Preserved activation of thyrotropin receptor antibody to stimulate thyroid function despite long-term treatment in euthyroid patients with Graves’ disease

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Abstract
Clinical evaluation was conducted to ascertain whether thyrotropin receptor antibody (TRAb) in the normal range may still be involved in the regulation of thyroid function after prolonged treatment for Graves’ disease. All patients (n = 33) were treated with antithyroid drugs for an average of 10.6 years and were under euthyroid conditions in which normal blood levels of tri-iodothyronine (T3) were significantly correlated with blood thyrotropin (TSH) levels, but not with titers of TRAb. A significant correlation was observed between TRAb titer and thyroid-stimulating antibody (TSAb) activity. In contrast, this correlation was not found in normal subjects. After administration of T3 (75 μg daily for 8 days), the patients showed increased levels of T3 with concomitant suppression of TSH levels. Under these conditions, linear regression analysis showed significant correlations of TRAb titer and TSAb activity with 24-h thyroid radioiodine uptake (r = 0.641 and 0.621 respectively, P < 0.01), in contrast to declining blood thyroxine levels. Moreover, the immunoglobulin G (IgG) of the patients precipitated to a greater extent than IgG from normal subjects a peptide consisting of the amino acid sequence near the terminus of the human TSH receptor. These findings indicated that TRAb at normal levels possessed significant unremitting activities on thyroid function despite long-term treatment in euthyroid patients with Graves’ disease.

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Introduction
Graves’ disease is an autoimmune anti-receptor disease and is characterized by abnormal immunoglobulin G (IgG) that can not only displace thyrotropin (TSH) binding to thyroid plasma membranes (TSH receptor antibody, TRAb), but can also stimulate thyroid functions (thyroid-stimulating antibody, TSAb) (1–3). Our previous observations (4–6) have suggested that the undeniable amino acid sequence at the N-terminal site of the extracellular domain of the TSH receptor is the immunogenic region for IgG in Graves’ disease but not in other thyroid disorders. Moreover, immunization with a peptide specific to Graves’ IgG was found to produce marked TSAb activity in rabbits (6). As this abnormal IgG contributes to increased thyroid gland function in the hyperthyroid state (7–10), assessment of TRAb enables physicians to make an accurate diagnosis of the disease. Under treatment with antithyroid drugs TRAb titers decrease to normal levels. Although the efficient duration of this treatment is still controversial (11), prolonged rather than short-term treatment may improve the remission rate of this disease (10). At the end of a course of long-term therapy, determination of TRAb may be important to forecast the patient’s prognosis (7–10, 12, 13), but this has not become standard practice due to difficulties in obtaining consistent results on testing (14, 15) or to insensitivity of the assay. Interesting results have come from Peakman et al. (16) who reported that T-lymphocytes remained activated in long-term remission of Graves’ disease. Furthermore, IgG of normal persons has been observed to be capable of binding the TSH receptor (17, 18). However, there is little information available as to whether TRAb in the normal range exhibits persistent functions of the thyroid gland and whether the patient’s IgG may continue to recognize the possible immunogenic region on TSH receptors. The present study was performed to obtain an insight into the characteristics of IgG in Graves’ patients receiving prolonged and effective treatment with antithyroid drugs.

