REVIEW

Animal models of Graves' disease

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Abstract

Graves' disease (GD) is an autoimmune condition in which goitre and hyperthyroidism are induced by thyroid stimulating antibodies (TSAB) which mimic the action of thyrotrophin (TSH). The target of the autoimmune response is the thyrotrophin receptor (TSHR) and, since its cloning, a number of differing approaches have been adopted in an attempt to develop an animal model of GD.

Methods in which synthetic peptides or fragments of the receptor produced in bacteria or insect cells have been injected into animals together with immunological adjuvants have had only limited success in inducing some of the signs and symptoms of GD. Genetic immunisation resulted in thyroiditis in the majority, but TSAB formation in only a minority, of treated inbred mice. Transfer of receptor *in vitro* primed T cells to syngeneic naive recipients, with priming either using a bacterial fusion protein or genetic immunisation, induced destructive thyroiditis in non-obese diabetic (NOD) mice but lymphocytic thyroiditis in BALBc mice. Furthermore, the orbits of 17/22 of the BALBc animals, but not the NOD animals, with thyroiditis had orbital changes similar to those seen in thyroid eye disease.

TSAB and elevated thyroxine levels were induced in AKR/N mice injected with fibroblasts expressing the full length human TSHR and murine major histocompatibility complex (MHC) class II homologous to the recipient mice. No thyroiditis was induced but preliminary results from a different group using the same protocol suggest that receptor autoantibodies and thyroid dysfunction could be transferred using T cells primed *in vitro* with the receptor and MHC-II expressing cells.

The majority of the studies described above have studied inbred mouse strains. In a novel departure, the NMR outbred strain has been treated by genetic immunisation with very promising results, including the induction of increased thyroxine levels in 4/30 female mice, accompanied by TSAB in addition to thyroiditis, and with signs of hyperactivity and orbital pathology.

This review discusses the various protocols together with the information regarding the pathogenesis of GD which each has contributed, and concludes with an evaluation of how close we are to mimicking this polygenic, multifactorial disease.

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Introduction

Graves' disease (GD) is an autoimmune condition characterised by hyperthyroidism, small goitre with uniform radio-iodine uptake, and eye signs such as proptosis. It is caused by antibodies to the thyrotrophin (TSH) receptor (TSHR), thyroid stimulating antibodies (TSAB), which mimic the action of TSH (reviewed in 1).

Graves' disease is polygenic and multifactorial (reviewed in 2) and, even though it is very common, patients present when the disease has advanced to the extent of causing clinical signs and symptoms, which is likely to be some time after the initiating immune events. Thus, it has long been recognised that fundamental progress in our understanding of GD would benefit from an appropriate animal model, with some or all of the animals demonstrating: (i) elevated thyroxine and/or reduced thyrotrophin levels; (ii) antibodies to the

TSHR, having biological activity, preferably TSAB; (iii) changes in thyroid architecture; (iv) lymphocytic thyroiditis; (v) clinical signs of hyperthyroidism such as weight loss; (vi) orbital changes akin to those of thyroid eye disease (TED), and (vii) lymphocytic infiltration in the pretibial skin.

There are very few spontaneous models of autoimmunity and none in which autoreactivity to the TSHR has been detected. The best examples for autoimmune thyroid disease are the obese strain chicken (3), the BB rat (4) and some colonies of non-obese diabetic (NOD) mice (5). In these examples, destructive thyroiditis is developed directed to thyroglobulin in the chicken and rat, and to thyroid peroxidase in the mouse.

