The Aetiology of Graves' Disease

The Scientific Basis of Medicine

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<**Opening titles**>

<**Professor R Hall to camera**>

Graves' disease is a useful omnibus term to describe the commonest variety of hyperthyroidism which is seen in non-endemic goitre areas.

<**Hall over close-up photographs of females with Graves' disease**>

The patient is usually a woman and shows the typical eye signs, the so-called Graves' ophthalmopathy. In addition there is often enlargement of the thyroid gland, there is a peculiar thickening of the skin, usually located over the front of the shins, localised or pretibial myxoedema and sometimes there is appearance of the fingernails which resembles clubbing, which is termed thyroid acropachy.

<**Hall to camera**>

The aetiology of the eye signs, of the localised myxoedema and of the thyroid acropachy is really quite poorly understood, and we shall not consider this further here. However, we have a considerable amount of information about the pathogenesis of the goitre and hyperthyroidism and this forms the basis of our talk.

<**Hall over close shot of thyroid gland in Graves' disease**>

The thyroid gland in Graves' disease is enlarged and vascular. Histologically it shows scalloping of the colloid, infolding of the epithelium and an increased height of the epithelial cells as well as a varying degree of lymphocytic infiltration of the thyroid, which is not shown on this slide. It is obviously a gland under intense stimulation.

<**Hall to camera and referring briefly to tables listing mechanisms which stimulate hyperthyroidism**>

Such stimulation could result from one or more of three main mechanisms: a primary thyroid abnormality, thyroid-stimulating hormone or other thyroid stimulators. So far as the first mechanism is concerned, Chopra and Solomon have postulated a primary thyroid abnormality of the TSH receptor, in which the receptor is in the permanent switched on state with activation of adenyl cyclase. However, there is no good evidence for this possibility, though we must bear in mind that some abnormality of the TSH receptor could be responsible for the hyperthyroidism of Graves' disease. Similarly, there is no evidence that TSH itself is responsible for the hyperthyroidism of Graves' disease.

Thyroid-stimulating hormone levels are actually reduced in Graves' disease with hyperthyroidism, though these are rather difficult to measure. The radioimmunoassay is not sufficiently sensitive to measure the low levels of TSH in hyperthyroidism. One can achieve this by extracting large volumes of thyrotoxic serum but this is a difficult technical procedure. One can measure TSH levels in hyperthyroid Graves' disease

directly by the highly sensitive cytochemical bioassay described by Chayen and Bitensky though this is a rather time-consuming procedure.

In addition, hyperthyroidism has occurred after hypophysectomy when there is no evidence of circulating TSH levels. In hyperthyroidism, the thyrotroph cells of the pituitary appear in a histologically inactive state. Occasionally, patients have been described who show hyperthyroidism on the basis of pituitary TSH stimulation, but these patients are rare. They show no eye signs of Graves' disease and there is no other ancillary evidence which would suggest that their hyperthyroidism is due to Graves' disease. They are the rare examples of pituitary TSH induced hyperthyroidism.

Now, what about other thyroid stimulators which might cause the hyperthyroidism of Graves' disease. Adams and Purvis in 1956 described another type of thyroid stimulator which we should discuss.

<**Hall over animated illustration of mouse**>

The original bioassay using the guinea pig was modified by McKenzie in Montreal who used the mouse as the assay animal. In this assay, thyroxin is injected to suppress pituitary TSH release and radioactive iodine is given to label the thyroid. When TSH is injected intraperitoneally, radioactive materials, iodine and thyroid hormones are released from the thyroid and these can be detected in venous blood samples removed from the tail vein or other sites.

<**Hall over graph showing blood radioactivity**>

This shows the blood radioactivity expressed as the percentage of the initial value against time. When TSH is injected, the blood radioactivity peaks at about 3 hours. When serum from a patient with Graves' disease is injected, however, the blood radioactivity peaks between 12 and 24 hours, hence this serum contains a thyroid stimulator with a delayed and prolonged action on the thyroid. This was designated long-acting thyroid stimulator, or LATS.

<**Hall to camera**>

It thus seemed reasonable to postulate that the major cause of the hyperthyroidism of Graves' disease was due to this long-acting thyroid stimulator. However, over the next few years a number of objections arose to the LATS theory.

<**Hall over tables listing points against the LATS theory**>

LATS was undetectable in the serum of many patients with hyperthyroid Graves' disease. There was poor correlation of LATS levels with the clinical and biochemical severity of the hyperthyroidism. There was T_3 suppressibility in the presence of LATS, a finding which was really very strong evidence against LATS being a thyroid stimulator. And finally, LATS was detectable in some normal relatives of patients with Graves' disease and in some patients with Hashimoto's disease.

