Chapter 2
Immunopathogenesis of Graves' Disease

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Abbreviations

Aire	Autoimmune regulator
APECED	Autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy
APS-1	Autoimmune polyendocrine syndrome type 1
cAMP	3′-5′-Cyclic adenosine monophosphate
CD4	Marker on helper T cells
CD25	Interleukin-2 receptor α chain
CD122	Interleukin-2 receptor β chain
CD8	Marker on cytotoxic T cells
CHO	Chinese hamster ovary cells
ELISA	Enzyme-linked immunoassay
ECD	Extracellular domain of the TSHR
Foxp3	Forkhead box P3 protein
LATS	Long-acting thyroid stimulator
LRD	Leucine-rich repeat domain of the TSHR
LH	Luteinizing hormone
SNP	Single nucleotide polymorphism
TBAb	TSH blocking antibody
TBI	TSH binding inhibition
Treg	Regulatory T cells
TSH	Thyrotropin

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TSHR	Thyrotropin receptor
TMD	Transmembrane domain of the TSHR
TSAb	Thyroid stimulating antibody
VNTR	Variable number of tandem repeats

Evidence That GD is an Autoimmune Disease

In 1835, Robert Graves described six pregnant women with diffuse goiter and hyperthyroidism [1]. Based on his paper, this condition is called "Graves' disease" in the UK and the US. Until 1956, the hyperthyroidism was believed to be of pituitary origin, presumably caused by thyrotropin (TSH). In that year, Adams and Purves reported that sera of Graves' patients contained a thyroid stimulating factor with a duration of action more prolonged than TSH, hence the term "long-acting thyroid stimulator" (LATS) [2]. The identification in 1964 that LATS was an immunoglobulin G molecule [3, 4] introduced the radical concept that autoimmunity can stimulate as well as destroy a target organ. Discovery of the TSH receptor (TSHR) in 1966 [5] was followed by the observations in 1970 and 1974 that LATS, like TSH, activated thyrocyte adenylyl cyclase and competed for TSH binding to the TSHR (reviewed in [6]). In addition to thyroid stimulating autoantibodies (TSAb), TSHR autoantibodies lacking agonist activity but capable of competing for TSH binding were found to be responsible for rare cases of autoimmune hypothyroidism (reviewed in [6]).

The role of TSHR antibodies in causing Graves' disease satisfied two of the Witebsky and Rose postulates for autoimmunity, namely the "direct demonstration of free circulating antibodies active at body temperature" and "recognition of the specific antigen (for this antibody)" [7]. Maternal transfer of TSHR antibodies leading to neonatal hyperthyroidism [8] provided powerful confirmation of the part played by TSHR autoantibodies. The other two requirements postulated by these eminent immunologists, involving immunization in animals, proved more difficult to fulfill. Unlike many autoantigens, only very small amounts of TSHR protein are present in the thyroid, precluding purification for use in immunization. Following its cloning in 1989 [9–11], recombinant TSHR protein was used to generate "antibodies against same antigen in experimental animals" (reviewed in [12]). However, TSHR antibodies induced by conventional immunization did not fulfill the final postulate, namely that the "experimental animal demonstrates same tissue changes in human."

In 1996, stimulatory TSHR antibodies and hyperthyroidism were induced in mice by injecting intact eukaryotic cells expressing the TSHR [13]. Building on this novel immunization approach, plasmid or adenoviral vectors were subsequently used to express the TSHR in vivo and induce TSAb and Graves'-like hyperthyroidism in mice or hamsters (reviewed in [14, 15]). These studies complement investigations in humans of Graves' disease, the commonest organ-specific autoimmune condition, with a prevalence of ~1 % [16].