TSHR based models have had to rely on introducing the receptor in some form, often in combination with strong immunological adjuvants. An added problem is the low level of receptor expression in the thyroid. One

solution was to obtain receptor by detergent extraction and TSH affinity purification of a human thyroid cell line, GEJ (6). When five different H-2 mouse strains received repeated immunisations with the receptor preparation, H-2s developed low levels of thyrotrophin binding inhibiting immunoglobulins (TBII), and mild thyroiditis was observed in H-2s, H-2b and H-2q mice. The disadvantage of this model is that, even with a cell line, limited quantities of antigen can be obtained and then only with considerable effort; the advantage of this model is its use of TSHR in a conformation able to bind TSH, a problem that troubled many of the earliest attempts using recombinant TSHR to develop an animal model of GD. More recent approaches have used antigens likely to comprise full length correctly folded receptor. The recombinant methods used include: (1) injection of synthetic receptor peptides; (2) injection of receptor protein produced in procaryotic cells; (3) injection of receptor protein produced in eucaryotic cells; (4) genetic immunisation; (5) injection of receptor transfected cells; (6) transfer of receptor *in vivo* primed T cells (priming via 1, 2, 4 or 5).

In this review an attempt will be made to evaluate how close we are to an appropriate model of GD, but it will begin with a brief reflection on the properties of the TSHR which may contribute to its autoantigenicity.

The thyrotrophin receptor as an autoantigen

The TSHR is a G-protein-coupled receptor, with the characteristic seven membrane spanning regions (MSR). It is a part of the glycoprotein hormone subfamily and, along with the receptors for luteinising hormone (LH) and follicle-stimulating hormone (FSH), has a large extracellular domain (ECD) which confers ligand binding specificity (reviewed in 7). Unlike the receptor for LH and FSH, the TSHR undergoes a post-translational cleavage with a metallo-protease (8), such that a proportion of surface receptor is in the form of two subunits, with the ECD linked to the MSR via (a) di-sulphide bridge(s). The TSHR has two unique insertions, one of 8 residues which is required for mature receptor to be expressed at the cell surface, and the second of some 50 amino acids, which does not seem to be essential. However, the 50 residue insert is the probable site of TSHR cleavage (reviewed in 9) and is highly immunogenic, as determined from hydropathy profiles and borne out by the development of monoclonal antibodies which bind linear sequences in this region (10–12). The immunogenicity may simply be the result of the carboxyl location in the ECD following cleavage of the receptor; in most proteins the N and C termini are immunogenic (reviewed in 13), and in the TSHR there is, indeed, a second 'hot-spot' for generated monoclonals at the amino terminus (14, 15). An alternative explanation is that a portion of the ECD or even the entire ECD may be released into the circulation either following

maturation or upon ligand mediated receptor activation, and there is some evidence for a circulating TSH binding protein (16, 17). Whether receptor fragments in the circulation might have a role in breaking peripheral tolerance remains conjectural, but there is evidence for widespread autoimmunity to the TSHR, defined as T cell epitopes (18), or in assays measuring direct antibody binding to the receptor (19), in normal individuals without GD.

Receptor induced animal models

Before discussing the recombinant approaches individually, it might be helpful to describe the techniques applied to investigate the immune response induced.

Antibodies to the TSHR have been measured using the following methods: (i) ELISA using receptor fusion protein: detects antibodies which bind but have no bioactivity; (ii) fluorescence activated cell sorting (FACS) analysis on cells expressing full length TSHR: also detects binding in the absence of bioactivity but more likely to reflect recognition of conformational epitopes; (iii) cAMP accumulation in cells expressing the full length TSHR: detects TSAB; (iv) antibodies measured as in (iii) but in the presence of TSH: detects thyroid blocking antibodies (TBAB); (v) inhibition of binding to TSHR either on membranes or intact cells: detects TBII.

Changes in thyroid weight, colour and size, which are evident macroscopically, have been reported. Thyroiditis is usually evaluated by histological examination of the gland following death; scoring methods can be applied to classify the severity. Immunocytochemical analysis of frozen tissue permits identification of the immune cell types present, and the cytokines they produce, which can also be expressed as percentages or ratios. Orbital pathology is assessed in the same way but with the addition of staining methods to detect specialised cells such as adipocytes.

