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Autoimmune thyroid disease: propagation and progression

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Abstract

Autoimmune thyroid disease is the archetype for organ-specific autoimmune disorders. Progress in treating these disorders lies in improvements of our understanding of the predisposing factors responsible, the mechanisms responsible for progression of disease, and the interaction between thyroid antigens and the immune system at the level of the T cell and antibody. In common with other autoimmune diseases, genetic, environmental and endogenous factors are required in an appropriate combination to initiate thyroid autoimmunity. At present the only genetic factors which have been confirmed lie in the HLA complex and *CTLA-4* or a closely linked gene. Identifying other predisposing genes will require large-scale family studies, or further insights into likely candidate genes. A number of environmental factors are known to predispose to autoimmune thyroid disease, including smoking, stress and iodine intake, while immunomodulatory treatments are revealing new pathways for disease emergence.

The thyroid cell itself appears to play a major role in disease progression, interacting with the immune system through expression of a number of immunologically active molecules including HLA class I and II, adhesion molecules, cytokines, CD40 and complement regulatory proteins. New techniques, in particular phage display libraries, are providing the methods with which to identify autoantibody diversity in autoimmune thyroid disease and to provide tools for mapping autoantigenic epitopes. Application of these techniques is likely to lead to an understanding of how TSH receptor antibodies interact with the receptor to cause Graves' disease and also to the identification of novel orbital autoantigens in thyroid-associated ophthalmopathy.

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Introduction

It is now 46 years since the original delineation of thyroid autoimmunity, first in rabbits immunised with thyroglobulin in adjuvant (1), and then in man, with the description of antibodies to thyroglobulin in the serum of patients with Hashimoto's thyroiditis (2). In the same *annus mirabilis*, the abnormal thyroid stimulator which turned out to be the cause of Graves' disease was also identified (3). As then, there are four important questions which have only partially been answered over the intervening period: (i) why does autoimmune thyroid disease (AITD) start; (ii) why does it progress; (iii) what is the nature of the interaction between thyroid autoantigens and the immune system; and (iv) how can these disorders be better treated? The answer to the last depends on clear answers being obtained to the first three questions, which is why treatment has not progressed greatly over the last 60 years, most tellingly in the case of thyroid-associated ophthalmopathy (TAO).

This paper is based on the Merck Lecture given at the European Thyroid Association meeting in Göteborg

2002, and as a result is biased largely to the work from my own group. Nonetheless, it may provide some insight into the way these four questions have been and continue to be addressed. Extensive historical reviews of the subject can be found elsewhere (4–6).

Predisposition

In common with probably all autoimmune disorders, the interplay of genetic, environmental and endogenous factors is required in the right combination to initiate thyroid autoimmunity. Combinations will vary between individuals, and some factors are shared by all AITDs, while others are unique to the individual disorders. In very general terms predisposing factors operate by disturbing immunological tolerance (Fig. 1), although these processes are far more easily followed in animal models than in man (7). Endogenous factors are often overlooked, but pregnancy is an important risk factor for Graves' disease and autoimmune hypothyroidism, as well as postpartum thyroiditis, and stochastic events during the gene

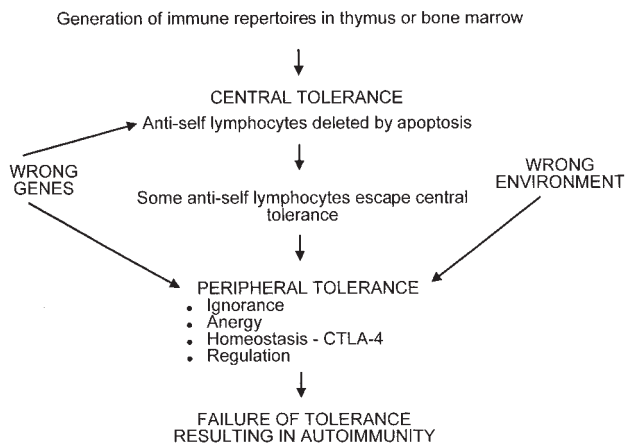


Figure 1 Interaction of genes and environment on the development of autoimmune disease.

rearrangements that create the repertoire of antigen-recognition molecules, namely antibodies and T cell receptors, may also have a crucial role in ensuring that autoreactive lymphocytes are or are not available to cause tissue injury.

Genetic factors

The evidence for a role for genetic susceptibility in AITD is compelling. Original clinical observations such as the presence of thyroid autoantibodies in half of the siblings of probands with autoimmune hypothyroidism (8) were followed by the identification of loci within the murine major histocompatibility complex (MHC) which were associated with a good or poor response to thyroglobulin immunisation (9). MHC class II alleles were later associated with Graves' disease (10) and a huge number of studies since have confirmed the

involvement of these HLA-D region genes in susceptibility. However, the effects are modest, not least as judged by the difficulty in confirming linkage rather than simple association (11), susceptibility varies between ethnic groups, and the major MHC specificity involved in Graves' disease in Caucasians, HLA-DR3, is also a risk factor for many other autoimmune diseases. Concordance studies in twins have shown a much bigger genetic effect than is conferred by the HLA alone (12).

All these observations imply the existence of other non-MHC loci, yet so far only polymorphisms of the *CTLA-4* gene have been confirmed as additional susceptibility markers. *CTLA-4* is a key immunomodulatory molecule expressed on T cells, capable of inducing anergy and terminating a T cell response when triggered by costimulatory molecules (Fig. 2). It is, therefore, a logical candidate for playing a role in autoimmunity, although there are genes encoding other immunoregulatory molecules in linkage disequilibrium with *CTLA-4*, so caution is still needed in ascribing any effect directly to this gene alone. Initially *CTLA-4* polymorphisms were associated with Graves' disease (13) but subsequently it became clear that the same effect was seen in autoimmune hypothyroidism (14) as well as in other related disorders including type 1 diabetes mellitus, Addison's disease and vitiligo. At present, therefore, we know of only two rather non-specific genetic loci in AITD that may operate by having generally enhancing immunological effects and we know of nothing that would explain tissue specificity.