Patients and methods

Subjects
Thirty-three patients with Graves’ disease (27–77 years old (average age 51.6 ± 2.4 years); male:female ratio...
6:27) were treated with methimazole (n = 22) and propylthiouracil (n = 11), the daily maintenance doses to maintain clinical euthyroid states being 5.7 ± 0.4 and 51.5 ± 5.5 mg respectively. All laboratory findings were within the normal range. The duration of treatment ranged from 3 to 19 years (average 10.6 ± 1.1 years). Eighteen normal healthy subjects (average age 49.9 ± 1.4 years; male:female ratio 3:15) were included in this study. All subjects gave their informed consent in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Thyroid function tests
Serum total thyroxine (T₄) and tri-iodothyronine (T₃) levels were measured using commercially available enzyme-immunoassay kits (Boehringer-Mannheim enzyme-immunoassay T₄ and T₃ kits, Boehringer–Mannheim, Tokyo, Japan). Serum TSH was measured using a radioimmunoassay kit (Daiichi radioimmunoassay TSH kit, Daiichi Radio-Isotope Co., Japan). The normal ranges of serum T₄, T₃, and TSH were within the normal range. This range is comparable to that of normal controls, they were designated in the study. All subjects gave their informed consent in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Determination of immunoglobulin
TRAb titers were measured using Smith’s kit (Baxter R.S.R. Ltd, Cardiff, UK) using labeled bovine TSH and porcine thyroid membranes as described previously (12), and TSAb activities were measured using porcine thyroid cells (8). The variations of these intra- and interassays were less than 12%. When TRAb titer and TSAb activity in individual patients, there were no significant relationships between these parameters (T₄ suppression rate vs TRAb titer and TSAb activity, r = 0.217 and 0.186 respectively). This reflects no activation of TRAb on T₄ secretion from the thyroid gland. In contrast, as shown in Fig. 3, significant positive correlations were observed in normal subjects (TSH vs T₃, \( r = -0.596, P < 0.05 \)). In Graves’ patients, neither TRAb titer nor TSAb activity was significantly correlated to thyroid hormone concentration (vs T₃ levels, \( r = -0.023 \), and \( r = -0.033 \) respectively). In contrast, a significant correlation was found between TRAb titer and TSAb activity (\( r = 0.504, P < 0.01 \)) as shown in Fig. 1. However, this significant correlation did not occur in normal subjects (TRAb vs TSAb, \( r = 0.036, P > 0.05 \)).

In patients with Graves’ disease, oral administration of T₃ caused an increase in blood T₃ levels, reaching the hyperthyroid state, followed by significant decreases in TSH, indicating the suppression of TSH secretion from the anterior pituitary. T₄ levels were also suppressed. Figure 2 shows the relationship between the rate of T₃-induced T₄ suppression and TRAb titer and TSAb activity in individual patients. There were no significant relationships between these parameters (T₃ suppression rate vs TRAb titer and TSAb activity, \( r = 0.217 \) and 0.186 respectively). This reflects no activation of TRAb on T₄ secretion from the thyroid gland. In contrast, as shown in Fig. 3, significant positive correlations were observed in normal subjects (TSH vs T₃, \( r = -0.596, P < 0.05 \)). In Graves’ patients, neither TRAb titer nor TSAb activity was significantly correlated to thyroid hormone concentration (vs T₃ levels, \( r = -0.023 \), and \( r = -0.033 \) respectively). In contrast, a significant correlation was found between TRAb titer and TSAb activity (\( r = 0.504, P < 0.01 \)) as shown in Fig. 1. However, this significant correlation did not occur in normal subjects (TRAb vs TSAb, \( r = 0.036, P > 0.05 \)).

Immunoprecipitation of the human TSH receptor-derived peptide
Detailed recognition by IgG of the TSH receptor was determined using a synthetic peptide corresponding to the amino acid sequence (amino acids 32–56) at the N-terminus of the extracellular domain of human TSH receptor, as described previously (4). Briefly, 50 μl IgG (0.5 mg), which were separated using a DEAE column (Bio-Rad Laboratories, Richmond, CA, USA), were incubated with 100 μl 125I-labeled synthetic peptide (approximately 20 000 c.p.m.) in phosphate-buffered saline (PBS; 0.01 mol/l PO₄, 0.15 mol/l NaCl, pH 7.5) containing 0.25% bovine serum albumin, and 150 μl incubation buffer (PBS containing 0.05 mol/l EDTA, pH 7.5). After incubation at 4 °C for 48 h, the precipitates were obtained by adding 500 μl 8 mg/ml bovine γ-globulin and 500 μl 30% polyethylene glycol (molecular mass 6000) followed by centrifugation at 2000 g for 30 min, and were counted for γ-rays. This assay was done as a single assay to avoid the inter assay variation. The intra-assay variation was 3.6%. Due to the limited amount of blood obtained, IgGs from 13 of the patients (aged 49.0 ± 4.1 years; male:female ratio 3:10) were examined and compared with those from 10 normal subjects (aged 48.8 ± 3.0 years; male:female ratio 3:7).
observed between the ratio of T₃-suppressive thyroid RIU and TRAb titer and TSAb activity in the patients (r = 0.641 and 0.621 respectively, P < 0.01), indicating that TRAb stimulates an iodide-transport function of the thyroid gland in euthyroid Graves’ disease. These data imply that Graves’ IgG might possess many different and separate actions on thyroid functions including iodide transport, thyroglobulin synthesis, thyroglobulin iodination, coupling reaction of iodotyrosines, iodothyronine formation and thyroid hormone secretion.

