Interactions between TSH binding inhibiting -
and adenylate cyclase stimulating -
antibodies in Graves' disease

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Abstract. It is well known that there exists a dissociation between TSH binding inhibiting antibody (TBIAb) and thyroid stimulating antibody (TSAb) in some patients with Graves' disease. The present studies were undertaken to investigate the interaction between TBIAb and TSAb quantitatively using porcine thyroid cells in order to determine whether the binding sites of TBIAb and TSAb are identical or independent from each other. TBIAb was determined using solubilized porcine thyroid membrane and TSAb using cultured porcine thyroid cells, and both were expressed as equivalent amounts of bovine TSH. In order to avoid interassay variations, all determinations were performed with a single batch of porcine thyroids. Serum samples were obtained from ten untreated patients with Graves' disease; two were negative for TBIAb and TSAb, two were TBIAb negative and TSAb positive, two were weak positive for both, two were strong positive for both, and two were TBIAb strong and TSAb weak positive. IgG from each patient were mixed with equal amounts of IgG of the other nine patients. In order to observe interactions, measured values for TBIAb and TSAb of mixed samples were compared to estimated values (addition of values of original samples). The correlation coefficient between measured values and estimated values for TBIAb was +0.964, and that for TSAb was +0.989. These results that TBIAb does not interfere with the activity of TSAb and vice versa suggest that at least in some patients with Graves' disease, TBIAb and TSAb are different antibodies which have different binding sites.

Since the identification of abnormal thyroid stimulators in the sera of patients with Graves' disease (Adams 1958), it has been suggested that these stimulators play an important role as pathogenetic factors in Graves' disease. Recently, many assays for thyroid stimulators have been developed; measurement of cAMP production using thyroid slices (Onaya et al. 1973; McKenzie & Zakarija 1976), crude membrane fractions (Yamashita & Field 1972; Orgiaizi et al. 1976) or cultured thyroid cells (Rapoport 1976; Kasagi et al. 1982; Valente et al. 1983), measurement of colloid droplet formation (Onaya et al. 1973) and measurement of \( T_3 \) release from thyroid slices (Laurberg et al. 1975; Hamada et al. 1983). On the other hand, it was reported that TSH binding inhibiting antibody (TBIAb) was detected in the sera of patients with Graves' disease, and TBIAb had good correlation with laboratory findings of untreated Graves' patients (Smith & Hall 1974; Mukhtar et al. 1975; Endo et al. 1978; Borges et al. 1982). Therefore, it has been suggested that TBIAb binds to TSH receptor and stimulates adenylate cyclase. However, it is well known that there exists a dissociation between TBIAb and TSAb in some patients with Graves' disease (Kuzuya et al. 1979, 1980; Sugenoza et al. 1979; Nagataki 1982; Bliddal et al. 1982; Konishi et al. 1983). The dissociation may be due to either agonistic or antagonistic action of TBIAb to adenylate cyclase stimulation, the presence of multiple antibodies which interfere with each other through the similar binding site, or multiple in-
dependent antibodies. The present studies were undertaken to investigate the interaction between TBIAb and TSAb quantitatively using porcine thyroids in order to determine whether the binding sites of TBIAb and TSAb are identical or independent from each other.

Materials and Methods

Patients
In order to investigate interactions between TBIAb and TSAb, sera from ten untreated patients were selected. Two of them were negative for both TBIAb and TSAb (Nos. 1, 2), two were TBIAb negative and TSAb positive (Nos. 3, 4), two were weak positive for both (Nos. 5, 6), two were strong positive for both (Nos. 7, 8), and two were TBIAb strong and TSAb weak positive (Nos. 9, 10).

Preparations of IgGs from serum samples
One ml of serum samples was mixed with a solution of polyethylene glycol (PEG; M.W. 4000). The final concentration of PEG of the mixture was 15%. After centrifugation at 2000 × g for 30 min, the pellet was dissolved with 1 ml of 10 mM Tris-HCl 50 mM NaCl pH 7.2 buffer for TBIAb assay or 1 ml of Hank's solution without NaCl containing 20 mM Hepes, pH 7.2 for TSAb assay (Kasagi et al. 1982), and undissolved material was removed by centrifugation (Shewring & Smith 1982).

Interaction between TBIAb and TSAb
Original values for TBIAb and TSAb were determined using 50 µl of sample IgG and 50 µl of normal IgG for TBIAb, and 100 µl of sample IgG and 100 µl of normal IgG for TSAb. In order to observe interactions, IgG from each patient was mixed with same amounts of IgGs of the other nine patients, and 200 µl or 100 µl of the mixed IgGs were subjected for TBIAb or TSAb, respectively. Measured values for TBIAb or TSAb of mixed samples were compared to estimated values which were calculated by the summation of original values.

Measurements of TBIAb
TBIAb was determined using solubilized porcine thyroid membrane (Nagataki 1982). 100 µl of IgG was added to 50 µl solubilized thyroid membranes (4 mg/ml) for 20 min at room temperature. [125I]TSH re-purified by crude human thyroid membrane (10000 × g sedimentation fraction of 800 g supernatant of homogenized thyroid tissue) in 100 µl of 10 mM Tris–50 mM NaCl 0.1% BSA pH 7.2 was then added and incubated for 60 min at 37°C. The volume of mixture was made to 750 µl with PEG in 1 mM NaCl so the final PEG concentration was 15%. After the mixture had been well shaken, the tubes were centrifuged at 3000 rpm for 50 min at 4°C, and the radioactivity of the pellet was counted.

