SUMMARY

Objective: The distinct properties of TSH receptor (TSH-R) autoantibodies (TRAbs) from patients with Graves’ disease (GD) are yet unexplained on a molecular level. In the present study, the authors compared serum concentration, affinity to the TSH-R, and binding sites on the TSH-R of stimulating (TSAb) and blocking (TBAb) TRAbs.

Methods and Patients: Four-step affinity purification using human recombinant TSH-R was performed with 22 TRAb-positive sera from GD patients (11 with only TSAb and 11 with only TBAb) and 5 control sera. Antibody concentration, TSH binding inhibition (TBII), and TSAb/TBAb activity of the purified TRAb were assessed. Labeled purified TRAbs were used for displacement studies with TRAb and an additional 30 patients and 10 control sera.

Results: TRAbs could be purified to 80-93% purity with recovery of the TBII and TSAb and TBAb activity. No TRAbs could be purified from healthy individuals. The mean ± SD concentration of TRAb was 17.3 ± 5.4 µg/IU for the TSAb sera (range: 9.6 – 25.9) and 18.2 ± 8.5 µg/IU for the TBAb sera (range: 4.6 – 29.2) respectively (P = 0.79). Affinity was in the picomolar range for both TRAb subtypes with mean ± SD dissociation constant of 167 ± 109 pM (60 – 410 pM) for TSAb and 253 ± 132 pM (80 – 410 pM) for TBAb (P = 0.12). Purified and labeled TSAb and TBAb showed a very similar binding pattern to the TSH-R in displacement studies with unlabeled TSAb/TBAb or unpurified patients sera, indicating that the binding sites on the TSH receptor were in close proximity of each other.

Conclusions: TSAbs and TBAbs in the serum of patients with GD have similar characteristics. They are of low concentration with high affinity for the TSH receptor and have also similar binding epitopes on the TSH receptor.

COMMENT

This interesting article deals with the following hitherto unanswered question: why do auto-antibodies to the TSH receptor exert stimulatory or blocking activities? Patients with Graves’ disease (GD) display circulating TRAbs (TSH-receptor antibodies) that are mainly TSAbs (stimulatory) and occasionally TBAbs (blocking). TSAbs activate the TSH receptor and cause hyperthyroidism, whereas occupancy of the receptor by TBAbs inhibits the action of TSH and can cause hypothyroidism. When TRAbs are measured in TBII assays (based on the
binding inhibition of radiolabeled TSH), the activities detected comprises both TSAbs and TBAbs. In present study, the authors used a method (recently developed in their lab) for the affinity purification of TRAb from human serum. Sera from 11 GD patients with a high TBII activity (all with TSAb activity and no TBAb activity) were selected for the TSAb group. Sera from 11 patients with autoimmune hypothyroidism and with a high TBII activity (obtained from Singapore) were selected to have a strong TBAb activity with no TSAb activity. They constituted the TBAb group of sera.

The main results can be summarized as follows. First, the concentration of TRAbs in patients with GD is ~ 18 µg/liter IgG per international WHO unit, with no difference between TSAbs & TBAbs (this represents a low concentration). Second, the affinity of both TSAbs & TBAbs to the TSH receptor is in the picomolar range (60 – 410 pM), again without difference between TSAbs & TBAbs (this represents a high affinity). Third, both TSAbs & TBAbs show a very similar binding pattern to the TSH receptor, indicating that binding on the receptor occurs in very close proximity, most likely in the TSH binding domain of the receptor which, according to present models of the TSH receptor, lies within the part called ‘the horseshoe’ of the ecto-domain.

If concentration, affinity for the receptor, and binding characteristics are so similar between TSAbs & TBAbs, what makes their so striking biological difference? A possible explanation was proposed recently by a study on the interaction of TSAb and TBAb monoclonal antibodies and TSH receptor mutants with single amino acid substitutions in the horseshoe part of the receptor (Sabine Costagliola et al. in ULB, Brussels). These authors showed that the TSAbs and TBAbs epitopes were in very close steric proximity but nevertheless distinct on the molecular level. Thus, a single amino acid difference could be the reason for the dramatic difference in biological activity. It remains to be shown that this explanation stands true for all sera of patients with Graves’ disease, Hashimoto’s disease, or patients with patterns of successive changes from one form of the autoimmune thyroid disease to the other.

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See Figure below

![Two representative saturation curves and Scatchard plots for affinity purified TSAb and TBAb.](image)