in 16 normal controls. Taking the lower limit of normal as mean-2SD for normal subjects, positive TBII were found in 18 (69.2%) out of 26 patients with untreated Graves' disease and 3 (12.0%) out of 25 patients with goitrous Hashimoto's thyroiditis (Fig. 1).

Fig. 1. Detection of TSH-binding inhibitor immunoglobulins (TBII) in normal controls and patients with untreated Graves' disease and goitrous Hashimoto's thyroiditis using conventional particulate TSH-receptors.

Fig. 2. Detection of TBII in normal controls and patients with untreated Graves' disease and goitrous Hashimoto's thyroiditis using Triton-solubilized TSH-receptors.

Detection of TBII by using Triton-solubilized receptors

By using soluble receptors, binding of [125 I]TSH was about 25%. TBII activities in 16 normal controls ranged from 93.2–108.3% ($100 \pm 3.9\%$; mean $\pm$ SD) in this assay. Thus TBII activity less than 92.2% (mean -2 SD) was considered to be positive, 20 (76.9%) out of 26 patients with untreated Graves' disease and 2 (8.0%) out of 25 goitrous Hashimoto's thyroiditis were positive for TBII (Fig. 2).

Relation between TBII activities measured by using particulate receptors and those by using soluble receptors

In Fig. 3, TBII activities in the patients with untreated Graves' disease measured by using soluble receptors were plotted against

Fig. 3. Relation between TBII detected by the assay using particulate receptors and those by the assay using solubilized receptors in untreated Graves' disease.

those measured by using particulate receptors.

A significant correlation was obtained ($r = 0.69$, $n = 26$, $p < 0.001$) between these two values. However, the results in a few IgGs were discordant in these two assays. Five IgGs which were positive for TBII using solubilized receptors were negative using particulate receptors, while 3 IgGs negative using soluble receptors were positive using particulate receptors. Only 3 IgGs (11.5%) were found negative by both assays.

Discussion

A radioreceptor assay of TSH using Triton-solubilized human thyroid receptors was applied for the detection of TBII activity in patients with untreated Graves' disease and goitrous Hashimoto's thyroiditis.

In previous studies using particulate receptors, the incidences of TBII in patients with untreated Graves' disease and in those with Hashimoto's thyroiditis have been reported to be from 57 to 93 percent and from 0 to 23 percent, respectively (Mukhtar *et al.*,

1975; Davies *et al.*, 1977; Schleusener *et al.*, 1977; Endo *et al.*, 1978; Strakosch *et al.*, 1978; O'Donnel *et al.*, 1978; Ozawa *et al.*, 1979; Sugeno *et al.*, 1979). The marked difference among the reported incidence figures could be due to the differences in procedures and amount or purity of IgGs used in the assay. One of the main factors limiting the detectability of TBII in the receptor assay appears to be the range of inhibition elicited by normal samples.

We showed that Triton-solubilized human thyroid TSH receptor retained all the basic properties of the membrane receptor and would provide starting material for receptor purification (Iida *et al.*, 1981). Therefore, in this study, we measured TBII activities by using solubilized receptors and compared the results with those obtained by the conventional assay using particulate receptors. In 26 patients with untreated Graves' disease, 77% showed detectable TBII activity by using soluble receptor in comparison to 69% by using the latter assay. As for goitrous Hashimoto's thyroiditis, 2 out of 25 patients were positive for TBII by using soluble receptor while 3 were positive by the conventional assay.

TBII activities in untreated Graves' disease measured by these two assay systems showed a significant correlation. The results support the concept that the TBII are interacting against the TSH receptor itself or its closely related structures. In spite of the generally increased detectability of TBII by using soluble receptors, there were few IgGs which were negative in the assay while showing definitely positive result using particulate receptors. These findings may reflect the heterogeneity in antibodies which interfere with the receptor binding of TSH. Thus, the possibility could not be excluded that TBII detected by using the particulate receptor might contain antibodies against membrane antigens not directly related to TSH receptors.

The present study revealed that TBII activity could be measured by the radioreceptor assay using soluble receptors with smaller

variability in [125 I]TSH binding in the presence of normal IgGs. We believe that further purification of the receptor would make the assay more reliable and specific for TSH receptor-related antibodies in autoimmune thyroid diseases.

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