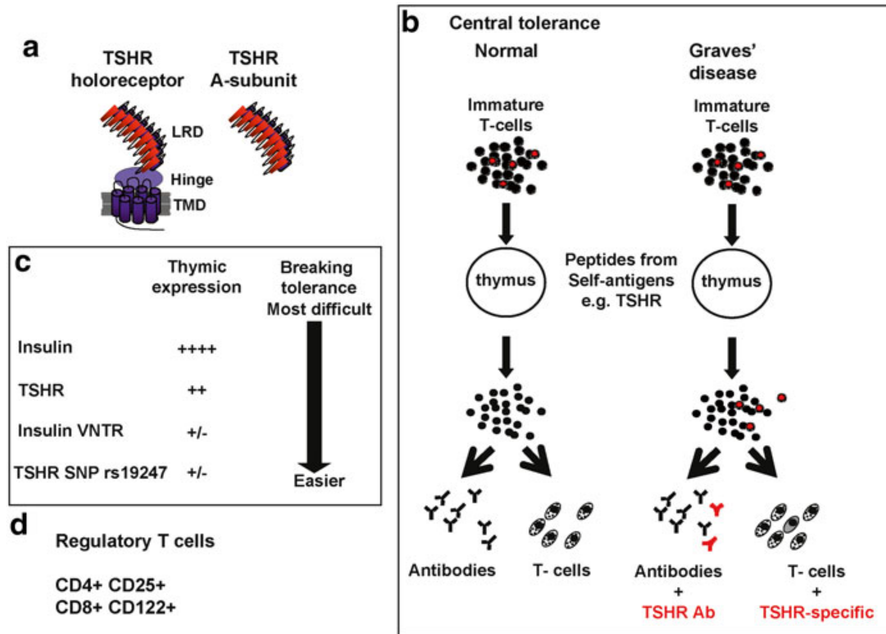

Fig. 2.1 (**a**) Schematic representation of the TSH holoreceptor including its transmembrane domain (*left*) and the TSHR A-subunit (*right*). (**b**) Central tolerance in a normal individual versus a patient with Graves' disease. (**c**) Relationship between thymic expression of insulin and the TSHR (and genetic variants) in relation to expectations for breaking self-tolerance. Insulin VNTR [29]; TSHR SNP rs19247 [30]. (**d**) Regulatory T cells; markers for subsets

Why Do TSHR Antibodies Develop?

TSHR antibodies develop in genetically susceptible individuals because of a breakdown in self tolerance to the TSHR, probably in association with environmental factors. Self-tolerance is a complex process. In addition, the characteristics of the TSHR itself play a role in the development of thyroid autoimmunity.

Characteristics of the TSHR: The TSHR, a member of the group A rhodopsin-like family, is a G-protein coupled receptor with seven membrane spanning domains. The extracellular portion, or domain (ECD) comprises a leucine-rich repeat domain (LRD) linked by a hinge region to the transmembrane domain (TMD). After synthesis and trafficking to the thyrocyte surface, some of the single polypeptide chain TSHR undergo intramolecular cleavage at two or more sites within the hinge region resulting in the loss of a C-peptide component. This posttranslational modification results in a two subunit structure with an extracellular A-subunit linked by disulfide bonds to a B-subunit comprising the remaining portion of the hinge region linked to the TMD (Fig. 2.1a, left panel).

In recent years, the molecular structures of the different TSHR components have been determined by X-ray crystallography or have been deduced by molecular modeling based on related molecules whose structures are known. Thus, the crystal structure of the TSHR LRD reveals it to comprise a slightly curved, oval-shaped tube with α-helix/β-strand repeats [17]. A fairly accurate structure for the TMD can be deduced by modeling [18] on the crystal structure of a related molecule, rhodopsin [19]. Until recently, the structure of the hinge region has been enigmatic. However, solving the crystal structure of the entire ECD (LRD plus hinge region) of the closely related FSH receptor [20] has enabled modeling of much of the TSHR hinge region [21, 22]. Because a portion of the TSHR hinge region comprises a poorly delineated "insertion" of approximately 50 amino acid residues relative to the gonadotropin receptors, this region cannot be modeled on the FSHR.

The loss of the C-peptide region during intramolecular cleavage of the TSH holoreceptor into disulfide-linked A and B subunits does not affect receptor function, either in its basal state or in response to ligand stimulation [23, 24]. However, disruption of the disulfide linkage, either by a specific enzyme or by continued proteolysis following intramolecular cleavage, leads to shedding of the TSHR A-subunit. There is strong evidence that the shed TSHR A-subunit rather than the membrane-bound holoreceptor (Fig. 2.1) is the autoantigen that induces the generation of the pathogenic autoantibodies that lead to hyperthyroidism in Graves' disease (reviewed in [25]). It is noteworthy that the other closely related members of the glycoprotein hormone receptor family, the luteinizing hormone- and follicle stimulating hormone receptors (LHR and FSHR, respectively), do not undergo intramolecular cleavage and shedding of a portion of their ectodomains. Unlike the TSHR, these gonadotropin receptors do not induce autoimmune responses in humans. The TSHR A-subunit is a heavily glycosylated soluble protein with a molecular weight of about 60 kDa. Perhaps unexpectedly in view of its central role in Graves' disease, the TSHR A-subunit is the least abundant of the three major thyroid autoantigens, the others being thyroglobulin and thyroid peroxidase.