As an alternative to the approach of examining the many other candidate thyroid and immunological genes that exist, recent endeavours have focused on genome-wide scans to identify additional loci which may shed new light on pathogenesis. In Caucasians,

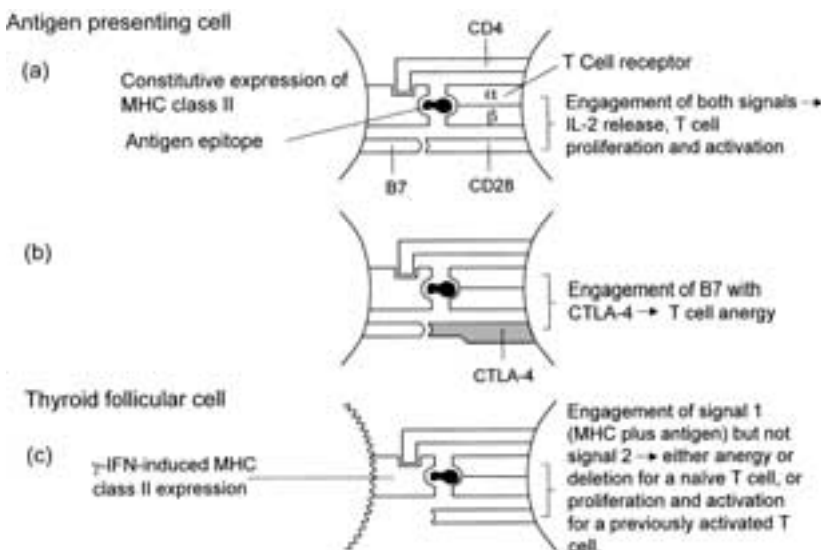


Figure 2 Alternative outcomes after antigen presentation: (a) normal presentation by a 'professional' antigen presenting cell, such as a dendritic cell, causing T cell stimulation; (b) engagement of B7 on the antigen presenting cell with CTLA-4 on the T cell leads to anergy; (c) the same outcome of anergy results if a non-professional antigen presenting cell, such as a thyrocyte, fails to provide costimulation via B7.

several loci have been identified by this approach. To summarise a large number of studies, the following chromosomal locations have been linked to Graves' disease: 14q31, 18q21, 20q11, Xp11 and Xq21, while 6p, 13q32 and 12q22 have been linked to Hashimoto's thyroiditis and AITD (15–18). It is apparent that relatively small numbers of families have been tested so far, and previous genome scan results in other diseases such as type 1 diabetes mellitus have shown the need to undertake studies of appropriate size in order to have sufficient power to avoid type 1 or type 2 statistical errors.

A thyroid genetics consortium was established in collaboration with Oxagen, Abingdon, UK in 1996, with five centres in the UK, two in continental Europe and two in Australasia, the aim being to collect 600 Caucasian sibling pairs affected by AITD. Such a sample size was judged necessary to achieve adequate statistical power based on a model of five interacting loci and a conservative λ_s (a measure of sibling risk) of five in AITD. The collection has just been completed, involving 558 families, totalling 871 sibling pairs, together with 935 unaffected relatives, and the genome analysis was conducted using 395 linked markers at 10 cM intervals. Initially we have focused on the previously reported loci (15–18) and none gave a maximum lod score of greater than 1.5, which was judged to be significant in this analysis. Of the two other known loci in AITD, namely MHC and 2q33 containing *CTLA-4*, only the latter showed linkage in the patients with Graves' disease (Thyroid Genetics Consortium, manuscript in preparation).

It should be borne in mind that linkage analysis is only suitable for detecting relatively large genetic effects, whereas association studies may pick up minor but significant effects, including those that may be less than 10% of the entire genetic contribution to disease. Whilst we hope that novel loci will be revealed by our continuing analysis of the remainder of the genome in these families, it already seems likely that there is no single novel gene conferring a large proportion of the genetic susceptibility to AITD, and that therefore a relatively large number of genes may contribute with effects similar to or less than the MHC and *CTLA-4*. An outstanding remaining question is whether Graves' disease and autoimmune hypothyroidism are genetically distinct; if so, an understanding of the basis for this distinction would be an important step in further understanding disease pathogenesis. The clinical course of patients who fluctuate between the two disorders, familial clustering and the late occurrence of spontaneous hypothyroidism in a proportion of Graves' patients treated with antithyroid drugs have previously suggested a partially shared pathogenesis, but it is increasingly striking how, apart from *CTLA-4*, there is a genetic separation between the two disorders (16, 19).

Environmental factors

As reviewed elsewhere, excess iodine intake is a risk factor for both Graves' disease and autoimmune hypothyroidism, as well as animal models of AITD (6, 20). Allowing for the biochemical Jod–Basedow and Wolff–Chaikoff effects, there is probably an additional, immunological basis to this adverse effect, which may lie at the level of autoantigen release or altered immunogenicity of thyroid antigens. Stress and drugs, especially agents such as α -interferon (α -IFN), may precipitate AITD in predisposed individuals through their effects on immunoregulatory mechanisms (6, 21). Smoking is a minor risk factor for Graves' disease and a major risk factor for TAO for reasons which remain unclear; one explanation for the adverse effect of smoking in TAO is that partial hypoxia increases the synthesis of glycosaminoglycans by retrobulbar fibroblasts, thereby exacerbating the extraocular muscle oedema and swelling which mechanically lies at the heart of the disease process (22).

