Background: The TSH receptor (TSHR) is constitutively active and is further enhanced by TSH ligand binding or by stimulating TSHR antibodies (TSHR-Abs) as seen in Graves’ disease. TSH is known to activate the thyroid epithelial cell via both Gαs-cAMP/protein kinase A/ERK and Gαq-Akt/protein kinase C coupled signaling networks.

Objective: The recent development of monoclonal antibodies to the TSHR has enabled the authors to investigate the hypothesis that different TSHR-Abs may have unique signaling imprints that differ from TSH ligand itself.

Methods: Sequential studies were performed, using rat thyrocytes (FRTL-5) as targets, to examine the signalling pathways activated by a series of monoclonal TSHR-Abs in comparison with TSH itself. Activation of key signalling molecules was estimated by specific immunoblots and/or enzyme immunoassays. Continuing constitutive TSHR activity in thyroid cells, deprived of TSH and serum for 48 h, was demonstrated by pathway-specific chemical inhibition.

Results: Under these experimental conditions, TSH ligand & TSHR-stimulating antibodies activated both Gαs & Gαq effectors. Importantly, some TSHR-blocking and TSHR-neutral antibodies were also able to generate signals, influencing primarily the Gαq effectors and induced cell proliferation. Most strikingly, antibodies that used the Gαq cascades used c-Raf-ERK-p90RSK as a unique signalling cascade not activated by TSH.

Conclusions: This study demonstrates that individual TSHR-Abs have unique molecular signatures which result in sequential preferences. Because downstream thyroid cell signalling by the TSHR is both ligand dependent and independent, this may explain why TSHR-Abs are able to have variable influences on thyroid cell biology.

COMMENT

The TSH receptor (TSHR) is a seven-trans-membrane receptor activating the thyrocyte via the classical G protein-coupled receptor effectors, namely Gαs & Gαq, and their complex downstream signalling modules. The TSHR has constitutive signalling activity which is further activated by TSH binding or by the unique stimulating auto-antibodies to the TSHR (TSHR-Abs) characteristic of patients with Graves’ disease.

The aim of present study was to test the hypothesis that different monoclonal TSHR-Abs might have unique signalling imprints at the level of the TSHR, altering, in turn, thyrocyte function in distinct patterns not seen with TSH itself. For this study, seven monoclonal TSHR-Abs were used (3 stimulating, 2 blocking, and 2 neutral antibodies) with FRTL-5 cells and CHO-cells transfected with the human TSHR.
With regard to the monoclonal stimulating TSHR-Abs, the main results were to show that these antibodies used signalling pathways similar to thyrocyte activation by TSH (the natural ligand of TSHR), with differences depending upon the strength of the stimulating signal generated by the antibodies. With regard to the monoclonal blocking TSHR-Abs, results showed that both antibodies resulted in signal activation with different pathway dominance. These findings suggest that blocking Abs may influence cell function by altering multiple signalling mechanisms in thyrocytes and may be considered as allosteric modulators causing either activation or inhibition of TSHR activity. Finally with regard to monoclonal neutral TSHR-Abs, results showed that one antibody suppressed multiple signalling modules (including cell proliferation), whereas the other antibody caused activation of several signalling modules. This was observed only with FRTL-5 cells (and not with CHO-cells). Both antibodies failed to activate cAMP production and, by definition, none of them inhibited TSH binding to TSHR. The authors concluded that the stimulating monoclonal antibodies used signalling pathways similar to TSH activation pathways, leading to cell activation and cell growth. Furthermore, both the blocking-type and neutral-type TSHR-Abs distinguished different signalling networks and resulted in variable signal responses, indicating that some may have the potential ability to act as weak agonists or inverse agonists. These interesting experimental observations help explain how TSHR-Abs may contribute to different clinical phenotypes seen in autoimmune thyroid diseases, such as Graves’ disease and Hashimoto’s disease.

(Daniel Glinoer, M.D.; Ph.D.)

See Figure below