Central tolerance: Central tolerance is based on negative selection of autoreactive T-cells in the thymus [26]. Immature T-cells generated in the bone marrow enter the thymus where they undergo processes of negative and positive selection (Fig. 2.1b). Stromal thymic medullary epithelial cells "ectopically" express a spectrum of peptides from self-proteins [27] and, in cooperation with dendritic cells, present them to immature T-cells (reviewed in [28]). T-cells that recognize self-peptides with high affinity are deleted from the repertoire [26]. In this "education" process, T-cells with moderate affinities for self-peptides are positively selected to undergo further differentiation and leave the thymus to become mature T-cells. In contrast, T- and B-cells with specificity for the TSHR may not be deleted in individuals subsequently susceptible to Graves' disease.

The degree of self-tolerance is related to the amount of autoantigen expressed in the thymus. Insulin, for example, is highly expressed in the thymus (Fig. 2.1c). A type I diabetes susceptibility locus in humans maps to a variable number of tandem repeats (VNTR) upstream of the insulin gene. This VNTR locus controls the level of intrathymic insulin expression and, by maintaining tolerance to insulin, is protective of disease [29].

Individuals homozygous or heterozygous for TSHR single nucleotide polymorphism (SNP) 179247, that is associated with Graves' disease, have significantly fewer thymic TSHR mRNA transcripts than individuals homozygous for the protective allele [30]. Thus, as for the insulin VNTR locus, lower intrathymic expression of the TSHR is likely to decrease central tolerance. As a result, T cells specific for the TSHR will not be deleted in the thymus (Fig. 2.1b, right). Persistence of these cells in the periphery, together with TSHR-specific B cells, will enhance the possibility of a triggering event leading to their activation and the generation of TSHR antibodies.

Autoimmune regulator (*Aire*): Intrathymic expression of a number of autoantigens is controlled by Aire and autoimmunity develops spontaneously in its absence (reviewed in [31]). In mice lacking one or both Aire alleles, thymic expression of insulin is reduced or absent [32, 33] but other autoantigens such as glutamic acid decarboxylase and α-fodrin in diabetes mellitus type 1 are unaffected by the absence of Aire [34, 35].

Patients with autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy (APECED) or autoimmune polyendocrine syndrome type 1 (APS-1) have mutations in Aire. In contrast, Aire mutations are not by themselves susceptibility genes for autoimmune thyroid disease (for example [36, 37]). Fifty percent of APECED patients in southern Italy had antibodies to thyroglobulin and thyroid peroxidase, as well as hypothyroidism in some patients [38]. However, Graves' disease has not been reported in APECED/APS-1 patients.

Regulatory T-cells: Deletion of auto-reactive T cells by central tolerance may not eliminate all self-reactive cells. Another potent mechanism for self-tolerance involves regulatory T cells (Treg) (Fig. 2.1d). Treg may be "natural" (constitutive) or inducible (involved in the adaptive immune response). Natural Treg develop in the thymus [39]. Both natural and inducible Treg are characterized by the expression of CD4, CD25 (the interleukin-2 receptor α chain) and the transcription factor Foxp3 (forkhead box P3 protein) (reviewed in [40]). Cell deletion studies showed that natural CD4+ CD25+ Treg regulate (for example) the development of autoimmune gastritis in BALB/c mice [41]. Another subset of Treg that express CD8 and CD122 (interleukin-2 receptor β chain) also controls auto-reactive effector T-cells in the periphery [42, 43].

Cytokines are involved in the effector mechanisms of Treg. For example, tumor necrosis factor or antibody to tumor necrosis factor regulate CD4+ CD25+ T-cells in Non-Obese diabetic mice [44]. In addition, CD8, CD122 expressing Treg generate interleukin 10 which suppresses production of interferon -γ as well as the proliferation of CD8 positive T cells [45].

B-cell tolerance: Immunoglobulin molecules expressed on the B-cell surface function as antigen receptors. If the rearranged immunoglobulin variable region genes have specificity for an autoantigen, B-cells can "edit" and replace their receptors with different antibody gene arrangements (for example [46]). Self-antigen-specific B-cells can be tolerized by other mechanisms including clonal deletion, anergy (functional inactivation) and perhaps competition for B cell growth factors.

Because T cells are required to stimulate B cells to proliferate and secrete IgG antibodies, tolerance mechanisms in B cells may be regarded as a secondary, or "fail-safe," mechanism. However, the increasingly recognized role of B cells as professional antigen presenting cells emphasizes the importance of silencing B cell autoreactivity even when the major players are T cells.

Tolerogenic dendritic cells: Plasmacytoid dendritic cells generate type 1 interferon in response to viral RNA or DNA. Their activities are complex: on the one hand, plasmacytoid dendritic cells are immunogenic because they have the ability to present antigens and induce naive T cells to differentiate. On the other hand, plasmacytoid dendritic cells can be tolerogenic by inducing deletion of CD8 positive cells and effector CD4 positive T cells. These cells contribute to both innate and adaptive immunity and should be considered as likely contributors to autoimmunity (reviewed in [47]).