Circulating thyroid hormone levels, thyroxine (T_4), tri-iodothyronine (T_3) and TSH, are measured by radioimmunoassay, which are often in-house methods adapted from the human equivalent.

The general health and well being of the animals, including changes in the rate of growth when compared with control animals, have also been observed.

Synthetic peptides

Several papers have claimed the induction of TSAB, TBAB, and TBII, and increased thyroxine levels in mice, rabbits and birds treated with synthetic receptor peptides (20–23). To my knowledge, lymphocytic thyroiditis was not reported in any of these studies and, in some cases, the antibody and hormone changes have subsequently become more questionable for the reasons described in the following section. In retrospect, it would have been surprising if this approach had been

successful in generating an animal model, but it remains a useful route for producing antibodies for validating receptor protein produced *in vitro* (24).

Recombinant receptor from procaryotic cells

We, and others, have produced recombinant receptor fusion proteins in bacteria. We employed the ECD (24) coupled to maltose binding protein (MBP), enabling us to hyperimmunise male and female BALBc mice with 50 or 100 µg doses of a purified receptor preparation. Our protocol uses the intra-peritoneal route and an adjuvant of alum and pertussis toxin, and always involves the study of test animals receiving ECD-MBP, and control mice treated with the fusion partner, MBP, alone. Initially, when studying pooled sera from male mice (25), we reported the induction of TBII in both groups early on in the immunisation schedule but this persisted only in the ECD-MBP group. When testing immunoglobulins (IgGs), the TBII were confirmed only in the receptor-treated mice, indicating that false positive TBII can be demonstrated using unfractionated sera. Similarly, reduced T₄ levels were present in both groups after the initial immunisations, but the MBP mice recovered, unlike the ECD-MBP mice whose thyroids also displayed increased vascularity and focal lymphocytic thyroiditis.

Subsequently, we extended the study to examine individual male and female BALBc mice (H-2d) hyperimmunised with ECD-MBP or MBP alone (26) and confirmed the induction of TBII and TBAB, accompanied by reduced circulating T₄, by the receptor antigen but not by MBP, in both males and females. Thyroiditis was induced in 50% of male and 100% of surviving female mice.

In mice of differing genetic background (27), particularly in the major histocompatibility complex (MHC), H-2b (C57) and H-2k (CBA) animals did not develop thyroiditis although receptor antibodies were induced to a similar degree to those in H-2d (BALBc) animals. In the same series of experiments, NOD mice, which have a unique H-2g haplotype, developed antibodies to the receptor, destructive thyroiditis and reduced T₄ levels, when treated with the ECD-MBP antigen. When comparing the phenotype of the lymphocytic infiltrate, B cells and immunoreactivity for interleukin (IL)-4 and IL-10 were found in the BALBc mice, whereas in the NOD mice there were very few B cells and immunoreactivity for interferon γ (INFγ) was found, indicating the Th2 and Th1 nature of the induced disease in BALBc and NOD mice respectively.

Attempts by other groups, even using the same hyperimmunisation protocol and strain of BALBc mice, failed to induce thyroiditis. This might be explained by environmental factors contributing to disease outcome, as in the case of transgenic mice expressing a myelin basic protein specific T cell receptor (28), which develop spontaneous allergic encephelomyelitis only when housed in a non-sterile facility.

More recently, it has been possible to induce thyroiditis with a similar protocol to ours but using an ECD truncated in the C terminus (29). The authors have interpreted this as being due to the removal of immunodominant non-thyroiditis epitopes, unmasking cryptic disease inducing T cell epitopes. This seems plausible, especially as our ECD-MBP fusion protein could be truncated in the C terminus due either to incomplete synthesis or degradation during purification, problems associated with proteins in which the fusion partner, which provides the selection for purification, is at the amino terminus.

