THYROID ANTIBODIES AND THYROIDITIS

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When patients with Hashimoto's thyroiditis were discovered to have a serum antibody to thyroid tissue[41] the study of thyroid disease gained a new perspective. Four years later, many of the questions raised are still unanswered, including the exact role of auto-immunity in the pathogenesis of thyroid disorders. Nevertheless, large contributions to clinical and pathological understanding have been made, both by the original workers at the Middlesex Hospital and by others who have confirmed and extended their observations. Several authors have reviewed this new body of knowledge, the most authoritative as well as the most recent survey being that of Roitt and Doniach.[40] The account which follows has been written with the needs of general physicians and surgeons in mind; for detailed information and a comprehensive review of the literature, the reader is referred to the earlier paper.

Auto-immunity

Auto-immunity is the formation by the organism of antibody to one of its own normal constituents. ('Immunity' in this usage strays far from its etymology, since auto-immunity can almost certainly be harmful.)

For a long time it was denied that auto-immunity was concerned in the causation of human disease or, indeed, that it occurred at all. Not only did the principle seem contrary to nature, but there was sound experimental evidence to indicate that each organism, near the beginning of its life, acquired immunological tolerance to its potentially antigenic* components (see Medawar, 1957). Thereafter, only when an alien substance enters the internal environment do immune defences come into play.

*Antigens, which are usually protein in nature, are substances capable of stimulating the formation of specific antibodies to themselves. A normal body constituent with this property is termed an auto-antigen.

Moreover, true auto-antibodies (as distinct from those to body proteins altered by, or combined with exogenous agents) could not be satisfactorily demonstrated in the serum of patients. Their frequently-asserted association with diseases of unknown aetiology remained, as in most instances it still does, largely speculative.

Auto-antibodies to the Thyroid Gland

Hashimoto's thyroiditis (synonyms: lymphadenoid goitre, struma lymphomatosa) is a condition occurring almost exclusively in older women, characterized by a hard goitre and a variable but often progressive tendency to hypothyroidism.

The essential clinical and pathological features were described some time ago,[25] but only recently was it recognized that the plasma protein pattern is often abnormal, notably in respect of an increased γ-globulin concentration.[9, 19a, 29a] Antibody activity is known to reside in the γ-globulin fraction of the blood protein, and this clue led Roitt and Doniach to suspect a long-continued auto-immune reaction.[41] They confirmed their hypothesis by allowing serum from a patient to react with an extract of human thyroid gland; a visible precipitate resulted, indicating the presence of antibody to a substance in the extract.[41, 13]

This was the first proof of an association between auto-immunity and a naturally occurring disease process. Experimental substantiation was already available, since it had a short while previously been shown that rabbits could be artificially immunized against their own thyroid tissue.[43, 52] Animals so treated developed histological changes in the portion of the gland left *in situ* resembling human thyroiditis, and thyroid antibody appeared in the blood. Final proof of the true auto-immune nature of the human antibody was obtained when a patient's serum

gave the specific reaction with an extract of her own thyroid gland.[53]

It followed that the antigen (or antigens) concerned was failing to conform to the law of immunological self-tolerance. To explain this, it was postulated that the substance was one which under normal conditions had no effective contact with extracellular fluid and hence with the immunity mechanism. Very probably it is the basement membrane attached to thyroid follicular epithelium which provides the insulating barrier, since its continuity has been shown to be deficient when thyroid antibody is present in the serum.[47]

The Auto-antigens

At least two thyroid gland components are potentially antigenic. One has been identified with reasonable certainty as thyroglobulin.[37, 53] The active thyroid hormones form part of this protein aggregate, histologically familiar as 'colloid', and are stored therein within the follicles until released by enzymatic breakdown.