<**Hall to camera**>

So, over the next few years, the LATS theory fell into disrepute. To take the story further from here, I'll introduce Dr Bernard Rees Smith.

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<**Dr Bernard Rees Smith to camera**>

Soon after the discovery of LATS, several laboratories started to work on trying to characterise the LATS activity in the serum of patients with Graves' disease. It was quickly shown that LATS activity was associated with the immunoglobulin fraction of serum. The immunoglobulin fraction of serum, of course, is responsible for antibody activity and the possibility immediately arose that LATS was a thyroid-stimulating antibody, conveniently abbreviated to TSAb. More detailed studies show that LATS activity was associated with the IgG class of immunoglobulins which have a molecular weight of about 150,000.

At about the same time that LATS activity was shown to be associated with IgG, immunochemists were working out the actual structure of the IgG molecule.

<**Smith over diagram showing the structure of the IgG molecule**>

The IgG molecule looks like this. It consists of two heavy chains and two light chains linked by disulphide bridges and by noncovalent bonds. Immunochemists were able to show that the antigen binding part of this molecule was located in this region here, and this region here. And when people working on the structure of LATS applied these techniques to LATS activity, they were able to show that the thyroid-stimulating site of the molecule was also located in this part of the molecule and in this part of the molecule. That is, the thyroid-stimulating activity of LATS was located in the same part of the IgG molecule as the antigen-binding activity of classical IgG antibodies. And these type of studies provided very good evidence that LATS was a thyroid-stimulating autoantibody.

<**Smith to camera**>

Further work showed that thyroid-stimulating antibodies stimulate the thyroid by interacting with specific receptor sites on the thyroid cell surface.

<**Smith refers to large mobile diagram of thyroid cell surface, uses indication stick**>

If we represent the thyroid cell surface like this, and the specific receptor sites for TSAb, and here, the thyroid-stimulating antibodies come along and interact with these specific receptor sites. This interaction leads to activation of the enzyme adenyl cyclase, the adenyl cyclase converts ATP to cyclic AMP, and the increased levels of cyclic AMP mediate the effects of the antibody.

In summary then, thyroid-stimulating antibodies are antibodies to specific receptor sites on the thyroid cell surface. And the binding of the antibody to the receptor leads to cell stimulation.

<**Smith to camera** >

The antibodies, of course, are antibodies to a receptor site on the surface of human thyroid cells and do not necessarily cross-react with thyroid cells from other species. Consequently, studies of thyroid-stimulating antibodies using experimental animals can sometimes give misleading results.

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<**Hall to camera**>

To overcome the problem of they phylogenetic specificity of these thyroid stimulators, a variety of assays have been developed using human thyroid tissue. The first of these was the LATS protector assay described by Adams and Kennedy.

<**Hall over animated illustration of mouse**>

The principle of the LATS protector assay is as follows. LATS+^{ve} Graves' serum is injected into the McKenzie bioassay mouse and causes thyroid stimulation. LATS+^{ve} Graves' serum mixed with human thyroid membranes then has no effect on the mouse bioassay system. If the thyroid membranes are preincubated with LATS-^{ve} Graves' serum, then these are mixed with LATS+^{ve} Graves' serum, the effect of the LATS+^{ve} Graves' serum is again manifest and there is thyroid stimulation.

<**Hall to camera**>

Thus, the LATS-^{ve} Graves' serum contains some material which interfered with the binding of LATS to thyroid membranes. This material was designated LATS protector

by Adams and Kennedy. Subsequent studies have shown that LATS protector was, in fact, a true human thyroid stimulator. The various points of evidence which support this view are as follows.

<**Hall over table listing points in support of LATS protector**>

LATS protector is present in virtually all patients with hyperthyroid Graves' disease. The titre of LATS protector correlates with the severity of thyroid function tests. LATS protector has been shown to have biological activity in man, both in vivo and in vitro. And LATS protector appears to be inactive in monkeys, in other words it is a true, human thyroid stimulator.

<**Hall over series of tables depicting assays developed for human thyroid stimulators**>

Since then, a variety of assays have been developed for human thyroid stimulators. The first of these, as we have discussed previously, is the McKenzie bioassay, using the mouse thyroid for LATS. Then, the Adams and Kennedy bioassay or LATS protector. Shishiba in Japan described a human bioassay system in which he used human thyroid slices, the end point being the number of colloid droplets formed. He designated this material human thyroid stimulator. Chopra measured the increase in cyclic AMP formation in human thyroid slices, this material was termed human thyroid adenyl cyclase stimulator. Our own bioassay depends on the inhibition of label TSH binding to human thyroid membranes, the so-called radioreceptor assay for thyroid-stimulating antibodies.

