INVITED COMMENTARY

Potential pathogenicity of autoantibodies to thyrotropin receptor in treated, euthyroid patients with Graves' disease

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Autoantibodies to the thyrotropin receptor (TSH-R) have a major aetiological role in Graves' disease (1). Since the first description of these autoantibodies as the long-acting thyroid stimulator in 1956, much progress has been made in understanding the TSH-R protein, following the cloning and amino acid sequence of the receptor in late 1989/early 1990 (reviewed in (2)). This has allowed a series of fundamental questions related to the TSH-R and the serum autoantibodies to be studied. The binding sites on the TSH-R that are recognised by different populations of anti-receptor autoantibodies have been identified (2, 3). Single amino acid alterations have been shown to be responsible for gain, loss, or both, of receptor function (4). The proteolytic cleavage sites on the receptor protein, together with the enzymes responsible for the shedding of the soluble domain from the surface of thyroid cells have been characterised (5–8). The availability of recombinant preparations of TSH-R protein, albeit in a non-functional form unable to bind TSH, have allowed a number of attempts to establish experimental animal models of Graves' disease (reviewed in (9)). This has finally culminated in a successful murine model resembling the hyperthyroid status induced by thyroid-stimulating antibodies (TSAb) (10). These recent advances, together with the increasing knowledge on the cell biology of TSH-R (11) are beginning to increase our knowledge at a molecular level on the role of TSH-R in autoimmune thyroid disease. Despite this, however, over the past decade there has been little progress with the identification and measurement of serum anti-TSH-R autoantibodies (12, 13). After the cloning of the human TSH-R, it was anticipated that sensitive and robust assay systems to measure serum anti-TSH-R autoantibodies would become readily available, which would allow the monitoring of patients with Graves’ disease and enable their response to treatment to be assessed accurately and easily. In reality, this has failed to occur for a number of reasons, in particular, the difficulties of high level expression of the receptor as a recombinant protein with preservation of its native structure and stability (14, 15). The routine clinical assays continue to use the radioreceptor binding assay (12) or bioassays, based upon cAMP stimulation (16), although the use of mammalian cell lines stably transfected with recombinant human TSH-R have made the assays easier to perform, with some improvement in sensitivity.

The TSH-R belongs to the family of G-binding glycoprotein receptors comprising a large N-terminal extracellular domain responsible for interacting with the hormone ligand and a seven transmembrane spanning region necessary for the process of signal transduction (2). A population of autoantibodies to TSH-R, that is heterogeneous in terms of both epitope recognition and, probably, affinity for the receptor protein, is manifest in thyroid autoimmunity (3). This, together with their low serum titre (17), compounds the difficulties of studying the autoantibodies. In Graves’ disease, a variety of TSAb, leading to hypersecretion of thyroid hormone, have been recognised in the serum. In combination with the stimulatory antibodies, there may also be present the much more rare TSH-blocking antibodies (TSBAb). To complicate matters further, there also exist neutral autoantibodies to the TSH-R that at present remain undetected by the available routine clinical assays. It is possible that the neutral antibodies may be involved in the extrathyroidal complications frequently observed in patients with Graves’ disease, such as ophthalmopathy and pretibial myxoedema. This heterogeneity of anti-TSH-R antibodies is the likely explanation for the lack of correlation between disease severity and titres of the serum antibodies in patients with Graves’ disease.

To unravel the structure–function relationship of the TSH-R and the mechanisms by which the TSAb and TSBAb mediate their different biological effects requires the determination of the regions on the TSH-R recognised by the autoantibodies. The limitation of maintaining TSH-R structure and function has meant that meaningful studies have been limited to those using either deletion mutants or using chimeras between the TSH-R and the related luteinising hormone/chorionic hormone receptor (LH/CG-R). Earlier studies focused on the importance of the large extracellular region of the TSH-R for the binding of TSAb and anti-TSH-R autoantibodies (2, 3). While the importance of the extracellular region in providing a number of spatially close contact residues for high-affinity binding of TSH and anti-TSH-R autoantibodies is well recognised, it is still not clear whether the three extracellular loops of the
receptor contribute to high-affinity binding of TSH, or to some or all of the population of anti-TSH-R autoantibodies. Initial studies indicated that, of the two unique insertions in the TSH-R relative to LH/CG-R at positions 38–45 and 317–366 (amino acid numbering from the first methionine), only the former insertion is crucial for binding of TSH and in inducing cAMP responses by TSH and TSAbs (2). Furthermore, Cys41 is the important residue in this insertion; substitution of this residue affects the binding of TSH, indicating an important intra-chain disulphide linkage in the receptor, the disruption of which results in a major effect on the folding and tertiary structure of the receptor polypeptide chain (18). Deletion of residues 295–306 and 387–395 towards the carboxyl region of the TSH-R produces negligible effects on TSAb activity, but specifically reduces the TSAb activity. Using replacements of LH/CG-R sequences in TSH-R chimeras led to the definition of the amino terminus as the important functional region for TSAbs and the carboxyl terminus as the region harbouring the sites for TSAbs. In particular, residues 25–30 (19), in addition to residues 90–165 (20), appear to be important for TSAb activity in patients with Graves’ disease. The latter finding also provides confirmatory evidence on the multiplicity of the antibodies with TSAb activity directed to the amino-terminal region of TSH-R. Further subdivision of the amino acid 90–165 region into residues 90–124 and 125–165 indicates that both the segments are necessary for TSAbs, suggesting that these amino acids are brought together to form an independent conformational epitope for binding and the consequent stimulation of cAMP activity (21). As far as the TSAbs are concerned, residues 261–370 appear to provide independent binding sites for these antibodies without affecting the TSAb activity.