Studies with fluorescein-labelled antibody suggest that thyroglobulin does not escape from the follicles unless there is destruction or disorganization of thyroid tissue.[50] Nor can it be demonstrated in the blood stream except after acute thyroid injury, e.g. by large doses of radioiodine.[36]

Another antibody is often present in the serum of patients with thyroid disease, distinguished from that to thyroglobulin by its property of utilizing complement on reacting with thyroid extract.[48] Differential centrifugation of such an extract readily separates thyroglobulin from the complement-fixing antigen, concentrating the latter in the 'microsome' fraction.[38, 4] The antibody-labelling technique has confirmed that the second antigen is normally intracellular, distributed diffusely throughout the cytoplasm of the thyroid epithelium after the manner of microsomes.[26] Though it has not yet been characterized, it seems to be related to glandular activity, being difficult to identify in normal thyroid tissue but present in abundance in goitres removed from thyrotoxic patients.[48, 3, 2, 42]

The possibility that other antigen-antibody systems are also involved in thyroid auto-immunity is by no means excluded.

Tests for Thyroid Auto-antibodies

The first demonstration of thyroid antibody depended on formation of a visible precipitate when the serum was brought into contact with a saline extract of thyroid tissue.[41] Precipitation-diffusion in agar gel[31, 32] is a convenient technique for the purpose and, with minor modifications, has been widely employed.[13, 21] The precipitating antibody was shown to be that directed against thyroglobulin.[13]

The precipitation test is simple to perform, but its practical value is limited by lack of sensitivity: a positive reaction requires a concentration of antibody protein great enough to yield visible precipitate on combination with antigen, and the level of circulating antibody is often below this threshold. An equally specific but much more sensitive method has been provided by the tanned-cell agglutination principle of Boyden.[5] This depends on the surface adsorption of soluble proteins by erythrocytes treated with dilute tannic acid and their subsequent agglutination in the presence of antibody to the particular protein adsorbed. Either semi-purified thyroglobulin[11] or the high-speed supernate of a thyroid extract is used to sensitize the tanned cells, a suspension of which is then added to serial dilutions of the serum to be tested.[54, 38, 34] By this method, antibody can be detected in a concentration some 40,000 times less than that required for a precipitin reaction.[34] By making quantitative studies possible, the technique has made a large contribution to thyroid immunology. An analogous method employing bentonite particles in place of red cells has been successfully used,[1] as have various other indirect means of demonstrating the thyroglobulin antibody,[39, 10, 19] including skin sensitivity to thyroid extract.[7]

Standard complement-fixation methods are satisfactory for detecting the CF antibody provided the antigen extract is prepared from thyrotoxic tissue and is not subjected to centrifugation at high speeds.[48] Results can be made semi-quantitative by testing serial dilutions of serum.

Site of Formation of Thyroid Antibodies

Indirect evidence suggests that the auto-antibodies are produced in and around the thyroid gland itself, and that their level in the circulation is the passive reflection of spill-over from this site. Thus auto-immunity is strongly correlated with the histological presence of lymphocytes and plasma cells, the antibody-producing function of which is well established.[8] In Hashimoto's disease, there is often enlargement of regional lymph nodes. The relationship holds not only in active thyroiditis but also in other thyroid diseases,[38] and probably in patients without overt thyroid disease who are found to have circulating antibody.[20] Round-celled infiltration is sometimes present when serology is negative, but this might be expected from variation in local balance between antibody and antigen; even in histologically active Hashimoto's disease serum antibodies cannot always be demonstrated, nor were they found in every rabbit with experimental

auto-immune thyroiditis.[43] In both thyrotoxic and nodular goitres, Schade *et al.*[45] found a highly significant association between lymphadenoid foci in the gland and serum antibody to thyroglobulin. (There was much less correlation with the CF antibody, which seemed to be related chiefly to the presence of thyrotoxicosis and which was rare in simple nodular goitre.)