<**Hall to camera**>

The term thyroid-stimulating antibodies can in fact be used as a generic term for all human thyroid stimulators. There have been few comparative studies of the different bioassay and other assay methods for the human thyroid stimulators. In addition, there is little information on the sensitivity of the different assays used. Dr Rees

Smith will now talk about our own experience with the human radioreceptor assay for thyroid-stimulating antibodies.

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<**Smith to camera, then refers to earlier large mobile diagram of thyroid cell surface, uses indication stick**>

If we consider the diagram of the thyroid cell surface, which we showed earlier, the surface of the cell is represented here, with receptors for thyroid-stimulating antibodies, shown here and here. Thyroid-stimulating hormone, conveniently abbreviated to TSH, also stimulates the thyroid by interacting with the receptor sites on the cell surface, shown here and here. The hormone binds to the receptor site; this activates the membrane enzyme adenyl cyclase, activation of the enzyme leads to increased levels of cyclic AMP, the increased levels of cyclic AMP mediate the effects of the hormone.

The interaction between TSH and its receptor can be studied directly by using hormone labelled with radioiodine. When thyroid-stimulating antibodies are added to this system, we find that they inhibit the binding of the labelled hormone to its receptor, indicating that the receptors for the hormone and the antibody are closely related. More detailed studies have shown that the two receptors are, in fact, identical.

The effects of the antibodies, the thyroid-stimulating antibodies, on the binding of labelled hormone to its receptor site can be used as a method of measuring the antibody. The assay method can be described as a competitive receptor binding assay in which the antibody and the labelled hormone compete for receptor sites on the thyroid cell surface.

<**Smith over series of graphs plotting thyroid stimulation**>

The type of data obtained with this system are shown here. Increasing doses of thyroid-stimulating antibodies inhibit the binding of labelled TSH to its receptor. When we use this assay to study thyroid-stimulating antibody activity in the sera of patients with Graves' disease, we obtain the following type of picture. Thyroid-stimulating activity is expressed as a TSAb index. A value high up on the scale here represents normal levels and a value down here, low on the scale, indicates high levels of TSAb. Here we have a group of normal subjects who have levels of TSAb in this range, and here we have a group of patients with Graves' disease, most of who have levels of TSAb outside the values found for the normal subject.

<**Hall to camera**>

You remember that levels of LATS did not correlate well with indices of thyroid function. How then do levels of TSAb, measured by the receptor assay, correlate with other indices of thyroid function? The next diagram shows this correlation.

<**Hall over diagram**>

Here, TSAb indices are shown on the y axis, increasing values being recorded downwards. These are plotted against thyroidal I131 uptake at 1 hour in patients with untreated Graves' disease and hyperthyroidism. As you will see, there is a significant correlation, and this is also statistically significant, the p value of less than 0.001.

<**Hall to camera**>

Although thyroid-stimulating antibodies can be detected in the majority of patients with hyperthyroid Graves' disease, they are also present in other groups of patients. They can be detected in patients with ophthalmic Graves' disease who are euthyroid or even hypothyroid, in patients with thyroid cancer and in some patients with Hashimoto's disease. Why then are these patients not hyperthyroid?

In some instances the hyperthyroidism is prevented by the presence of extensive autoimmune thyroid disease, though from thyroid antibody studies, this explanation is

not valid in some patients. Evidence has accumulated for the presence of biologically inactive thyroid-stimulating antibodies in patients particularly who show a suppressibility of the thyroidal iodine uptake by triiodothyronine. What happens then to the control of thyroid function in patients with thyroid-stimulating antibodies? The next diagram shows normal control of thyroid function.

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<**Hall over diagram showing normal thyroid function, then thyroid function in a patient with thyroid-stimulating antibodies**>

Thyrotropin-releasing hormone, used in the hypothalamus, acts on the pituitary to enhance the production of thyroid-stimulating hormone. This increases thyroidal iodine uptake and hence, thyroid hormones are produced which act on the tissues and, in a negative feedback way, on the pituitary and on the hypothalamus. What is the situation in the patient with thyroid-stimulating antibodies?

Here, thyroid-stimulating antibodies act on the thyroid, these increase thyroidal iodine uptake, there is increased formation of thyroidal hormones – at a tissue level this causes clinical hyperthyroidism, and at a hypothalamic pituitary level, it causes inhibition of pituitary TSH secretion.

<**Hall to camera**>

A number of clinical applications are now emerging for the receptor assay for thyroid stimulating antibodies.

