Thyroid Stimulating Immunoglobulins

The Watson Smith Lecture 1983

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Although the manifestations of Graves' disease involve many different tissues, it has been recognised since 1887[1] that its life-threatening features result from excessive thyroid function. Investigations into the pathogenesis of Graves' disease have, therefore, been primarily concerned with the causes of the unregulated production of thyroid hormones, in spite of such manifestations as severe eye signs which are often associated with, but not caused by, thyroid overactivity. This brief review follows the interrelationships between contemporary advances in physiology and pathology and hypotheses concerning the nature of Graves' disease. The conditions of autonomous hyperfunctioning thyroid adenoma, thyrotoxicosis arising from hyperfunction in multinodular toxic goitre and the influence of iodide administration on thyroid function are not discussed[2].

After the discovery of pituitary thyroid stimulating hormone (TSH)[3], assays were used to investigate the levels of TSH in the blood and urine of patients with Graves' disease[4], because the histological changes induced in the thyroid by TSH treatment of experimental animals closely resembled those in the thyroid of untreated Graves' disease[5]. Even the earliest histiometric assays were able to show that, whereas there was increased TSH in the body fluids of patients with myxoedema, no increase over control levels in normal subjects could be found in untreated thyrotoxicosis[6,7]. These results must have puzzled their instigators, as the negative feedback for TSH control of thyroid function[8], which explains them, had not yet been postulated. Moreover, when histiometric assays were refined and quantitated more precisely, both De Robertis (1948)[9] and Purves and Griesbach (1949)[10] produced evidence for thyroid stimulating activity in the circulation of some patients with Graves' disease.

The Discovery of the Long Acting Thyroid Stimulator (LATS)

A new observation, allowing these apparently conflicting results from histological assays to be reconciled, came with the advent of improved bioassays for thyroid stimulating activity in serum which depended on the availability of radioactive isotopes of iodine. In Dunedin, New Zealand, Adams and Purves showed that, in suitably prepared guinea pigs, it was possible to demonstrate clear differences in the time courses of thyroid stimulation by pituitary extracts rich in TSH and the stimulation by serum from some patients with Graves' disease. Whereas the effects of TSH were relatively brief in duration, reaching a peak 2 to 3 hours after intravenous injection, the responses to sera from some patients with thyrotoxicosis were slower in onset and the time of maximal effect was later and tended to plateau, rather than peak, approximately 16 hours after intravenous injection[11]. Because of its prolonged time-course of action (compared with TSH) the thyroid stimulating activity in serum from patients with thyrotoxicosis was attributed to the presence of an abnormal 'long acting thyroid stimulator', abbreviated to LATS.

The principles underlying the Purves and Adams guinea pig assay[12] were based on new knowledge of the physiological negative feedback control of thyroid function and of the influence of dietary iodide on thyroidal radioiodine uptake. Experimental animals, depleted of iodide by appropriate dietary iodide restriction, were injected with radioiodine and subsequently treated with thyroid hormone. After a few days, the animals could be shown to have the iodotyrosines in their thyroid glands freely labelled with radioiodine, yet little radioactivity was discharged into the peripheral circulation because of suppression of endogenous pituitary TSH release. Thyroid stimulation, after the injection of TSH or test sera, was demonstrated by the discharge of some of the thyroid store of radio-labelled hormone into the peripheral blood so that peripheral blood radioactivity increased in proportion to the dose of stimulator injected. McKenzie (1958)[13] transferred the principles of the assay to mice and his assay immediately became the most widely used bioassay for the detection of thyroid stimulating activity.

At this stage, roughly 25 years ago, it was thought that the problem of understanding the pathogenesis of the thyroid overactivity of Graves' disease was on the point of solution. However, it was soon appreciated that many of the early results with this type of assay had failed to take adequate account of the non-specific effects which fre-

quently follow the injection of serum into the test animals[14] and it was concluded, ultimately, that only a minority of patients with Graves' disease have undoubted LATS activity in their serum. Yet the 'LATS-negative' patients suffered from Graves' disease of comparable severity, with similar clinical manifestations.