Further evidence in favour of thyroglobulin antibody production being mainly or wholly local is the sharp fall in titre observed after subtotal thyroidectomy.[13, 34] Moreover, passive transfer experiments in the monkey indicate that the mere presence of antibody in the circulation does not affect the intact thyroid gland,[38] nor do thyroid cells in tissue culture appear to be damaged by addition of thyroglobulin antibody to the medium.[35]

Possible Causes of Thyroid Auto-immunity

It would seem indisputable that some form of thyroid injury must precede the development of auto-immunity. But only rarely is the nature of this primary lesion obvious, and in such cases the antibody level has usually been low, e.g. in thyroid carcinoma.[46] The possibility of occult virus infection has been much considered, but, though auto-antibodies have been detected in the blood after thyroiditis complicating mumps, their appearance has been evanescent and progressive auto-immunity of the Hashimoto type has apparently not resulted.[18] Similarly, in de Quervain's thyroiditis, a subacute form in which virus infection has been implicated,[17] antibodies are absent or of low titre only.[12] If the infective theory is true, presumably the thyroid insult needs to be of long duration or repetitive, like an artificial immunization schedule, or else it must be postulated that some individuals have an abnormal liability to develop a chain-reaction between antibodies and thyroid tissue. A self-perpetuating mechanism of the latter sort, which might ultimately lead to complete thyroid destruction, was originally suggested by Doniach and Roitt[13] as the explanation of progressive auto-immunity, but their view has been modified in the light of the knowledge that auto-immunity may sometimes be a limited and temporary process.[38] It nevertheless remains true that not a few cases are encountered clinically with high antibody levels where an infective aetiology seems possible, in which the onset is acute or subacute with pain and tenderness in the neck and is followed by the rapid development of myxoedema over the course of a few months. Other antigen-antibody systems than those so far identified may be relevant to the cytotoxic process; in particular the significance needs to be assessed of the 'cytotoxic factor' in Hashimoto sera shown by Pulvertaft *et al.*[35] to inhibit the early growth of thyroid cells in tissue culture.

An alternative suggestion is that hyperthyroidism comes first, overstimulation of the gland by pituitary thyrotropic secretion being responsible for antigen release and the subsequent immune response. (An abnormal iodine-containing protein has been detected in the serum of some patients with Hashimoto's disease.[33]) This would explain the occurrence of toxic symptoms at the onset of Hashimoto's disease,[49] the frequent presence of auto-antibody in thyrotoxicosis,[38] and the fact that without treatment about 10% of thyrotoxic patients eventually become myxoedematous.[16] According to this idea, primary hyperthyroidism is sometimes — especially in females—modified by excessive antibody response into the syndrome of chronic thyroiditis, a reaction which might teleologically be regarded as a crude attempt at self-cure. Students of thyroid histology have more than once called attention to the occurrence of transitional patterns between classical thyrotoxicosis and lymphadenoid goitre.[22]

A third possibility, not necessarily inconsistent with either of the foregoing, is that thyroid auto-immunity, or the predisposition towards it, is determined genetically. Familial examples of classical lymphadenoid goitre are rare, but they undoubtedly occur;[15] the present authors recently observed the condition developing at different times in two sisters, in one of whom it took the rapidly progressive, subacute form initiated by pain and tenderness in the thyroid region. Moreover, disorders of thyroid function generally are well-known to run in families[28] which may display instances of clinically opposite syndromes, e.g. thyrotoxicosis and myxoedema.[44] How fundamental is the association of auto-immunity with these cases has yet to be determined. But evidence accumulates that such an association exists. Thus Hall *et al.*[24] recently examined sera from 38 siblings of eight propositi with auto-immune thyroid disease and high antibody levels; though only a small minority of the sibs had clinically apparent thyroid disturbance, one-half proved to have thyroid antibodies in the circulation, in many instances at higher titre than the 5% of 'false positives' found in control studies on hospital patients. This proportion strongly suggests that the development of auto-immunity depends on a genetically controlled factor, the nature of which is still unclear.