TSHR Antibodies: Immunological Markers of Graves' Disease

T cells specific for the TSHR, their cytokine responses and relationship to MHC antigens, have been described [48–50]. Autoantigen-specific T cells are, of course, critical for the generation of IgG class autoantibodies. However, TSHR antibodies, the direct cause of hyperthyroidism, are the indisputable immunological markers of Graves' disease.

Unlike autoantibodies to other autoantigens that are measured by ELISA or Western blots, TSHR autoantibodies cannot be measured by such assays with sufficient sensitivity or specificity for clinical use. Instead, assays for TSHR autoantibodies can be separated into two types: assays involving competition for ligand binding to the TSHR and bioassays using intact cells in tissue culture. Beginning approximately 40 years ago, each type of assay has undergone extensive modifications (Table 2.1).

Competition assays: The "first generation" of competition assays involved inhibition by TSHR autoantibodies for radiolabeled TSH binding to porcine thyroid membranes or membrane extracts [51, 52]. Second generation assays included the use of porcine TSHR or recombinant human TSHR in solid phase rather than in solution and TSH ligand [53, 54]. In the "third generation" assay, a tagged human monoclonal TSHR autoantibody replaced TSH [55]. Both second and third generation assays provide comparable excellent sensitivity and specificity [56].

Bioassays: Bioassays for TSHR stimulating antibodies (TSAb) measure the ability of TSHR antibodies or TSH to increase intracellular cAMP levels in thyroid cell monolayers, initially using human thyroid cells [57, 58]. Subsequent assays employed a rat thyroid cell line [59] porcine thyroid cells [60] and, more recently, Chinese hamster ovary (CHO) cells expressing the recombinant human TSHR [61]. The sensitivity of the cultured thyroid cells assays was increased by using IgG or serum diluted in hypotonic medium [62] or in medium containing polyethylene glycol [63]. In some assays cAMP is detected indirectly by means of a light generating reporter molecule [64].

Table 2.1 Development since 1974 of assays for TSHR autoantibodies

Competition for ligand binding to the TSHR (*TBI*)				
Species	*TSHR source*	*Ligand*	*Format*	*References*
Porcine	Membranes	TSH	Suspension	[51]
	Membrane extracts	TSH	Suspension	[52]
Porcine	Membrane extracts	TSH	Solid phase	[53]
Human	Recombinant	TSH	Solid phase	[54]
Porcine	Recombinant	M22	Solid phase	[55]
Bioassays for TSHR stimulating antibodies (TSAb)				
Species	*Source/type*	*Serum/IgG*	*Signal*	*References*
Human	Thyroid monolayers	Serum/IgG	cAMP	[57, 58]
Rat	Thyroid cell line	Serum/IgG	cAMP	[59]
Porcine	Thyroid monolayers	IgG	cAMP	[60]
Human	CHO cell monolayer	Serum/IgG	cAMP	[61]
		Serum	Light[a]	[64]
Human-rat	CHO cell monolayer	Serum/IgG	Light[a]	[65]
TSHR-LHR				
Bioassays for TSH blocking antibodies (*TBAb*)				
Similar to TSAb but performed without and with a standard low TSH concentration				

[a]cAMP-dependent luciferase reporter gene
CHO chinese hamster ovary cells

Because both TSH blocking antibodies (TBAb) and TSAb will be positive in the TBI assay, only the bioassay can be used to specifically detect the former type of autoantibody. The recent use of a bioassay with CHO cells expressing a chimeric (TSH-LH) recombinant receptor to specifically detect TSAb and exclude TBAb [65] does not have a theoretical basis for such a property [66, 67], as confirmed in practice [68].

Nomenclature: The competition assays are most simply described as "TSH binding inhibition (TBI)" assays. TBII (TSH binding inhibitory immunoglobulin) is unnecessarily complex and TRAb (TSHR antibody) does not distinguish the competition assays from the bioassays. Although TSH is no longer used in some TBI assays, the term TBI can still describe competition for a TSHR autoantibody. Regarding the bioassays, TSAb (thyroid stimulating antibody) rather than the older term TSI (thyroid stimulating immunoglobulin) is preferable for consistency with TBAb (TSH blocking antibodies). The commonly used term TSBAb (TSH or thyroid stimulating blocking antibody), a tongue twister, is redundant because TSH is inherently a stimulator.