Recombinant receptor from eucaryotic cells

A major drawback using receptor produced in bacteria was the lack of correct folding and glycosylation, and it was hoped that eucaryotic expression in insect cells, using baculovirus vectors, might offer a solution to this problem. Certainly, the human ECD produced using this system is superior to the antigen from bacteria in terms of absorbing out TBII activity from GD patients' sera (30), but there have been no convincing demonstrations of direct TSH binding to a receptor protein expressed using baculovirus. Nevertheless, useful information has been obtained, with both the human and murine ECD, when injected into animals in combination with various adjuvants. Prabhakar and colleagues (31) studied four different strains of mice treated with the human ECD and observed that antibodies to the receptor, having no biological activity, were induced in all the mice. In contrast, increased thyroxine levels were present only in the BALBc mice and, despite the absence of thyroiditis, there were changes in the gland, such as budding, indicative of hyperactivity. The same antigen, used by the same group but this time injected into rabbits, induced TBII (32). Perhaps reasoning that an induced immune response to a heterologous antigen is not sufficient to claim autoimmunity, Davies' group have expressed the murine ECD in insect cells and obtained monoclonal antibodies, some of which had TBII and TBAB activities when measured *in vitro* on Chinese hamster ovary cells expressing the human TSHR (33). Furthermore, when BALBc mice were treated with the same murine antigen and an adjuvant comprised of alum and pertussis toxin (34), TBII and TBAB were induced and mice had reduced levels of T₃ accompanied by increased TSH, but no signs of thyroiditis or thyroid destruction.

Genetic immunisation

This novel method involves immunisation with the cDNA for the full length human TSHR cloned in a eucaryotic expression vector, usually into the anterior tibialis muscle (35). It is assumed that the cDNA is taken up into the myocytes at the site of injection and, subsequently, expressed at the surface of these cells.

Myocytes do not express MHC-class II or the co-stimulatory molecules necessary to activate T cells. Consequently, there must be a phase, perhaps triggered by inflammation of the muscle, in which professional antigen presenting cells become involved, maybe by phagocytosing fragmented receptor released from myocytes. However, if this is the whole story, why is it not possible to induce thyroiditis and TSAB using synthetic peptides administered with adjuvants capable of stimulating inflammation at the injection site?

Groups of female BALBc mice received three injections of 100 µg TSHR in pcDNAIII, or the empty vector alone, at 3 week intervals (36). The cDNA was administered in either PBS or 25% sucrose, or following pretreatment of the muscle with cardiotoxin. Fourteen out of fifteen mice treated with receptor cDNA developed antibodies to the TSHR, measured by FACS, and the majority contained TBII and TBAB activities. One serum contained TSAB, resulting in an 800% increase in cAMP production which persisted for 18 weeks. Thyroid hormone levels remained normal throughout the experiment. All mice displayed severe thyroiditis with many infiltrating B cells, but, rather than showing thyroid destruction, there were signs of epithelial thickening and budding.

The method was then applied to the NMRI outbred strain of mice, with very exciting results (37). Thirty male and thirty female mice underwent the genetic immunisation protocol and virtually all developed receptor antibodies detectable by FACS. Nine out of thirty males displayed signs of hypothyroidism, with TBAB and reduced T_4 . Four out of thirty females developed stable hyperthyroidism, with circulating TSAB accompanied by increased T_3 and T_4 but undetectable TSH. In addition, thyroiditis and orbital changes (please see section on *Transfer of receptor in vivo primed T cells* for details) were induced.

Injection of receptor transfected cells

One very promising approach has involved treating mice with fibroblasts which express an MHC-class II molecule and the full length, functional human TSHR (38). The mouse strain used was the AKR/N which is H-2^k, homologous to the RT4.15HP murine fibroblast cell line. Recipient mice and cells used as immunogen are MHC-class I identical. The TSHR was introduced by transfection into the RT4.15HP cells or the parent line lacking the MHC-class II, and surface expression of MHC molecules was confirmed by FACS analysis. Furthermore, the transfected TSHR was shown to be functional in terms of TSH-mediated cAMP production.

