Thyroid glands in patients with Graves' disease are sources of thyrotropin-binding inhibitory (TBI) activity

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Abstract. In order to investigate the main sources of production of Graves' immunoglobulins, 4 women with Graves' hyperthyroidism, which relapsed after withdrawal of methimazole (MMI) therapy, were selected for this study. The patients underwent subtotal thyroidectomy after pre-operative treatment with MMI and Lugol's solution. Seven blood samples were obtained in each patient during surgery: 1) a peripheral vein, immediately before neck incision; 2) the carotid artery; 3) and 4) the left and right inferior thyroid veins, respectively, before manipulation of the thyroid; 5) and 6) the left and right inferior thyroid veins, respectively, after surgical handling of the gland; 7) a peripheral vein at the end of operation.

Thyrotropin-binding inhibitory (TBI) activity was measured in all samples by a radioligand method. Serum TSH was also measured in those samples.

There was a substantial increment of TBI in the thyroid veins compared with the activity in the carotid artery. The mean TBI was significantly higher after surgical handling of the thyroid lobes. The two lobes from each gland secreted differing levels of TBI, whereas the TSH concentrations were similar in all samples from each individual patients.

We conclude that at least part of the TBI activity in patients with Graves' disease comes from the lymphocytic infiltration of the glands, and that differences in antibody production between the thyroid lobes may explain the difference in TBI activity in their respective thyroid veins.

It is well-known that in the sera of patients with Graves' disease there are abnormal immunoglobulins (TSAb) which bind the TSH receptor at the follicular cell (Kishihara et al. 1981; de Bruin & van der Heide 1984). Thyrotoxicosis results from the interaction of TSAb with thyroid cell membrane receptors (Manley et al. 1974; Hall et al. 1975).

The site of antibody production in vivo has not been clearly established. It has been reported that in Graves' disease peripheral blood lymphocytes produce thyroid stimulating IgG in vitro after specific stimulation with thyroid extract and after non-specific polyclonal activation (Knox et al. 1976; McLachlan et al. 1978; Sugenoja et al. 1978). However, several reports suggested that the target organ itself is the site of antibody synthesis in autoimmune thyroid diseases (McLachlan et al. 1979; Weetman et al. 1982).

Subtotal thyroidectomy often leads to a permanent remission of hyperthyroidism. It would be expected that circulating lymphocyte-derived antibodies should produce recurrence of the hyperthyroidism, through the stimulation of growth and function of thyroid remnants. This rarely happens, supporting the concept that autoreactive clones – actively synthesizing thyroid autoantibodies – may be primarily located within the thyroid.

For these reasons, we have studied the thyrotropin-binding inhibitory (TBI) activity during surgery on thyrotoxic patients in order to search for increments of activities in their thyroid vessels. Thus, we have reported preliminary evidences that Graves' immunoglobulins were augmented in the...

Patients and Methods

Patients
Four women with Graves' hyperthyroidism, which re¬
lapsed after withdrawal of methimazole (MM1) therapy, were selected for this study. The patients, aged 22–37 years, underwent subtotal thyroidectomy after pre¬
operative administration of MM1 and Lugol's solution, established in order to reach euthyroidism at the time of operation.

Samples
Seven blood samples were obtained in each patient during surgery from 1) a peripheral vein, immediately before neck incision; 2) the carotid artery; 3 and 4) the left and right inferior thyroid veins, respectively, before manipulation of the thyroid; 5) and 6) the left and right inferior thyroid veins, respectively, after surgical handling of the gland; 7) a peripheral vein at the end of operation. Serum was separated and stored at -20°C until assay.

Assays
TBI activity was measured in intact serum samples ac¬

All samples from each individual patients were mea¬
sured in a single assay in order to validate the comparison among them, avoiding inter-assay variation.

Fresh of frozen (-70°C) thyroid tissues from subjects who underwent thyroidectomy for Graves' disease, or histologically normal thyroid tissues from subjects who underwent thyroidectomy for common thyroid diseases, were used as TSH receptor sources. The material was gently homogenized with 3 vol of cold 10 mM Tris-HCl buffer, pH 7.5, containing 0.1% BSA. Homogenates were centrifuged at 700 x g for 5 min at 4°C. The supernatant fraction was then recentrifuged at 15,000 x g for 5 min at 4°C. The final pellet was resuspended in the same buffer and utilized as thyroid membrane.