Perhaps the most striking example of a disturbance in immunoregulation causing AITD is the emergence of Graves' disease in a third of patients with multiple sclerosis treated with the humanised anti-CD52 monoclonal antibody Campath-1H (23). Unlike the precipitation of disease caused by α -IFN, this effect does not seem to be due to an exacerbation of subclinical thyroid disease, nor is there an underlying association between multiple sclerosis and thyroid autoimmunity, based on a recent large survey of almost 200 multiple sclerosis patients (R Roxburgh *et al.*, unpublished observations). One explanation for this adverse effect of anti-CD52 treatment is that the lymphocytes which reappear after depletion are biased away from a Th1 and towards a Th2 phenotype, leading to suppression of the autoimmune response in multiple sclerosis, but permitting the development of the antibody-mediated Graves' disease.

An alternative reason might be some effect of the monoclonal antibody on CD4⁺ CD25⁺ regulatory T cells, akin to the effects of thymectomy and sublethal irradiation, which cause specific autoimmune disorders such as thyroiditis and diabetes in genetically susceptible rats (24). Little, so far, is known about the role of these cells in AITD, although one can confidently predict a rapid change in this situation as their importance in peripheral tolerance is increasingly established (25). However, abnormalities in this T cell subset seem unlikely to account for previous observations of a T suppressor cell defect in AITD based on allogeneic cultures (26), as CD4⁺ CD25⁺ T regulatory cells require MHC-restricted specific antigen presentation for their activation (although they are then non-specific in their effects), and certainly would not correspond to the proposal that the suppressor defect is in the CD8⁺ compartment (27). Understanding how Graves' disease arises after Campath-1H treatment, or indeed how

spontaneous remission occurs in some patients with AITD, especially postpartum thyroiditis, should give crucial cues for the development of new treatments aimed at restoring normal immunoregulation.

How the autoimmune process progresses

It is a commonplace observation that thyroid autoantibodies and focal thyroiditis occur with much greater frequency than the full-blown autoimmune diseases of hypothyroidism and Graves' disease. At least one explanation for the progression of the autoimmune process is the involvement of thyroid cells themselves in this chain reaction. Rather than being the innocent victims of an unchecked and disordered immune system, it is increasingly obvious that the target cells interact with the immune system, often in ways that teleologically would seem to be defensive and protective, and yet which go awry and exacerbate autoimmunity under particular circumstances. Since multiple steps in this interaction exist, the potential for genetic and environmental factors to operate increases, leading to the diversity seen in the rates of disease progression.

The first inkling of this complicity came with the demonstration of MHC class II expression, in the form of HLA-DR molecules, by thyroid cells from patients with AITD but not in healthy controls (28). In a separate paper, Bottazzo and colleagues described the potential that this phenomenon created, whereby thyroid cells could become thyroid autoantigen presenting cells, capable of initiating or perpetuating AITD (29). It quickly became apparent that the only stimulus able to induce MHC class II expression on thyroid cells was the T cell cytokine, γ -IFN, that normal thyroid cells responded exactly as did AITD thyroid cells to this

stimulus, and that, in animal models of AITD, class II expression by thyroid cells always followed the appearance of lymphocytic infiltration of the gland (30–32). Class II expression did not, therefore, initiate the process but could it exacerbate AITD by enhancing antigen presentation? Moreover, could thyroid cells stimulate naïve $CD4^+$ T cells that required costimulatory signals for their activation (Fig. 2)?

We have been unable to detect expression of the main costimulatory molecule, B7 (CD80), on thyroid cells *in vivo*, or *in vitro* after stimulation with multiple cytokines (33). Thyroid cells can present peptide antigens to T cell clones that are not B7-dependent but cannot do so when there is a requirement for B7-mediated costimulation (34). In the latter case, the presentation of antigen by thyroid cells without B7 results in T cell anergy, which is mediated by both Fas-dependent and -independent pathways (35). Thus, thyroid cells induce peripheral tolerance in naïve T cells by MHC class II expression, but this defence mechanism backfires when thyroid autoimmunity is initiated by professional antigen presenting cells capable of supplying costimulation (Fig. 3). Under such circumstances, class II expression becomes a liability, leading to disease exacerbation by increasing antigen presentation.

At the same time as inducing MHC class II expression, γ -IFN increases MHC class I expression by thyroid cells, with the potential to increase their susceptibility to recognition by cytotoxic $CD8^+$ T cells, believed to be responsible for much of the tissue destruction in autoimmune hypothyroidism. Perhaps more significantly, thyroid cell expression of intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-3 (LFA-3) is enhanced by γ -IFN, tumour necrosis factor (TNF) and interleukin-1 (IL-1), which can be shown to have a direct enhancing effect on

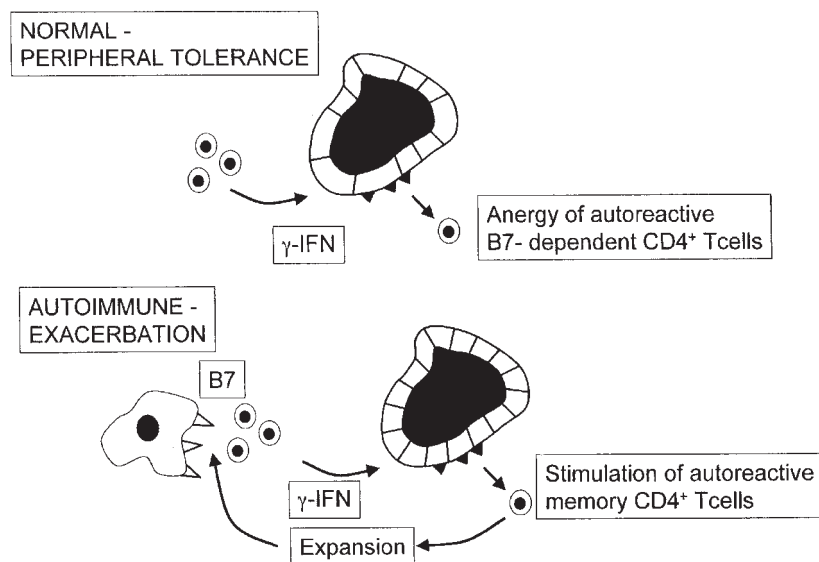


Figure 3 Effect of class MHC II expression on thyrocytes. In the upper panel, transient expression of class II may serve to induce peripheral tolerance in potentially autoreactive T cells, for example during viral thyroiditis. In the lower panel, previously activated T cells, no longer requiring a costimulatory signal, will proliferate in response to class II-positive thyrocytes presenting thyroid autoantigens, thereby perpetuating the autoimmune process.