<**Hall over tables and graphs listing the link between high levels of thyroid-stimulating antibodies and Graves' disease**>

If we have a hyperthyroid patient who has no eye signs of Graves' disease, the finding of thyroid-stimulating antibodies is a useful indication that this patient has, in fact, Graves' disease. If we have a patient with the eye signs of Graves' disease

without hyperthyroidism, ophthalmic Graves' disease, where often the eye signs are unilateral and the differential diagnosis is ophthalmic Graves' disease or orbital tumour, then again the presence of thyroid-stimulating antibodies indicates that the patient has Graves' disease.

Further indications for the use of receptor measurements of thyroid-stimulating antibodies is a pregnant woman with Graves' disease. If a pregnant woman with Graves' disease has highly positive levels of thyroid-stimulating antibodies, her child is at risk for the development of neonatal hyperthyroidism.

We now have evidence to support the view that patients who have high levels of thyroid-stimulating antibodies, at the end of a course of anti-thyroid drugs, are at risk for relapse of their hyperthyroidism. This diagram shows some of the results of these studies, carried out by Dr Davies and ourselves in Newcastle.

Thyroid-stimulating antibodies are measured on the y axis. A group of patients who remained euthyroid two months after the termination of a six-month course of anti-thyroid drugs are shown here. As you will see, the thyroid-stimulating antibodies were low or absent. In a group of patients who relapsed within two months after the termination of a six-month course of anti-thyroid drugs, the thyroid-stimulating antibodies were significantly higher. That is, it is possible, with further studies, to separate out patients who are at risk to relapse after a course of anti-thyroid drugs.

<**Hall to camera, and over tables listing other thyroid autoimmune diseases**>

It is worthwhile placing Graves' disease in the spectrum of the organ-specific thyroid autoimmune diseases. These include: Hashimoto's disease, myxoedema and lymphocytic thyroiditis. These diseases are characterised by the presence of circulating thyroid antibodies and by a variable degree of lymphocytic infiltration of the thyroid.

The thyroid organ-specific autoimmune diseases are only one of the group of other organ-specific autoimmune diseases which include: Addison's disease affecting the

adrenal cortex, pernicious anaemia due to gastric autoimmunity and premature ovarian failure, often associated with Addison's disease.

These diseases are characterised by greater frequency in females by a familial aggregation and by a seriological and clinical overlap in the patient and their families. A number of other conditions, not necessarily autoimmune, are associated with the organ-specific autoimmune diseases. These include: vitiligo and premature greying of the hair, allergic alveolitis, Sjögren's syndrome and chronic hepatitis. There are a number of other minor associations such as renal tubular acidosis, which may or may not be significant.

<**Hall to camera**>

Although we have described the presence of thyroid-stimulating antibodies in Graves' disease, and suggested that they are responsible for the goitre and hyperthyroidism in these patients, there seems little doubt that the underlying abnormality must be some defect of the immune control mechanism. In particular, the T lymphocyte system, and this is under intensive study at the present time.

Some interesting facts have emerged from studies of the histocompatibility system. In Caucasian patients with hyperthyroid Graves' disease, there seems to be an increased frequency of HLA-b8. In the Japanese, however, the HLA type BW-35 is more frequent in patients with Graves' disease. It's now considered that genes which code for these particular HLA antigens may be associated with certain immune-response genes which control the production of cell populations of suppressor thymic lymphocytes. If there is deficiency of suppressor thymic lymphocytes, then B lymphocytes may be proliferating and producing the effector thyroid-stimulating antibodies.

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<**Hall over tables summarising main points of lecture, camera returns intermittently to him**>

So, in summary, in Graves' disease, antibodies react with the TSH receptor and cause thyroid stimulation.

We might then ask whether this interaction between the thyroid and the immune system is unique. However, this is not the case; in myasthenia gravis antibodies react with the acetyl choline receptor and result in the muscular paralysis which is seen in this condition. Also, in diabetes mellitus with acanthosis nigricans, a very rare variant of the condition, antibodies have been described which react with the insulin receptor and this results in extreme insulin resistance.

Thus, we now have three types of disease in which there is an interaction between the immune system and hormone receptors, resulting in some disease process. In Graves' disease, an antibody reacts with the receptor to cause hyperthyroidism. In myasthenia gravis an antibody reacts with the acetyl choline receptor to cause muscular paralysis. In the rare form of diabetes mellitus with acanthosis nigricans, an antibody reacts with the insulin receptor to cause extreme insulin resistance.

I think there is little doubt that in the future we shall see other types of this interaction between the humeral immune system and hormone receptors causing a variety of diseases.

<**End credits**