[125I]hTSH (from New England Corp.) was used as the radioligand tracer, and 1 IU of commercial TSH (Thytopar®, from Armour Pharmaceutical Co.) was used in each assay as the 'cold' hormone for assessing the specific binding of radioactive TSH. A 'pool' of 10 serum samples of normal subjects was used in the standard tubes for calculating the 100% binding of [125I]hTSH to thyroid membranes. U of TBI activity were calculated as follows:

\[
\text{U} = \frac{(\text{CPM ['pool']} - \text{CPM [sample]}) \times 100}{(\text{CPM ['pool']} - \text{CPM [cold TSH]})}
\]

Since TBI activities in normal subjects and in patients with non-autoimmune thyroid disorders are lower than 30 U, we took this value as the cut-off point of negative from positive TBI. In this radioligand assay, 72% of patients with Graves' disease had positive TBI (> 30 U), the intra-assay variation being less than 5% (unpublished data).

In the present study, venous TBI activity was also expressed in an alternative manner, which we called 'fractional TBI activity' (see below):

\[
\text{Fractional TBI activity} = \frac{\text{U of TBI (vein)}}{\text{U of TBI (artery)}}
\]

Serum TSH was also measured (RIA) in the samples obtained during surgery. Normal values: < 7 µU/ml.

Statistics
Differences in TBI activity between samples were ana¬
yzed according to the t-test for paired values.

Results

TBI activity in the carotid artery
All patients showed positive TBI activity in the carotid artery, with values as follows: patients 1, 2, 3 and 4 = 48, 75, 50 and 43 U, respectively.

Fractional TBI activity in the thyroid veins
Fig. 1 shows the increasing TBI activity in the thyroid veins as compared with that in the carotid artery. Although there was a slight decrease of TBI activity in the veins from the two lobes in connection with gland manipulation, the mean TBI was significantly higher after surgical handling of the thyroid lobes. The TBI activity from the individual lobe differed from that obtained from the contra¬lateral one.

TBI activity in peripheral veins
Before surgery, no differences were observed be¬
tween the TBI activities in the peripheral vein and the carotid artery in the 4 patients studied. How¬
ever, when the blood from these veins was drawn at the end of operation, variable degrees of fractional TBI increments were observed: 0.75, 0.04, 0.36 and 0.30 for patients 1, 2, 3 and 4, respectively.

Serum TSH
There were no differences between the TSH con¬
centrations in the thyroid veins and in the carotid artery measured in the individual patient. TSH values were: 1.2, 5.1, 2.3 and 6.4 µU/ml for patients 1, 2, 3 and 4, respectively.
of thyroid tissue which contains large quantities of lymphocytes (Teng et al. 1980). The TBI (Arques-
oros et al. 1983) and TSAb increments in the blood from the thyroid veins (Kendall-Taylor et al. 1984) are in agreement with the concept that thyroid lymphocytes are involved in organ-specific antibodies production (McGregor et al. 1979; McLach-
lan et al. 1979).

In this report we not only demonstrate the increasing TBI activity in the thyroid veins, but also present evidences that surgical handling of the glands, in most cases, leads to a sudden pouring of Graves’ immunoglobulins into the blood stream. Although it is not possible to assess whether the TBI activity comes from the thyroid lymphocytes or from the IgG attached to the follicular cell surface, it is unlikely that the antibody-receptor complex could be broken by mechanical procedures. In fact, Weetman & McGregor (1984) pointed out that Graves’ antibodies usually decline slowly and progressively after thyroidectomy, pre-
ceded by a transient increase occurring in the first few postoperative days. Our data fit with these findings and give an alternative explanation of the transient rise in TSAb found after subtotal thyroidectomy. Weetman & McGregor (1984) proposed a ‘dual response to thyroidectomy: first, the removal of a major source of autoantibody synthesis, and second, the release of autoantigen from the dam-
aged remnant which stimulates autoantibody production at extrathyroidal sites.

Since the TSH concentrations in all samples from every one of our patients were similar, it can be assumed that the TBI activities in the thyroid veins were not influenced by the circulating levels of serum TSH.

We conclude that at least part of the TBI activity comes from the thyroid lymphocytes and that surgical handling favours the release of Graves’ immunoglobulins to the circulation. Differences in antibody production between the thyroid lobes may explain the difference in TBI activity in their respective thyroid veins.

References


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