Female mice were injected with 10⁷ fibroblasts, i.p., a total of six times at intervals of two weeks, and they were examined two weeks following the final injection. The fibroblasts expressed MHC-class II, or human TSHR or both. A variety of investigations were performed including measurement of TBII and circulating thyroid hormone levels in individual mouse sera, TSAB and

TBAB determination in paired protein A purified mouse IgGs, and the gross and microscopic morphology of the thyroid gland. The majority of mice receiving cells expressing MHC-class II and receptor developed TBII. About 20% had increased thyroxine levels and this was shown to be accompanied by TSAB activity. In contrast, TBII positive sera in animals with normal thyroid hormone levels displayed TBAB activity. No TBII, TSAB or TBAB, or changes in circulating thyroid hormone were induced in mice receiving cells expressing either TSHR or MHC-class II alone, and the thyroids of these animals appeared normal.

In contrast, the thyroids of the animals receiving TSHR and MHC-class II expressing fibroblasts were macroscopically enlarged and, microscopically, they displayed changes in architecture e.g. hypertrophy and hypercellularity with follicular cells protruding into the lumen, changes which are similar to those seen in hyperthyroidism. However, there was no lymphocytic infiltration.

Of interest, the induction of TBII and TBAB might be expected to result in reduced circulating thyroid hormone levels. The authors have not commented on the fact that the five mice having the lowest thyroxine levels are in this category, although the induction of increased and reduced T_4 levels using this protocol has been noted more recently (please see below).

In a second series of experiments, the non-MHC genetic control of the induced disease was investigated in five different strains of mice, all H-2^k (39). When treated with fibroblasts expressing MHC-class II and the TSHR, the majority of animals developed TBII, irrespective of the strain. Furthermore, the CBA and C3H strains had circulating TBII even when the fibroblasts expressed only receptor and not MHC-class II. When a larger series of AKR/N and C3H animals were compared, elevated T_4 levels, which were said to be associated with detectable TSAB although no data were reported, were found only in C3H mice receiving fibroblasts expressing both receptor and MHC-class II.

From these experiments, in which TBII and increased T_4 levels were induced in mice receiving cells co-expressing the TSHR and MHC-class II, it was concluded that aberrant class II expression in human thyrocytes is necessary for the development of GD. Non-MHC genes play a more limited role.

Subsequently, the same authors, using this protocol in the original AKR/N mice (40), investigated whether certain regions of the TSHR might be necessary or sufficient to induce disease. In previous chimera studies, in which portions of the TSHR were substituted with the equivalent part of the LH receptor, the amino terminal of the protein was found to be required for binding and specific recognition by TSAB. A range of such chimeras lacking residues 9–165 (mc1+2), 90–165 (mc1) and 261–370 (mc4) were transfected into the RT4.15Hp and parent cell lines, and characterised by FACS analysis as described for the wild type (WT) receptor

above. Demonstration of chimeric receptor at the surface compared with WT might have been a useful analysis to confirm comparable expression, although all types of receptor expressing cells induced *in vitro* proliferation of splenocytes which had been primed *in vivo* with the same receptor, showing that all constructs contain T cell epitopes.

TBII were induced in mice receiving MHC-class II expressing fibroblasts transfected with the WT and mc4 receptors but not with the mc1 or mc1+2 constructs. Elevated T₄ levels, associated with TSAB, were present in 2/9 WT and MHC-class II recipients but not in any of the other treated or control groups.

Based on the TBII results, it was concluded that the N terminal segment of the receptor is critical, not only as an epitope for human TSAB but also in the induction of TSHR antibodies in a murine model of GD. However, considering the lack of TSAB in animals receiving the mc4 construct, the participation of the carboxyl end of the ECD should not be overlooked.