Recovery of LATS with Immunoglobulin G

In spite of the discouragingly low prevalence of LATS in Graves' disease, the effects of the most potent sera were so striking that several groups persisted in their attempts to purify the activity from large serum samples and were encouraged to persist when it was shown in the early 1960s that LATS activity could be recovered with immunoglobulin G (IgG)[15,16]. By this time, of course, Roitt *et al.* (1956)[17] and Trotter, Belyavin and Waddams (1957)[18] had identified thyroid autoantibodies to thyroglobulin and thyroid microsomes respectively.

Analysis of Structure-Activity Relationships in LATS-IgG

The analysis of the structure of IgG, either by enzymatic hydrolysis into Fab,(Fab')2 and Fc fragments, or by breaking the disulphide bonds between the pairs of heavy and light chains, confirmed that the thyroid stimulating groupings in LATS-IgG were located in the Fd portion of the heavy chain[15,16,19]. The analogy with the location of antigen binding sites in other well characterised experimental antibodies seemed complete.

LATS-absorbing Activity (LAA)

Once the IgG nature of LATS was accepted, speculation that LATS-IgG might be a thyroid autoantibody, with the unique property of stimulating the thyroid, led logically to a search for the relevant thyroidal antigen. It was soon established that the thyroid stimulating activity in LATS-containing sera could be specifically absorbed by extracts of human thyroid[16]. The solubilisation of this binding activity for LATS, which came to be known as LATS absorbing activity (LAA), allowed some progress in its purification. Smith recovered LAA activity in a 4S protein[20,21], which was further purified by Dirmikis[22], who studied the influence of freezing and slow thawing on the release of LAA from human thyroid homogenates; other species of thyroid did not yield LAA when extracted in an identical manner[23].

In the ten years that had now elapsed since LATS was first described, the major obstacle to its more widespread acceptance as an important pathogenetic influence in Graves' disease remained the low prevalence of LATS-positive sera in patients with thyrotoxicosis. Strangely, it was through further study of the binding of LATS to LAA that a way around this difficulty emerged.

Discovery of LATS-protector

In studies of LATS absorption, many different laboratories commented on the considerable variation in the susceptibility of LATS activity in different sera to be absorbed by LAA. When different sera of comparable LATS potency were incubated with a fixed concentration of human thyroid extract rich in LAA, it was common experience to find that the extent to which LATS activity was bound could vary from total removal to apparent total resistance (Fig. 1). The explanation for this variability

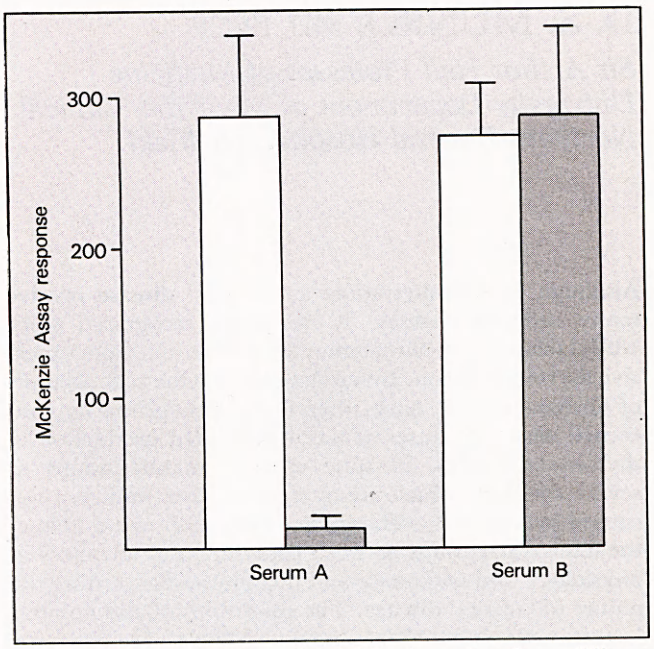

Fig. 1. *The variability of LATS absorption from different sera. LATS activity from serum A was readily bound by LATS absorbing activity (LAA). In contrast, LATS activity in serum B was unaffected after similar incubation with the same concentration of LAA.*
LATS activity in unabsorbed serum □
LATS activity after incubation with LAA ■