The IgG from patients with Graves’ disease precipitated the synthetic TSH receptor-related peptide to a significantly greater extent than normal IgG (1565 ± 49 vs 1207 ± 40 counts per min, P < 0.01), as shown in Fig. 4.

**Discussion**

The present study demonstrated that TRAb titer in the normal range showed a significant positive correlation with some thyroid functions after prolonged treatment with antithyroid drugs in Graves’ disease. Although in untreated hyperthyroid patients increased TRAb titers have been shown to be related to blood concentrations of thyroid hormones (9, 12), no significant correlation

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**Figure 1** Relationship between TRAb titer and TSAb activity in long-term treated euthyroid patients with Graves’ disease.

**Figure 2** Relationship between TRAb titer and TSAb activity and T₃-induced T₄ suppression rate. (a) T₄ suppression rate vs TRAb titer; (b) T₄ suppression rate vs TSAb activity.

**Figure 3** Relationship between TRAb titer and TSAb activity and thyroid RIU rate after T₃ administration. (a) Thyroid RIU vs TRAb titer; (b) thyroid RIU vs TSAb activity.
TSAb activities showed no correlation with T3-induced TRAb or TSAb in euthyroid Graves' disease. TRAb and TSAb from the anterior pituitary but not by activities of either thyroid hormone levels were regulated by TSH secreted as observed in normal subjects, the data implying that significant inverse correlation with blood levels of TSH was found between TRAb titer and thyroid hormone levels in the euthyroid patients examined here. In contrast, the blood concentrations of T3 showed a significant inverse correlation with blood levels of TSH as observed in normal subjects, the data implying that thyroid hormone levels were regulated by TSH secreted from the anterior pituitary but not by activities of either TRAb or TSAb in euthyroid Graves' disease. TRAb and TSAb activities showed no correlation with T3-induced T4 suppression. As the suppression of T4 levels after T3 administration reflects a decrease in T4 secretion from the thyroid gland (21), the present results suggest that thyroid hormone secretion from the thyroid gland was also basically unconnected to the activities of TRAb or TSAb in euthyroid Graves' disease.

The existence of a positive relationship between TRAb titer and thyroid hormone levels in the euthyroid patients examined here. In contrast, the blood concentrations of T3 showed a significant inverse correlation with blood levels of TSH as observed in normal subjects, the data implying that thyroid hormone levels were regulated by TSH secreted from the anterior pituitary but not by activities of either TRAb or TSAb in euthyroid Graves' disease. TRAb and TSAb activities showed no correlation with T3-induced T4 suppression. As the suppression of T4 levels after T3 administration reflects a decrease in T4 secretion from the thyroid gland (21), the present results suggest that thyroid hormone secretion from the thyroid gland was also basically unconnected to the activities of TRAb or TSAb in euthyroid Graves' disease.

The present results also show that the recognition activities of patients' IgG for the possible immunogenic region on the extracellular domain of the human TSH receptor were preserved, although their connection with TRAb titer and TSAb activity remains to be resolved. The peptide utilized here was synthesized on the basis of the strong turn potential indicated by Chou-Fasman secondary protein structure, the heightened antigenicity of the individual amino acid composition, and sequential amino acids with low homology to the amino acid sequence of luteinizing hormone/chorionic gonadotropin receptor, as described previously (4). Recognition of this peptide sequence is specific to IgG from Graves' patients, but is not recognised by those from Hashimoto's thyroiditis, subacute thyroiditis, or goitrous patients (5). These observations are compatible with the findings of Wadsworth et al. (24) who reported that the amino acid sequence near the amino terminus of the TSH receptor was an important site for the function of TSAb. Furthermore, we observed that immunization with a peptide identical to that tested here produced significant TSAb activity in rabbits (6). In conjunction with these observations, the present study supports the concept that TRAb still recognizes the TSH receptor even in euthyroid patients after prolonged treatment.

The present observations are in agreement with the previous results that T-lymphocytes in peripheral blood were activated in Graves' patients with long-term remission as compared with normal controls (16). Taken together, the present results support the possibility that the activated immunological mechanisms (3, 16) are responsible for activation of peripheral IgG with a specific immunogenic region on the TSH receptor that may, in turn, be connected with thyroid stimulation in Graves' disease after long-term treatment.

It will be of interest to assess whether the patients observed here relapse or go into remission after withdrawal of treatment.

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