This model has recently been confirmed and extended (41) using the same AKR/N mouse strain, which received the RT4.15HP cell line transfected with the human TSHR (in a slightly different expression vector), administered i.p. a total of 8 times, and the mice were examined 6, 12 and 18 weeks after the first injection. In the basic protocol, most animals developed TBII, and elevated T₄ levels, defined as being > 5, were induced in 5/20 recipients of fibroblasts expressing receptor and MHC-class II; a further two mice died prior to levels being measured. One mouse had elevated TSH and reduced T₄ levels but the thyroid was normal as it was in the euthyroid and control animals. The five animals with increased T₄ had enlarged thyroids with signs of hypertrophy and colloid droplet formation but no lymphocytic infiltration. When the protocol was modified by including alum, a Th2 adjuvant, increased T₄ levels and goitres were found in 9/19 mice together with an earlier onset (9 compared with 11 weeks), whilst 2 mice had increased TSH indicative of hypothyroidism. In contrast, the inclusion of complete Freund's, a Th1 adjuvant, resulted in a slower onset of hyperthyroidism with 10/31 mice having increased T₄ levels by 14 weeks. This last group comprised male and female animals and no difference was observed in the incidence or severity of induced disease.

Epitope mapping by ELISA revealed that 9/13 sera from mice receiving fibroblasts expressing receptor and MHC-class II, recognised a peptide for residues 97–116 of the N terminal of the ECD, but peptides for residues 322–371 in the carboxyl part of the ECD also showed high absorption by experimental but not control sera.

Transfer of receptor *in vivo* primed T cells

The earliest reported transfer of receptor primed T cells used synthetic peptides shown to be T cell epitopes for GD patients in the *in vivo* priming step of DBA mice (23).

Treatment with 3/4 T cell epitope peptides elicited weak TSAB activity, and T cell lines were developed from one TSAB positive and one TSAB negative mouse. When these were transferred to naive syngeneic mice, following a period of *in vitro* priming with receptor peptides, weak TSAB activity was present in 2/4 mice receiving the line from the TSAB positive donor but no TSAB activity was present in the four mice receiving the line from the TSAB negative donor.

We have used unfractionated T cells and a CD4+ enriched population to transfer disease to syngeneic BALBc and NOD recipients. The *in vivo* priming step could be performed using the receptor produced in bacteria (ECD-MBP) or by genetic immunisation but in both cases it was followed by an *in vitro* priming period using ECD-MBP. In our first study (42), BALBc and NOD recipients were examined 16 days after transfer of syngeneic receptor primed T cells and both strains of mice displayed thyroiditis similar in phenotype to that induced in the donors using ECD-MBP, i.e. Th2 in the BALBc mice and Th1 with thyroid destruction in the NOD mice. Neither strain had developed antibodies to the receptor in the recipient animals at this early stage although these were present in the donor mice. In more recent experiments (43), we examined the kinetics of disease induction, again using unfractionated T cells and a CD4+ enriched population, at 4, 8 and 12 weeks after transfer. In addition, since we had found evidence for TSHR transcripts and protein in orbital tissue from TED patients (44, 45), the mouse orbits were examined. In both BALBc and NOD recipients the Th2 and Th1 nature, respectively, of induced thyroiditis was confirmed and found to persist for the 12 week duration of the experiment. At 4 weeks, TSHR antibodies, including TBII, had been induced in both strains and these, too, persisted throughout the experiment. Changes in thyroid hormone levels were more difficult to evaluate, especially in the BALBc mice. In NOD recipients of TSHR primed T cells, thyroid hormone levels were reduced, as might be expected from the destructive thyroiditis induced in this strain. Four weeks after transfer, BALBc recipients of TSHR primed and control non-primed T cells had reduced T₄ levels, which slowly recovered in the latter. At eight and twelve weeks, some BALBc recipients of receptor primed T cells had increased T₄, relative to the control non-primed recipients.