both cytotoxicity and other interactions with lymphocytes, as both molecules are important in the binding of lymphocytes to targets (36, 37). Others have shown that thyroid cells express CD44 which acts as a homing receptor for hyaluronan, mediates leukocyte rolling, the first step in tissue homing, and may (like ICAM-1) induce lymphocyte activation under certain circumstances; expression of CD44 by thyrocytes is presumably also altered by cytokines as lymphocytes can upregulate expression (38).

Another pathway by which lymphocytes may be activated in AITD is through their CD40 ligand (CD154) binding to CD40 expressed on thyroid cells (39). Again, CD40 is upregulated by the cytokines γ -IFN, IL-1 and TNF, known to be produced by the thyroid lymphocytic infiltrate, and ligation of CD40 on thyroid cells may have additional direct effects including the release of cytokines such as IL-1. Many cytokines are now known to be produced by thyroid cells especially after stimulation with IL-1, including IL-1, IL-6, IL-8, IL-12, IL-13 and IL-15 (40–42); recently we have also found expression of IL-17 and IL-18 mRNA by thyroid cells stimulated with IL-1, γ -IFN or TNF (R Ajjan *et al.*, unpublished observations).

Thyroid cells, like most nucleated cells, are relatively resistant to the lytic effects of complement, resulting from the expression of a number of inhibitory proteins which prevent the assembly of complement membrane attack complexes. The most important of these proteins is CD59, which shows increased expression in AITD secondary to cytokine stimulation of thyrocytes (43–45). Although thyrocytes are spared from lysis by this mechanism, sublethal effects of complement activation may still be potent in exacerbating the autoimmune process, as thyroid cells are metabolically impaired and release cytokines, prostaglandins and reactive oxygen metabolites when subjected to sublethal complement attack (45). These responses are attenuated by antithyroid drugs, which are actively concentrated by thyroid cells, and this effect may account for the thyroid-specific immunomodulatory activity of these agents, which cause a fall in thyroid autoantibodies and a permanent remission in up to half of Graves' patients so treated (46).

In summary, thyroid cells actively participate in the autoimmune process in AITD (Fig. 4). Upregulation of MHC class II and complement inhibiting proteins by thyroid cells may be viewed teleologically as protective responses, especially through the mechanism of peripheral tolerance, but it is difficult to see how expression of cytokines, chemokines, adhesion molecules and CD40 can be anything other than harmful. Moreover, this is by no means a complete list of immunologically relevant molecules expressed by thyroid cells in AITD. For instance, thyroid cells can produce nitric oxide (47) and much recent attention has been focused on Fas (CD95) and Fas ligand expression by thyroid cells, which may play a crucial role in thyrocyte apoptosis

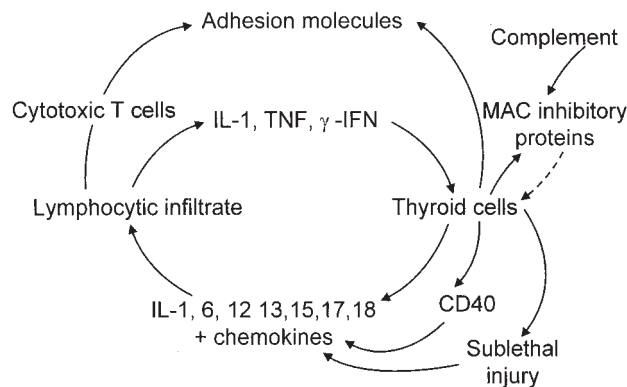


Figure 4 Interactions between the lymphocytic infiltrate and thyroid cells in autoimmune thyroid disease. MAC, membrane attack complex.

and thus in their killing by cytotoxic T cells, but it is also possible that any Fas ligand expression may help in the tolerisation of Fas⁺ T cells, creating a condition of protection from autoimmunity which has been termed 'immunological privilege' (48, 49).

Autoantigen recognition by T cells

Defining the nature of the interaction between the antigen receptors of the immune system, namely the T cell receptors (TCR) and immunoglobulin antibodies, and autoantigenic epitopes is not merely an arcane exercise in the pursuit of knowledge. Some forms of specific immunotherapy, already successful in animal models of autoimmune disease, depend on interference with this interaction and further understanding should shed new light on pathogenesis. CD4⁺ T cells recognise short antigenic peptides presented by MHC class II molecules using a TCR that is a heterodimer of an α and β chain, whose antigen recognition structure is encoded by variable (V) α and β genes. Considerable interest was aroused when the V α usage of thyroid-infiltrating T cells in Graves' disease was shown to be heavily restricted, with around 4 out of a possible 18 V α gene families being utilised (50, 51). V α usage varied between patients but, nonetheless, such restriction implied a narrow autoimmune response, and selective depletion of these T cells might therefore offer a safe and relatively specific means of treatment. In our patients, and those of other groups, however, no such V α restriction has been evident, even in IL-2 receptor-positive CD4⁺ T cells which largely contribute the activated, antigen-responding population (52, 53).