of LATS in its susceptibility to binding came from the same laboratory of the New Zealand Medical Research Council in Dunedin that originally discovered LATS. When seeking to standardise the protein concentrations for studies of the binding of LATS to LAA, Adams and his colleagues used a sample of LATS-negative serum from a patient with Graves' disease as a diluent for a particularly potent serum which was their LATS standard. To their surprise, they found that after this dilution, LATS binding to LAA was blocked by an unidentified activity in their LATS-negative serum. Because of its mode of detection, this new activity was thought of as 'protecting' LATS from binding to LAA and, hence, was named LATS-protector (LATS-P)[24]. Further study showed that LATS-P activity was also associated with IgG and that it was found, with rare exceptions, solely in sera from patients with Graves' disease. The group in Dunedin quickly set about establishing that the prevalence of LATS-P in untreated Graves' disease was very much higher than for LATS. They also showed that there was a strong positive correlation between the level of LATS-P and the extent to which

thyroid function was stimulated in Graves' disease, as reflected in radioiodine clearance studies in their patients[25]. By infusion of plasma rich in LATS-P into normal human volunteers, a direct action on their thyroids was confirmed[26]. A similar effect had been shown earlier by Arnaud and others[27].

In the wake of these reports there was a further period of debate about the nature of LATS-protector. However, the present consensus view of the relationship between LATS and LATS-protector is that both are members of a common population of human thyroid stimulating autoantibodies, which show a variable degree of cross-reactivity with other mammalian thyroids[28,29]. Thus, when the thyroid stimulating antibody cross-reacts with the mouse, it is active in the McKenzie bioassay system and appears as LATS activity, but LATS-protector, although specifically binding to LAA with the same characteristics as LATS, does not cross-react with the mouse and is, therefore, LATS-negative in the McKenzie system. In support of this concept, serum surveys established that, although LATS-protector activity frequently occurs without associated LATS in sera from patients with thyrotoxicosis, those with LATS activity invariably have an excess of LATS-P. Less than 10 per cent of all patients with untreated Graves' disease have no assayable LATS-P activity[30].

Although the discovery of LATS-P greatly strengthened the general hypothesis that thyroid stimulating immunoglobulins were of importance in Graves' disease, not many laboratories have embarked on extensive use of the elaborate bioassay system needed for its measurement. Two biological standards are required, one for LATS activity and the other for LAA. The assay for LATS-protector involves, firstly, a preliminary screen to exclude the presence of LATS and, secondly, the selection of concentrations of standard LATS activity and a preparation of LAA which almost, but not wholly, annuls the LATS activity in the standard serum. The selection of such conditions is necessary to achieve optimum sensitivity for the detection of blocking of LATS binding by LATS-P. This assay has, therefore, much in common with the techniques widely used in immunology for the detection of blocking antibodies. The McKenzie assay system is used in the final stage to measure the unbound standard LATS activity, which is a most unusual role for a bioassay.

A very real difficulty with research into the role of LATS-P has been one of interpretation; it could not be assumed that LATS-P activity, which bound to LAA *in vitro,* was necessarily a true human thyroid stimulator of relevance to Graves' disease. The answer to this question could only come from clinical correlations between LATS-P assays and the natural history of Graves' disease and its treatment.

Alternative *in vitro* Assay Methods

It is not surprising that, faced with the inherent variability of bioassay methods in whole animals and the lack of direct evidence of thyroid stimulation in the LATS-P assay, several groups have developed alternative *in vitro* assay systems. Such assay systems for thyroid stimulation frequently employ human thyroid tissue and include histological studies of colloid droplet resorption[31], cyclic AMP accumulation in human thyroid slices[32,33] and adenylate cyclase activation in human thyroid membranes[34,35].