These studies are beginning to provide a picture of the surface conformational regions of TSH-R recognised by TSH and the anti-TSH-R autoantibodies present in autoimmune thyroid disease. Large areas in the amino-terminal region of TSH-R contribute amino acids to form conformational epitopes for at least two, if not more, different populations of antibodies with TSAb activity. Amino acids in the carboxyl region of the extracellular domain of TSH-R provide independent conformational epitopes for blocking antibodies. In contrast, the high-affinity binding and activity of TSH is dependent upon interaction with multiple, discontinuous amino acid residues scattered throughout the extracellular region of TSH-R. In addition, for high-affinity binding of pathogenic autoantibodies to TSH-R, there appears to be a strict requirement for faithful glycosylation in the form of complex carbohydrate side chains on the TSH-R protein, which may have a subtle role in the correct maintenance of the tertiary structure of the autoantigenic epitopes (22, 23).

Although our present knowledge on the TSH-R and the pathogenic autoantibodies has increased dramatically, the precise number of antibodies to closely different epitopes on the TSH-R remains to be defined. The availability of such knowledge will depend on the isolation of individual specificities of antibodies from different groups of patients, most probably as human IgG-secreting B cell clones (24), or from the hyperthyroid murine model (10). Such approaches will allow the dissection of the neutral class of anti-TSH-R autoantibodies, which so far have been difficult to validate. Until such progress has been made (25), populations of anti-TSH-R autoantibodies that show pathogenicity at different clinical stages of the disease will continue to be described. It is the description of another such population of pathogenic autoantibodies by Akuzawa et al. in this issue of European Journal of Endocrinology that is of great interest (26).

Akuzawa et al. have examined the continuing effects of TSH-R antibodies (TRAb) in 33 patients with Graves’ disease, who have undergone prolonged treatment with anti-thyroid drugs for a mean of 10.6 years (3–19 years). Tri-iodothyronine-suppression testing demonstrated correlations between 24-h thyroid uptake of radioiodine, TRAb titre ($r = 0.641$) and TSAb activity ($r = 0.621$). The authors interpret the data to indicate that there is residual activity of TRAb in Graves’ patients who have remained euthyroid over a prolonged period of time. In support of this, TSAbs directed to the amino-terminal region of the TSH-R were identified. The inference from this is that patients with continued TSAb activity are at risk of relapse after the cessation of anti-thyroid drug treatment.

From the clinical point of view, there are few consistent predictors of relapse in patients when anti-thyroid treatment is discontinued. The rate of decrease in TSAb activity over 12 months after treatment may predict relapse (27). However, a large vascular goitre and suppressed serum TSH after the cessation of medical treatment are both likely to be associated with relapse (28, 29). The authors state that the measurement of TRAb has not been found to be of clinical value in predicting relapse. Certainly, conflicting data have been reported (28, 30, 31). Serum concentrations of TRAb and TSAb activity are not consistently increased in patients with Graves’ disease, and assessment of these parameters has had little effect upon clinical practice. The suggestion that prolonged treatment with anti-thyroid drugs improves remission rates is not borne out in practice: approximately 50% of patients suffer relapse, regardless of the dosage or duration of anti-thyroid drug treatment (32).

It would be of interest to know the TSAb and TRAb measurements at the start of anti-thyroid drug treatment, in the context of the clinical presentations of the patients (goitre size, thyroid hormone concentrations, thyroid autoantibody titres, presence of eye disease). Presumably, the TSAb concentrations and TRAb activities decreased during anti-thyroid drug treatment. This may partly reflect the natural history of the disease,
as patients also go into remission with β-blocker treatment and, in the long term, a proportion of patients become hypothyroid (33). Full TSH suppression must be shown to have occurred before any conclusions can be drawn about the stimulating effects of TRAb on thyroid uptake of radioiodine. No data have been given for normal subjects. If complete suppression of TSH was achieved in the controls, then thyroid uptake of radioiodine should have been eliminated. It is unclear whether or not intrinsic TSH activity was fully abolished in the patients before the uptake of radioiodine was assessed.

Overall, subject to the caveats detailed above, the data suggest the possibility that there may be continuing stimulatory activity of TRAb in some patients, despite long-term treatment with anti-thyroid drugs. Whether or not this will bear any relationship to subsequent disease activity remains to be demonstrated. The TRAb assays that are available at present are unable to differentiate different subpopulations of TRAb which, as mentioned above, are likely to have differential effects upon disease activity. The routine measurement of TRAb is unlikely to be of clinical value until the specific antibodies can be identified and measured using sensitive assays. It may then be possible to predict the long-term course of disease activity, associated complications such as ophthalmopathy, and their responses to different treatment modalities. The aim must be to optimise treatment for the individual patient on evidence-based criteria, unlike present practice which is largely dependent upon local preference. The ability to define clearly those patients who should receive medical treatment (with recommendations for both anti-thyroid drug dosage and duration of treatment), surgery, or radioiodine treatment may be expected to provide the first major advance in the management of Graves' disease for a number of decades.

References


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