This unrestricted picture is not unexpected. At least three major and possibly several minor thyroid autoantigens exist, and there are multiple peptide epitopes in each. Indeed, the key lesson from studies on the epitopes of thyroid peroxidase (TPO) and thyrotrophin-receptor (TSH-R) recognised by T cells is that there is

no obvious immunodominant region (54–56), with again disappointing implications for the development of specific immunotherapy using peptide epitope antagonists. This lack of restriction in the CD4⁺ T cell response in AITD is the result of disease duration, causing ‘determinant spreading’, in which the initial response against a single epitope in an antigen becomes diversified as other T cells are attracted to the site, and cryptic epitopes within the antigen, and even epitopes of other antigens, are presented to these recruited T cells in the course of the inflammatory response. It is unlikely that, even with the development of predictors of disease, we will be able to intervene early enough in the CD4⁺ response in AITD to use treatments based on inhibiting this response at the TCR-peptide epitope level.

TPO and TSH-R autoantibodies

A series of pioneering studies by Rapoport, McLachlan and colleagues has utilised phage display technology to establish human monoclonal Fab antibody fragments against TPO from patients with AITD (57, 58). Using the thyroid as a major site of thyroid autoantibody synthesis, surgical specimens from patients with AITD were used to obtain a library of immunoglobulin DNA sequences by reverse transcription-polymerase chain reaction which were then expressed in phage plasmids that could be screened for expression of antigen-binding Fabs by panning on solid phase-bound TPO. The yield of adherent phage expressing appropriate Fabs could then be enhanced by successive rounds of panning before cloning, sequencing and expression. A surprising degree of heavy and light chain V gene restriction was found, and the Fabs were also shown to recognise primarily one of two broad domains in TPO. Our own studies have confirmed these findings even when lymph node-derived lymphocytes are used from patients with AITD, a site where maximum diversification of the immunoglobulin repertoire might be expected (59, 60).

However, the key targets for such studies now are TSH-R antibodies, and developing human monoclonal TSH-R antibodies has been termed the Holy Grail of research in AITD (61). This interest is sparked by the need to understand these unusual antibodies which have such obviously potent pathological effects despite their low serum concentration relative to other autoantibodies. Besides permitting epitope mapping, monoclonal TSH-R antibodies could be used to dissect out the intracellular signalling pathways in thyroid cell activation and growth and would also permit analysis of immunoglobulin V gene usage. There is already evidence that TSH-R antibodies are relatively restricted in both heavy and light chains, implying an oligoclonal origin that could perhaps be targeted therapeutically (62–64). So far, no antibodies which satisfy the stringent criteria established by Rapoport and McLachlan

Table 1 Criteria for establishing that monoclonal TSH-R stimulating antibodies match those causing Graves’ disease (from McLachlan and Rapoport (61)).

1	IgG class
2	Detectable at ng/ml concentration
3	Activity removed by adsorption with specific but not control antigen
4	Activity present after complete purification (to remove non-specific artefacts in assays)
5	Heavy and light chains should be expressed and recombinant Fab should retain activity

(Table 1) have been obtained. The reasons for this failure include the rarity of TSH-R-specific B cells, even in the thyroid and especially after treatment with antithyroid drugs, and the difficulty of expressing the receptor in a native state that can be used to bind antibodies, for instance, as expressed by phage display libraries (65).

However, although the Holy Grail of this quest remains elusive, its pursuit has led to several new developments. At present, the only method for detecting TSH-R stimulating antibodies remains bioassay, with all the vagaries that this imposes, coupled with low and slow throughput. To screen large numbers of potentially stimulating antibodies, new assay methods have been developed including a cell line transfected with the TSH-R coupled to a cyclic AMP-luciferase signalling system, allowing rapid read-out of cyclic AMP production in a luminometer (66). Others have developed transfected cell lines with the TSH-R expressed on the cell via a glycosylphosphatidylinositol (GPI) anchor; high levels of surface receptor allow these cells to be used in sensitive flow cytometry assays (67, 68). Such cells can be used to show the presence of non-immunoglobulin (Ig) G class TSH-R antibodies in patients with TAO who do not otherwise appear to have autoimmunity against the receptor, thus narrowing the odds that the immune system in all (or almost all) patients with TAO recognises TSH-R, hence making this a prime autoantigen in the orbital autoimmune response (69).

Whether TSH-R is the only autoantigen shared between thyroid and orbit remains a moot point, yet understanding the autoantigens involved in ophthalmopathy is central to improving treatment. Using a kind of reverse approach to that employed to obtain autoantibody Fabs, we have started to analyse this question by expressing a library of potential antigens from orbital tissue in phage, which can then be panned on immunoglobulins from patients with TAO, thus enriching for novel orbital antigens if there are antibodies to these in patient sera. The possibility of success with this approach can be seen from the recent cloning of the melanin-concentrating hormone receptor from phage-displayed melanoma cell antigens screened on vitiligo sera; this receptor is the first autoantigen in vitiligo associated with an autoantibody

with a functional and therefore potentially pathogenic effect, as such antibodies interfere with ligand binding (70). Given the decades of controversy over the nature of the autoantigens in TAO, resolution of this issue might be regarded a second Holy Grail in AITD; it would be a curious coincidence if both goals could be achieved by application of the same technology, although this hope must be guarded given the difficulties encountered so far in these two areas.

Future developments in treatment

Treatment for autoimmune diseases can be classified broadly as generalised or specific. Glucocorticoids are a good example of the former. They have a wide spectrum of immunological activities and although undoubtedly effective in most autoimmune disorders, their side-effects, including the suppression of beneficial immune responses, restrict their use. As previously described, more specific therapy targeting T cell receptors or epitopes is unlikely to be successful in AITD, but the success of monoclonal antibodies targeting TNF in rheumatoid arthritis (71) suggests that similar approaches using cytokine or cytokine receptor antagonists, or using suppressive cytokines like IL-10 may be more successful (72).

Redundancy in the activity of various cytokines will make targeting the individual cytokines difficult, but such treatment would seem especially suited to TAO in which IL-1, TNF and γ -IFN seem to have key roles. As already described, antithyroid drugs have a thyroid-specific immunomodulatory action which lies, at least in part, in their ability to reduce the release of proinflammatory molecules from thyrocytes. Relapses after treatment are particularly likely in patients with allergic disorders, and in those with elevated IgE and IL-13 levels (73), indicating that such drugs could be more effective if given with agents to inhibit Th2 responses, provided this did not lead to deviation of the autoimmune response to a destructive thyroiditis caused by Th1 cells.