The Concept of Auto-immune Thyroiditis

Almost all patients with Hashimoto's disease have thyroid antibodies in their serum, though there are occasional exceptions. Antibody to thyroglobulin, which can be estimated quantita-

tively, is present in about 85%, in very high concentration in the great majority of recent, untreated cases.[38, 34] A high anti-thyroglobulin titre—or a positive precipitin test—in a patient with an unexplained goitre is presumptive evidence of Hashimoto's disease or a clinical variant of it, such as the subacute form. (Though, since thyroid carcinoma has been known to arise against a background of pre-existing chronic thyroiditis,[29] the necessity for adequate biopsy where carcinoma is suspected is not obviated; the authors have recently encountered such an association in a woman whose family displayed a high incidence of auto-immune thyroid disease.) It seems justifiable as well as convenient to group these conditions under the term 'auto-immune thyroiditis',[38, 12] provided this is not taken to imply that auto-immunity is necessarily the sole factor in their pathogenesis.[6]

There is little doubt that non-goitrous or 'spontaneous' myxoedema must be included under the same head. It was soon apparent that precipitating thyroid antibody was not specific to Hashimoto's disease but was occasionally also present in ordinary myxoedema.[13, 21, 48] This finding was amplified by later studies with the tanned-cell method, from which it appeared that thyroglobulin antibody was present little if any less frequently than in thyroiditis but was usually in much lower concentration; the levels closely approximated those found in patients with surgically treated and inactive Hashimoto's disease.[34] These results, which were confirmed by skin-sensitivity tests with intradermal thyroid extract,[7] have lent strong support to the hypothesis that myxoedema is the end-result of thyroid auto-immunity.[13] Histological examination of the thyroid gland remnant in myxoedema has shown changes resembling those of chronic thyroiditis[3, 14] and favours such a basic unity between the two conditions. In myxoedema the phase of thyroid enlargement either does not occur, perhaps because destruction of the gland outstrips compensatory enlargement, or else passes unnoticed by the patient. Intermediate syndromes, in which the gland is small, but hard and readily palpable, are now being recognized with increasing frequency.[12, 27]

The relationship of thyrotoxicosis to these conditions is *sub judice*. The evidence that thyroid over-activity precedes auto-immune thyroiditis is at least highly suggestive. But it is also clear that as a rule patients who present with clinical hyperthyroidism do not later develop Hashimoto's disease or myxoedema, despite the fact that between one and two-thirds can be shown to possess auto-antibodies in the blood.[38, 34a] As Roitt and Doniach point out, auto-immunity in such cases remains low-grade and non-progressive, as do the associated lymphadenoid changes in the gland. Nevertheless, in patients operated upon for thyrotoxicosis a relationship exists between histological lymphoid infiltration of the surgical specimen and incidence of post-operative myxoedema.[51, 22, 23] The finding of thyroid antibody in thyrotoxicosis should probably therefore be accorded clinical significance, at least in deciding the most appropriate form of treatment. Evidence in support of this view was obtained by the present authors when they reviewed a series of patients with post-operative myxoedema and found that 80% had circulating thyroid antibody, a significantly greater proportion than in control thyrotoxic patients whether treated or untreated.[34a]

Summary and Conclusions

The subject of auto-immunity in relation to thyroiditis is reviewed. At least two antigens in thyroid tissue are concerned, one being thyroglobulin and the other an unidentified intracellular constituent associated with the microsomes. The auto-antibodies to these antigens are probably formed by lymphoid cells in and around the thyroid gland itself. The cause of thyroid auto-immunity is unknown in the vast majority of cases, but there is increasing evidence that a genetic factor may play an important part. The term 'auto-immune thyroiditis' can be justifiably used to describe Hashimoto's disease and allied syndromes, including primary myxoedema, since thyroid antibodies are present almost constantly in these conditions and may well be of importance in their pathogenesis.

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