These methods have not been widely applied either. For example, colloid droplet resorption in human thyroid maintained *in vitro* has only been reported from one laboratory[31]; the use of human thyroid tissue slices for assays of thyroid stimulating immunoglobins is confined to McKenzie and Zakarija[36]; only Beck and her colleagues have published many studies concerning the activation of adenylate cyclase in human thyroid membranes[37].

There are also several other assay systems which follow *in vitro* stimulation of thyroid tissue from other species. Such assays depend on cross-reactivity of the human immunoglobulin with the target tissue for positive results[38,39].

The Inhibition of Binding of TSH to Human Thyroid Membranes

A different approach to the assay of these immunoglobulins of Graves' disease depends on the inhibition of binding of radioiodinated TSH to human thyroid membranes and this has been widely applied. Manley, Bourke and Hawker (1974)[40] in Brisbane, first established that IgG from patients with Graves' disease could block the binding of highly purified bovine TSH to guinea pig thyroid membranes. This type of experiment could only be done when bovine TSH of adequate purity became available; knowledge of methods for the radioiodination of peptide hormones and experience in the study of hormone receptor interactions were also necessary.

The principles of this assay were applied to human thyroid membranes by Smith and Hall and their assay, which is relatively simple, has now been adopted by many laboratories[41].

Recent advances in this technique, utilising solubilised TSH receptors and thereby circumventing nonspecific interfering effects from normal IgG, will undoubtedly increase its popularity[42]. It must be remembered, however, that this type of assay, like the LATS-P assay, does not measure thyroid activation directly.

Clinical Correlations between LATS-P assays and the course of Graves' disease

In spite of the tediousness of the assay method, the discovery of LATS-P aroused great interest because, in contrast to the relatively low prevalence of LATS activity in Graves' disease, it was soon established that more than 90 per cent of patients have LATS-protector activity and that the activity is specifically associated with a history of Graves' disease[30]. The difficulties of interpretation have already been discussed. Could it reasonably be assumed that LATS-P activity, which bound to LAA in a manner indistinguisable from LATS, was a true human thyroid stimulator of relevance to Graves' disease? Care-

ful clinical correlation between patients and assay results soon showed that, in addition to being almost exclusively confined to the serum of patients with Graves' disease, those patients who had both LATS and LATS-P activity invariably had LATS-P in much the greater concentration. It was also of interest to find that, like LATS activity, LATS-P was found in very high concentrations in many patients suffering from the rare skin manifestation of Graves' disease known as localised myxoedema, often in the pretibial region[43].

In studies of the liability to relapse after a course of antithyroid drug treatment, LATS-P was shown to be a good predictor of relapse when the assay remained positive after a course of treatment with carbimazole which had controlled thyroid overactivity for at least 12 months[44]. Treatment with antithyroid drugs, either alone or followed by subtotal thyroidectomy[45], led to a steady decline in LATS-P levels but there was a variable pattern after radioiodine, some patients showing a tendency for LATS-P to rise in a transient and erratic manner in the six months following administration of therapeutic doses of ^{131}iodine[46]. A similar phenomenon had been observed for LATS by Pinchera and colleagues some years previously[47]. When LATS and LATS-P were assayable in the same patient, changes invariably ran in parallel.