Other approaches which offer promise include blockade of costimulation and induction of anergy through CTLA-4. Understanding more about the interaction of genes and environment might yield entirely novel pathways, some of which might be as simple as they are unexpected, such as the recently identified need to avoid smoking in relation to the development of TAO. I remain optimistic that treatment for Graves' disease will improve over the next 46 years, as a result of the progress made since the discovery of thyroid autoimmunity in 1956.

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References

- Rose NR & Witebsky E. Studies in organ specificity. V Changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. *Journal of Immunology* 1956 **76** 417–427.
- Roitt IM, Doniach D, Campbell PN & Vaughan Hudson R. Autoantibodies in Hashimoto's disease (lymphadenoid goitre). *Lancet* 1956 **ii** 820–821.
- Adams DD & Purves HD. Abnormal responses in the assay of thyrotrophin. *Proceedings of the University of Otago Medical School* 1956 **34** 11–12.
- Weetman AP & McGregor AM. Autoimmune thyroid disease; developments in our understanding. *Endocrine Reviews* 1984 **5** 309–355.
- DeGroot LJ & Quintans J. The causes of autoimmune thyroid disease. *Endocrine Reviews* 1989 **10** 537–562.
- Weetman AP & McGregor AM. Autoimmune thyroid disease: further developments in our understanding. *Endocrine Reviews* 1994 **15** 788–830.
- Goodnow CC. Pathways for self-tolerance and the treatment of autoimmune diseases. *Lancet* 2001 **357** 2115–2121.
- Hall R, Owen SG & Smart GS. Evidence for genetic predisposition to formation of thyroid autoantibodies. *Lancet* 1964 **ii** 187–188.
- Vladutiu A & Rose NR. Autoimmune murine thyroiditis: relation to histocompatibility (H-2) complex. *Science* 1971 **174** 1137–1139.
- Bech K, Lumholtz B, Nerup J, Thomson M, Platz P & Ryder LP. HLA antigens in Graves' disease. *Acta Endocrinologica* 1977 **86** 510–516.
- Allahabadi A & Gough SCL. The different approaches to the genetic analysis of autoimmune thyroid disease. *Journal of Endocrinology* 1999 **163** 7–13.
- Brix TH, Kyvik KO & Hegedüs L. What is the evidence of genetic factors in the etiology of Graves' disease? A brief review. *Thyroid* 1998 **8** 727–732.
- Yanagawa T, Hidaka Y, Guimaraes V, Soliman M & DeGroot LJ. CTLA-4 gene polymorphism associated with Graves' disease in Caucasian population. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 41–45.
- Kotsa K, Watson PF & Weetman AP. A CTLA-4 gene polymorphism is associated with both Graves' disease and Hashimoto's thyroiditis. *Clinical Endocrinology* 1997 **46** 551–555.
- Tomer Y, Barbesino G, Greenberg DA, Concepcion E & Davies TF. Mapping the major susceptibility loci for familial Graves' and Hashimoto's diseases: evidence for genetic heterogeneity and gene interactions. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 4656–4664.
- The International Consortium for the Genetics of Autoimmune Thyroid Disease, Tomer Y, Barbesino G, Greenberg DA, Concepcion E & Davies TF. A new Graves' disease-susceptibility locus maps to chromosome 20q11.2. *American Journal of Human Genetics* 1998 **63** 1749–1756.
- Vaidya B, Imrie H, Perros P, Young ET, Kelly WF, Carr D, Large DM, Toft AD, Kendall-Taylor P & Pearce SHS. Evidence for a