Fig. 1.
Dose response curve: TBIAb assay is shown on the left and TSAb assay is shown on the right figure.
Table 1.

Measured values for TBIAb and TSAb of the mixture samples which were prepared by mixing IgG fraction of each patient with equal amount of that of the other 9 patients.

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Results

TBIAb and TSAb in 10 untreated patients with Graves' disease. The mean ± sd of values for TBIAb and TSAb of ten normal subjects were 0 ± 0.2 mU/ml and 0 ± 4.5 µU/ml equivalent amounts of bovine TSH, respectively. Values for TBIAb and TSAb of ten untreated patients with Graves' disease are shown in Table 1. There was no significant correlation between these values for TBIAb and those for TSAb.

Values for TBIAb and TSAb of mixture samples

Table 1 shows values for TBIAb and TSAb of the samples mixed with IgG of the other nine patients. There was no significant correlation between TBIAb and TSAb in these mixed samples. In order to observe interactions, these measured values of the mixed samples for TBIAb or TSAb were compared to estimated values which were calculated by the summation of original values. For example, when IgG of patient 5 was mixed with that of patient 8, measured values for TBIAb and TSAb were 7.0 mU/ml and 92 µU/ml, respectively, and estimated values were 6.2 mU/ml (2.1 + 4.1) and 92 µU/ml (20 + 72), respectively. The correlation between measured values and esti-
Estimated values for TBIAb

Estimated values for TSAb

Fig. 2.

a (Left): Relation between measured values and estimated values for TBIAb of the mixture samples which were prepared by mixing IgG fraction of each patients with equal amount of that of the other 9 patients. Correlation coefficient is \( r = 0.964 \).
b (Right): Relation between measured values and estimated values for TSAb of the same mixture samples. Correlation coefficient is \( r = 0.989 \).

Discussion

In the present study performed in ten untreated Graves' patients with various values for TBIAb and TSAb, remarkably close relationships for both TBIAb and TSAb were observed between measured values of the mixed IgG and estimated values calculated by the addition of the original values (\( r: +0.964 \) for TBIAb, \( r: +0.989 \) for TSAb), indicating that TBIAb does not interfere with the activity of TSAb and vice versa. As shown in Fig. 1, clear dose response relationships were obtained from 0.1 to 10 mU/ml of TSH for TBIAb assay and from 0.01 to 1.0 mU/ml of TSH for TSAb, and values of all original and mixed samples determined in the present study were within the range of accurate determinations.

The dissociation between TBIAb and TSAb among patients with Graves' disease (Kuzuya et al. 1979; Sugenoya et al. 1979) and changes of dissociation in individual patient during the course of disease (Gossage et al. 1983; Madec et al. 1984) have been reported. Although it was suggested that other antibodies leading to such a dissociation might appear during therapy (Bliddal et al. 1982), interactions between TBIAb and TSAb in Graves' patients have not been demonstrated. The dissociation between TBIAb and TSAb in patients with Graves' disease could be explained in various ways. 1) If TBIAb and TSAb are the same antibody, the dissociation may be due to either agonistic or antagonistic action of the antibody to TSH receptor, or a single antibody binds to two receptor sites with different affinity. 2) If TBIAb and TSAb are different antibodies, the dissociation could be explained by the different dose or affinity to a single receptor, or the dissociation may be due to different binding sites which interfere with each other or are independent from each other. However, if TBIAb does not interfere with the activity of TSAb and vice versa as shown in this study, it is very likely that TBIAb and TSAb are different antibodies and have different binding sites.

One method to explore the mechanism of this heterogeneity is to produce monoclonal antibodies against TSH receptor. Monoclonal antibodies against the TSH receptor have been produced in some institutes (Valente et al. 1982; Ealey et al. 1984) using mouse or human myeloma cells. According to their results, some monoclonal
antibodies have both TBIAb and TSAb activities, while others have only TBIAb or TSAb activity. However, their results did not show the distribution of various monoclonals in patients with Graves’ disease. Our results did not exclude the existence of antibodies with both TBIAb and TSAb in Graves’ patients. However, in some patients with a dissociation of TBIAb and TSAb, their TBIAb and TSAb are very likely to be different antibodies which react with different binding sites.

As for the binding sites of TSH-receptor antibodies or the structure of TSH receptor, it has been postulated that there exist two subunits and α subunit binds to TSH and β subunit does not, and β subunit has close relation to adenylate cyclase activity (Valente et al. 1982; Ealey et al. 1984; Buckland et al. 1985). Although in the present experiment, the correlation among TSH subunits and TSH-receptor antibodies were not studied, the different binding sites proposed in the present study could be the α and β subunits of TSH-receptor.

From the results of this study, it is concluded that TBIAb does not interfere with the activity of TSAb and vice versa and that at least in some of patients with Graves’ disease, TBIAb and TSAb are different antibodies which have different binding sites.

References


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