Neonatal Graves' disease

The most convincing evidence, concerning the relevance of LATS-P to the pathogenesis of thyrotoxicosis, comes from studies on the rare syndrome of neonatal Graves' disease. It is recognised that mothers who have a history of Graves' disease may give birth to children who suffer from a self-limiting form of the illness. This phenomenon is rare, probably occurring in less than one per cent of all such mothers. In a collaborative study with other centres, Dirmikis and others[48] made observations in the last trimester of 93 pregnancies occurring in mothers with a history of Graves' disease. Twelve pregnancies from this highly selected group resulted in the birth of a child suffering from neonatal Graves' disease. In this report, the prevalence of neonatal Graves' disease is very greatly inflated, as several of the mothers had given birth to earlier children who had similarly been afflicted with neonatal Graves' disease, resulting in a keen interest in subsequent pregnancies. Thus, there was a very strong bias towards including patients in whom the phenomenon was already known to occur. When arrayed in descending order of potency for LATS and LATS-P, it was clear that these 12 pregnancies resulting in the birth of children with neonatal Graves' disease were all associated with the maternal level of LATS-P in excess of 20 units/ml (compared to our internal laboratory standard). It has also been shown that the duration of neonatal Graves' disease is related to the persistence of LATS-P in the circulation of the newborn infant and, moreover, when this falls below a critical level of 20 units/ml, antithyroid drug treatment can safely be withdrawn.

Taken together, these clinical correlations suggested very strongly that LATS-P activity was, indeed, of pathogenetic significance in the thyroid overactivity of Graves' disease. In general, the trends of change in LATS-P levels after various forms of treatment in adult thyrotoxicosis are very similar, no matter which of the assay methods for immunoglobulin-associated activities is employed[49,50]. When detailed comparisons were made by different assays of the same serum sample, however, there were frequent discrepancies between the results[51]. The contrast between the broad correlation between groups of patients showing similar changes with different assay systems and the discrepant results seen in detailed studies on individuals, is difficult to evaluate. It may be that these assay systems look at a range of similar, but not identical, immunoglobulins which interact with closely related components of the thyroid cell surface. The occasional discrepancy may well result from differences in the specific interaction between an immunoglobulin and its epitope which have a critical effect in some, but not all, assays.

The Concept of Antibodies to Hormone-receptors

In 1960 Simpson proposed that defective neuromuscular transmission in myasthenia gravis could be due to a blocking antibody which was occupying the receptor for acetyl choline in striated muscle[52]. Thirteen years elapsed before this hypothesis was demonstrated to be true by Patrick and Lindstrom[53] who induced an experimental form of myasthenia gravis by immunising rabbits with highly purified receptors for acetyl choline, made from the electric organ of eels, which is a particularly rich source of these receptors. Ingenious exploitation of the interaction between certain snake venoms and acetyl choline receptors from human striated muscle has provided an assay which confirmed that there is a circulating blocking antibody in patients with myasthenia gravis[54]. Detailed analysis of the way in which the naturally occurring receptor antibodies influenced striated muscle function has revealed, surprisingly, that different mechanisms apply to the actions of different antibodies. For example, one mechanism by which the antibodies interfere with the striated muscle function is to accelerate the rate of receptor internalisation and another is to cause lysis of cells by a complement-dependent cell destructive process. Advances in understanding of the structure of the receptor for acetyl choline has allowed the development of monoclonal antibodies acting against different components of the receptor, which contains several potentially antigenic sites[55]. Another point of interest is that the titre of acetyl choline receptor antibody did not relate clearly to the severity of the disease, although, when remission was induced by immunosuppressive treatment, the level of antibody invariably declined[56].

Another disease in which antibodies to hormone receptors have been studied is diabetes mellitus. Originally, attention was focussed on patients with extreme insulin resistance and in whom such antibodies were first identified. However, with relevance to the problems of antibodies in Graves' disease, there is a small number of patients in whom insulin receptor antibodies apparently provoke insulin-like action and cause hypoglycaemia[57].

A point of contrast with Graves' disease is that the interaction of these immunoglobulins with the insulin receptor is 'bivalent'; 'univalent' fragments are inactive[58]. For a long time it was thought that the phenomenon of insulin receptor antibodies was extremely rare but there is a recent report[59] which suggests that, when they are sought in newly diagnosed juvenile diabetics, more than half of untreated patients can be shown to have significant insulin receptor antibodies.

A third disease in which receptor antibodies have recently been demonstrated is myxoedema. Interestingly, these TSH-blocking antibodies first came to light through the study of transient neonatal hypothyroidism[60] but, more recently, there have been reports of similar TSH receptor blocking antibodies in myxoedema[61].