- new Graves' disease susceptibility locus at chromosome 18q21. *American Journal of Human Genetics* 2000 **66** 1710–1714.
- 18 Imrie H, Vaidya B, Perros P, Kelly WF, Toft AD, Young ET, Kendall-Taylor P & Pearce SHS. Evidence for a Graves' disease susceptibility locus at chromosome Xp11 in a United Kingdom population. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 626–630.
 - 19 Hunt PJ, Marshall SE, Weetman AP, Bunce M, Bell JI, Wass JAH & Welsh KI. Histocompatibility leucocyte antigens and closely linked immunomodulatory genes in autoimmune thyroid disease. *Clinical Endocrinology* 2001 **55** 491–499.
 - 20 Ruwhof C & Drexhage HA. Iodine and thyroid autoimmune disease in animal models. *Thyroid* 2001 **11** 427–436.
 - 21 Chiovato L & Pinchera A. Stressful life events and Graves' disease. *European Journal of Endocrinology* 1996 **134** 680–682.
 - 22 Metcalfe RA & Weetman AP. Stimulation of extraocular muscle fibroblasts by cytokines and hypoxia: possible role in thyroid-associated ophthalmopathy. *Clinical Endocrinology* 1994 **40** 67–72.
 - 23 Coles A, Wing M, Smith S, Coraddu F, Greer S, Taylor C, Weetman A, Hale G, Chatterjee VK, Waldmann H & Compston A. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. *Lancet* 1999 **354** 1691–1695.
 - 24 Thornton AM & Shevach EM. Suppressor effector function of CD4⁺ CD25⁺ immunoregulatory T cells is antigen non-specific. *Journal of Immunology* 2000 **164** 183–190.
 - 25 Taams LS, Smith J, Rustin MH, Salmon M, Poulter LW & Akbar AM. Human anergic/suppressive CD4⁺ CD25⁺ T cells: a highly differentiated and apoptosis-prone population. *European Journal of Immunology* 2001 **31** 1122–1131.
 - 26 Volpé R. The immunoregulatory disturbance in autoimmune thyroid disease. *Autoimmunity* 1988 **2** 55–72.
 - 27 Iitaka M, Aguayo JF, Iwatani Y, Row VV & Volpé R. Studies of the effect of suppressor T lymphocytes on the induction of antithyroid microsomal antibody-secreting cells in autoimmune thyroid disease. *Journal of Clinical Endocrinology and Metabolism* 1988 **66** 708–714.
 - 28 Hanafusa T, Pujol-Borrell R, Chiovato L, Russell RCG, Doniach D & Bottazzo GF. Aberrant expression of HLA-DR antigen on thyrocytes in Graves' disease: relevance for autoimmunity. *Lancet* 1983 **ii** 1111–1115.
 - 29 Bottazzo GF, Pujol-Borrell R, Hanafusa T & Feldmann M. Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. *Lancet* 1983 **2** 1115–1119.
 - 30 Todd I, Pujol-Borrell R, Hammond LJ, Bottazzo GF & Feldmann M. Interferon- γ induces HLA-DR expression by thyroid epithelium. *Clinical and Experimental Immunology* 1985 **61** 265–273.
 - 31 Weetman AP, Volkman DJ, Burman KD, Gerrard TL & Fauci AS. The *in vitro* regulation of human thyrocyte HLA-DR antigen expression. *Journal of Clinical Endocrinology and Metabolism* 1985 **61** 817–824.
 - 32 Cohen SB, Dijkstra CD & Weetman AP. Sequential analysis of experimental autoimmune thyroiditis induced by neonatal thymectomy in the Buffalo strain rat. *Cellular Immunology* 1988 **114** 126–136.
 - 33 Tandon N, Metcalfe RA, Barnett D & Weetman AP. Expression of the costimulatory molecule B7/BB1 in autoimmune thyroid disease. *Quarterly Journal of Medicine* 1994 **87** 231–236.
 - 34 Lombardi G, Arnold K, Uren J, Marelli-Berg F, Hargreaves F, Inami N, Weetman AP & Lechler R. Antigen presentation by IFN- γ treated thyroid follicular cells inhibits IL-2 and supports IL-4 production by B7-dependent human T cells. *European Journal of Immunology* 1997 **27** 62–71.
 - 35 Marelli-Berg FM, Weetman AP, Frasca L, Deacock SJ, Inami N, Lombardi G & Lechler RI. Antigen presentation by epithelial cells induces anergic immunoregulatory CD45RO⁺ T cells and depletion of CD45RA⁺ T cells. *Journal of Immunology* 1997 **159** 5853–5861.
 - 36 Weetman AP, Freeman M, Borysiewicz LK & Makgoba MW. Functional analysis of intercellular adhesion molecule-1-expressing human thyroid cells. *European Journal of Immunology* 1990 **20** 271–275.
 - 37 Tandon N, Makgoba MW, Gahmberg CG & Weetman AP. The expression and role in adhesion of LEA-3 and ICAM-2 on human thyroid cells. *Clinical Immunology and Immunopathology* 1992 **64** 30–35.
 - 38 Jungheim K, Caspar G, Usadel KH & Schumm-Draeger PM. Expression of intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 and homing factor CD44 after engraftment of Graves' lymphocytes in xenotransplanted human thyroid tissue in athymic nude mice. *Thyroid* 2001 **11** 831–837.
 - 39 Metcalfe RA, McIntosh RS, Marelli-Berg F, Lombardi G, Lechler R & Weetman AP. Detection of CD40 on human thyroid follicular cells: analysis of expression and function. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1268–1274.
 - 40 Weetman AP, Bright-Thomas R & Freeman M. Regulation of interleukin-6 release by human thyrocytes. *Journal of Endocrinology* 1990 **127** 357–361.
 - 41 Weetman AP, Bennett GL & Wong WLT. Thyroid follicular cells produce interleukin-8. *Journal of Clinical Endocrinology and Metabolism* 1992 **75** 328–330.
 - 42 Ajjan R, Watson PF & Weetman AP. Detection of IL-12, IL-13 and IL-15 mRNA in the thyroid of patients with autoimmune thyroid disease. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 666–669.
 - 43 Weetman AP, Cohen SB, Oleesky DA & Morgan BP. Terminal complement complexes and C1/C1 inhibitor complexes in autoimmune thyroid disease. *Clinical and Experimental Immunology* 1989 **77** 25–30.
 - 44 Weetman AP, Freeman MA & Morgan BP. Thyroid follicular cell function after non-lethal complement membrane attack. *Clinical and Experimental Immunology* 1990 **82** 69–74.
 - 45 Tandon N, Morgan BP & Weetman AP. Expression and function of membrane attack complex inhibitory proteins on thyroid follicular cells. *Immunology* 1992 **75** 372–377.
 - 46 Weetman AP, Tandon N & Morgan BP. Antithyroid drugs and the release of inflammatory mediators by complement-attacked thyroid cells. *Lancet* 1992 **340** 633–636.
 - 47 Kasai K, Hattori Y, Nakanishi N, Manaka K, Banba N, Motohashi S & Shimoda S. Regulation of inducible nitric oxide production by cytokines in human thyrocytes in culture. *Endocrinology* 1995 **136** 4261–4270.
 - 48 Giordano C, Stassi G, De Maria R, Todaro M, Richiusa P, Papoff G, Ruberti G, Bagnasco M, Testi R & Galluzzo A. Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. *Science* 1997 **275** 960–963.
 - 49 Bretz JD & Baker JR Jr. Apoptosis and autoimmune thyroid disease: following a TRAIL to thyroid destruction? *Clinical Endocrinology* 2001 **55** 1–11.
 - 50 Davies TF, Martin A, Concepcion ES, Graves P, Cohen L & Ben-Nun A. Evidence of limited variability of antigen receptors on intrathyroidal T cells in autoimmune thyroid disease. *New England Journal of Medicine* 1991 **325** 238–244.
 - 51 Davies TF, Concepcion ES, Ben-Nun A, Graves P & Tarjan G. T-cell receptor V gene usage in autoimmune thyroid disease: direct assessment by thyroid aspiration. *Journal of Clinical Endocrinology and Metabolism* 1993 **76** 660–666.
 - 52 McIntosh RS, Tandon N, Pickerill AP, Davies R, Barnett D & Weetman AP. IL-2 receptor-positive intrathyroidal lymphocytes in Graves' disease: analysis of V α transcript microheterogeneity. *Journal of Immunology* 1993 **151** 3884–3893.
 - 53 Caso-Pelaez E, McGregor AM & Banga JP. A polyclonal T cell repertoire of V-alpha and V-beta T cell receptor gene families in intrathyroidal T lymphocytes of Graves' disease patients. *Scandinavian Journal of Immunology* 1995 **41** 141–147.
 - 54 Tandon N, Freeman M & Weetman AP. T cell responses to synthetic thyroid peroxidase peptides in autoimmune thyroid disease. *Clinical and Experimental Immunology* 1991 **86** 56–60.
 - 55 Tandon N, Freeman M & Weetman AP. T cell responses to synthetic TSH receptor peptides in Graves' disease. *Clinical and Experimental Immunology* 1992 **89** 468–473.