When examining the orbits, all the NOD recipients of primed and non-primed cells displayed normal histology with intact, well organised muscle fibre architecture. The orbits of BALBc recipients of primed (but not non-primed) T cells appeared strikingly different. The muscle fibres were disorganised and separated by periodic-acid-Schiff positive oedema. There was accumulation of adipose tissue and infiltration by immune cells, especially mast cells. These changes were observed in 17/25 BALBc recipients of receptor primed cells and did not correlate with TBII or T₄ levels. However, orbital changes were

Table 1 Comparison of the signs and symptoms of Graves' disease induced by the various protocols.

	TBII	TBAB	TSAB	T ₄ , T ₃ , TSH	Thyroiditis	Eye signs
Peptides	+	+	–	Mixed	–	ND
Procaryotic	+	+	–	Euthyroid	+ focal Th2	ND
Eucaryotic	+	+	–	Mixed	–	ND
cDNA inbred	+	+	–	Euthyroid	+ focal Th2	ND
cDNA outbred	+	+	+	T ₄ ↑ TSH↓	+ focal Th2	+
Fibro + class II	+	+	+	T ₄ ↑ TSH↓	–	–
Transfer prot	+	+	–	Euthyroid	+ Th2	+
Transfer cDNA	+	+	–	Euthyroid	+ Th2	+
Transfer class II	+	+	–	T ₄ ↓	–	–

TSAB, considered positive only when more than 1 animal developed persistent antibodies giving at least a doubling of the cAMP levels; ND, not done; Fibro, fibroblasts transfected with TSHR and MHC class II; prot, transfer of T cells *in vivo* primed with ECD-MBP. +, Present; –, absent.

observed only in mice having the most severe thyroiditis, with 25–30% of the gland occupied by interstitium, which also correlated with the most skewed Th2 response: B:T cell ratio 1.6–1.9 and IL-4:INF γ ratio >2.5.

Finally, passive transfer to naive recipients was performed using either spleen or i.p cells from receptor + class II fibroblast-treated mice (41). The sole criterion for successful transfer was thyroid hormone status. Of a total of 8 animals receiving cells from hyperthyroid donors, only one recipient was transiently hyperthyroid and one was hypothyroid. When transferring hypothyroid donor cells, all four recipients became hypothyroid. We have reported that transfer of non-primed control splenocytes can reduce thyroid hormone levels, particularly in some strains of mice. Thus, it might be unwise to claim transfer of disease on the basis of change in thyroid hormone levels alone.

Concluding comments

It would seem that it is very easy to induce receptor antibodies lacking biological activity in almost any mouse strain. TBII (and to a lesser extent TBAB) and reduced T₄ levels are relatively easy to induce, especially in BALBc mice which can also develop hyperthyroxinaemia.

On the other hand, TSAB and thyroiditis (and consequently orbital pathology akin to TED) are very difficult to induce. TSAB require administration of receptor in the correct conformation to bind TSH, whether via the cDNA or cells transfected with the full length TSHR. Thyroiditis, which is the hallmark of an autoimmune response induced to the endogenous receptor, may require a combination of genetic background and environmental factors. Orbital pathology seems to depend on a Th2 response to the thyroidal TSHR but does not require TSAB.

As shown in Table 1 which compares the signs and symptoms of GD induced by the various protocols, the most promising is the most recent, i.e. genetic immunisation of outbred mice. Furthermore, this protocol offers a unique opportunity of identifying the genes

implicated in the loss of tolerance to the TSHR, leading to thyroiditis with or without accompanying eye disease. This protocol is unusual in its use of outbred animals but humans are outbred and can, indeed, be induced to develop GD. Patients with multiple sclerosis (MS), who were treated *in vivo* with a monoclonal antibody (Campath-1H) to CD52, resulting in elimination of >95% of circulating lymphocytes, displayed considerable improvement of their MS (46). Eighteen months after treatment, T cell numbers had returned to 35% and B cells to 180% of pretreatment values, but 12/34 patients had developed GD with hyperthyroidism and antibodies to the TSHR. Campath-1H results in immune deviation away from Th1, hence the improvement in MS but, unfortunately, it allows the expansion of a Th2 response with production of TSAB leading to GD.

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