Is Graves' Disease a Receptor-Antibody Disease?

It is always tempting to group diseases through common pathogenetic mechanisms and most workers on the thyroid stimulating immunoglobulins of Graves' disease would now discuss their research in terms of the action of antibodies on the TSH receptor. Even those who are enthusiastic about designating the thyroid stimulating immunoglobulins of Graves' disease as TSH receptor antibodies, must concede that there are differences between Graves' disease and the other diseases with antibodies to cell membrane receptors. For example, this is the only disease in which stimulating activity associated with IgG is found in the vast majority of cases throughout the course of the illness. There is a clear contrast with, for example, diabetes mellitus where the stimulatory actions of insulin receptor antibodies are rare and have only been described in a handful of cases. The more common insulin receptor antibody is solely blocking in its action. Techniques which measure the immunoglobulins of Graves' disease, by inhibiting the binding of TSH to thyroid membranes, appear to point very clearly to the antibody having an action directed at the TSH receptor[62]. However, our knowledge of the structure of the TSH receptor remains very imperfect. The binding interaction between highly purified pituitary TSH and thyroid receptors and the binding interaction of the immunoglobulins of Graves' disease with these receptors is probably very different, the latter being a much more stable union. Furthermore, the purified binding protein for thyroid stimulating immunoglobulins, LAA, cannot easily be shown to bind TSH; recent work has suggested that there is a binding action between pituitary TSH and LAA, but very special conditions are required to demonstrate it[63]. It has been postulated that LAA may be a fragment of the intact human TSH receptor.

In addition to a stimulatory effect on thyroid hormone synthesis and secretion induced by thyroid stimulating immunoglobulins, there may be other immunoglobulins more specifically associated with promoting thyroid growth[64]. It is known that TSH is not the only growth influence on the thyroid; other factors, such as epidermal growth factor (EGF), can also stimulate tritiated thymidine uptake in cultured thyroid cells[65]. It is also known that there are specific receptors (distinct from those for TSH) for EGF on the thyroid cell surface[66]. Thus, the prediction made by Carnegie and McKay in 1973[67] that cell function might be perturbed in many different ways by the interaction of immunoglobulins with different components on cell surfaces could well be valid for human thyroid disease. There is, however, no evidence at the moment that EGF receptors are involved with the actions of growth stimulating immunoglobulins and antibodies to the EGF receptor could not be found in one study in Graves' disease[68].

Conclusions

Over the past 27 years, there has been steadily increasing evidence which supports the idea that circulating immunoglobulins have an important pathogenetic role in Graves' disease. Although we still lack a simple stimulatory assay for routine use, there can be little doubt that the thyroid overactivity of Graves' disease results from the interaction between these immunoglobulins and the thyroid cell surface to induce stimulation by mechanisms very similar to those which operate when the thyroid is stimulated by TSH. In myxoedema, it is easy to accept the concept that a blocking antibody acting on the TSH receptor could induce thyroid failure. So far as stimulation of the thyroid by immunoglobulins is concerned, however, it is evident that the first interaction between antibody and TSH receptor must have features which differ from the action of TSH itself.

The most immediate requirement is improved knowledge of the structure of the human TSH receptor, so that monoclonal antibodies can be raised to its components and used to compare their actions on thyroid cells with the effects of TSH and the naturally occurring antibodies of Graves' disease.

Work on these lines is already reported[69] using monoclonal antibodies raised by Kohn and his colleagues[70]. So far, such studies can only be done by cytochemical bioassay in guinea pig thyroids. It may be that we shall soon be able to analyse the precise actions of the immunoglobulins of Graves' disease on our patients' thyroids and even to explain the effects of this disease on the other, extrathyroidal, tissues which are frequently involved.

Thereafter, it will be possible to focus on those factors which underlie the generation of stimulating autoantibodies and to explore further the roles of cell mediated immunity and genetic influences in the pathogenesis of Graves' disease.

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