- 56 Soliman M, Kaplan E, Yanagawa T, Hidaka Y, Fislalen M-E & DeGroot LJ. T-cells recognize multiple epitopes in the human thyrotropin receptor extracellular domain. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 905–914.
- 57 Chazenbalk GD, Portolano S, Russo D, Hutchison JS, Rapoport B & McLachlan SM. Human organ-specific autoimmune disease. Molecular cloning and expression of an autoantibody gene repertoire for a major autoantigen reveals an antigenic immunodominant region and restricted immunoglobulin gene usage in the target organ. *Journal of Clinical Investigation* 1993 **92** 62–74.
- 58 Rapoport B, Portolano S & McLachlan SM. Combinatorial libraries: new insights into human organ-specific autoantibodies. *Immunology Today* 1995 **16** 43–49.
- 59 McIntosh RS, Asghar MS, Kemp EH, Watson PF, Gardas A, Banga JP & Weetman AP. Analysis of IgG κ anti-thyroid peroxidase antibodies from different tissues in Hashimoto's thyroiditis. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 3818–3825.
- 60 Guo J, McIntosh RS, Czarnocka B, Weetman AP, Rapoport B & McLachlan SM. Relationship between autoantibody epitopic recognition and immunoglobulin gene usage. *Clinical and Experimental Immunology* 1998 **111** 408–414.
- 61 McLachlan SM & Rapoport B. Monoclonal human autoantibodies to the TSH receptor - The Holy Grail and why are we looking for it? *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 3152–3154.
- 62 Zakarija M. Immunochemical characterisation of the thyroid-stimulating antibody in Graves' disease: evidence for restricted heterogeneity. *Journal of Clinical and Laboratory Immunology* 1983 **10** 77–85.
- 63 Williams RC, Marshall NJ, Kilpatrick K, Montano J, Brickell PM, Goodall M, Ealey PA, Shine B, Weetman AP & Craig RK. Kappa/lambd immunoglobulin distribution of Graves' thyroid stimulating antibodies. Simultaneous analysis of CA gene polymorphisms. *Journal of Clinical Investigation* 1988 **82** 1306–1312.
- 64 McIntosh R, Watson P & Weetman AP. Somatic hypermutation in autoimmune thyroid disease. *Immunological Reviews* 1998 **162** 219–231.
- 65 Weetman AP, Yateman ME, Ealey PA, Black CM, Reimer CB, Williams RC, Shine B & Marshall NJ. An investigation of thyroid stimulating antibody activity between different IgG subclasses. *Journal of Clinical Investigation* 1990 **86** 723–737.
- 66 Watson PF, Ajjan RA, Phipps J, Metcalfe R & Weetman AP. A new chemiluminescent assay for the rapid detection of thyroid stimulating antibodies in Graves' disease. *Clinical Endocrinology* 1998 **49** 577–581.
- 67 DaCosta CR & Johnstone AP. Production of the thyrotropin receptor extracellular domain as a glycosylphosphatidylinositol-anchored membrane protein and its interaction with thyrotropin and autoantibodies. *Journal of Biological Chemistry* 1998 **273** 11874–11880.
- 68 Costagliola S, Khoo D & Vassart G. Production of bioactive amino-terminal domain of the thyrotropin receptor via insertion in the plasma membrane by a glycosylphosphatidylinositol anchor. *FEBS Letters* 1998 **436** 427–433.
- 69 Metcalfe R, Jordan N, Watson P, Gullu S, Wiltshire M, Crisp M, Evans C, Weetman A & Ludgate M. Demonstration of immunoglobulin G, A, and E autoantibodies to the human thyrotropin receptor using flow cytometry. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 1754–1761.
- 70 Kemp EH, Waterman EA, Hawes BE, O'Neill K, Gottumukkala RVSRK, Gawkrodger DJ, Weetman AP & Watson PF. The melanin-concentrating hormone receptor 1, a novel target of autoantibody responses in vitiligo. *Journal of Clinical Investigation* 2002 **109** 923–930.
- 71 Mackay IR. Tolerance and autoimmunity. *British Medical Journal* 2000 **321** 93–96.
- 72 Batteux F, Trebeden H, Charreire J & Chiochia G. Curative treatment of experimental autoimmune thyroiditis by *in vivo* administration of plasmid DNA coding for interleukin-10. *European Journal of Immunology* 1999 **29** 958–963.
- 73 Komiya I, Yamada T, Sato A, Kouki T, Nishimori T & Takasu N. Remission and recurrence of hyperthyroid Graves' disease during and after methimazole treatment when assessed by IgE and interleukin 13. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 3540–3544.

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