Characteristics of TSHR Antibodies

TSHR antibodies that stimulate the receptor (TSAb) are present at very low concentrations in serum [69–71]. In contrast, TSH blocking antibodies (TBAb) are present at much higher concentrations [71, 72]. The immunological properties of TSHR

antibodies are summarized in Table 2.2. The data include observations for serum TSHR antibodies as well as for human monoclonal TSHR antibodies [73–75]. TSHR antibodies are predominantly IgG although although IgA- and IgE-class TSHR antibodies have been observed by flow cytometry [76]. It should be noted that TSHR antibodies in sera as well as human monoclonals have extremely high affinities, as would be expected for antibodies that compete with TSH for binding to its receptor.

TSHR Autoantibody Epitopes

Features of TSHR autoantibody epitopes: Given the complexity of the TSHR structure, it is not surprising that defining the binding sites (epitopes) of autoantibodies to the TSHR has been a difficult task. The crowning achievements in this endeavor have been the recent determinations of the crystal structures of an human TSAb (M22) [17] and an human TBAb (K1-70) [77] in complex with the LRD component of the TSHR ECD (see above). These breakthroughs were facilitated by the cloning of monoclonal human TSAb and TBAb from the B-cells of patients with Graves' disease [73–75]. This information supports the deduction from earlier studies involving chimeric substitutions between the receptors for TSH and luteinizing hormone (LH) that human TSHR autoantibodies in humans, like most antibodies to complex globular proteins [78], do not recognize "linear" epitopes, but rather conformational epitopes formed by discontinuous segments in the amino acid sequence [66, 79]. Unlike human TSHR autoantibodies, some monoclonal TSHR antibodies generated by immunizing mice with TSHR protein do recognize linear epitopes, but these lack biological activity. Consistent with human autoantibodies, monoclonal mouse TSAb such as KSAb1 and KSAb2 [80] do not recognize linear epitopes.

Determination by X-ray crystallography of the molecular interactions between a human TSAb and human TBAb with the TSHR LRD also puts to rest one of the long standing mantras in the field that TSAb recognize the N-terminus and TBAb the C-terminus of the TSHR ECD. This view was generally held despite contrary evidence from chimeric receptor studies [66]. Indeed, the crystallography data (albeit from single TSAb and TBAb) reveal that the TSAb and TBAb epitopes largely overlap and that the TBAb epitope is actually situated more towards the TSHR N-terminus than the TSAb. Moreover, this information invalidates the theoretical basis for a new TSHR antibody bioassay originally purported to distinguish between TSAb and TBAb (see above). Of course, structural data from single human monoclonal TSAb and TBAb do not exclude the possibility that other TSHR autoantibodies have nonidentical epitopes. The view we favor, based on experimental evidence [67], is that the TSAb epitope(s) is more restricted to a "sweet spot" on the receptor that leads to receptor activation, whereas TBAb epitopes are more diverse. In order to block TSH binding by steric hindrance, an antibody can bind to a wide range of sites that overlap only partially with the TSH binding site.

Table 2.2 Characteristics of human TSHR antibodies

Serum or MAb	Concentration in serum	Affinity (Kd)	IgG subclass	Light chain	References
TSAb (common; characteristic of Graves' disease)					
Serum	50–500 ng/ml	$1.5–3.3 \times 10^{-11}$ M	IgG1, IgG4	Kappa, lambda	[71, 95, 96]
M22	–	2.0×10^{-11} M	IgG1	Lambda	[73]
K1-18		2.5×10^{-11} M	IgG1	Kappa	[75]
TBAb (in rare hypothyroid patients)					
Serum	1.7–27 μg/ml	$1.4–3.3 \times 10^{-11}$ M	IgG1, IgG2, IgG3	Kappa, lambda	[71, 97]
5C9	–	2.5×10^{-11} M	IgG1	Kappa	[74]
K1-70	–		IgG1	lambda	[75]

It should be emphasized that determining the precise amino acids comprising the epitope in the TSHR LRD of a monoclonal TSAb does not explain how TSAb activate the TSHR. This understanding will require knowledge of the three-dimensional structure of the entire TSHR, including the orientation of its individual components and, perhaps, its quaternary structure. The TSHR is known to multimerize [81, 82] but the number and arrangement of the protomers in this complex are unclear. It is known that there is partial steric hindrance to TSAb interaction with the TSH holoreceptor on the cell surface [72, 83]. High-affinity interactions between antibodies such as TSAb and the TSHR are largely determined by antigen-driven affinity maturation (mutagenesis) in the complementarity determining regions of the heavy and light chains. However, other regions of TSAb (such as the framework region) may also impinge on the TSHR, and not only at LRD residues revealed by the crystal structure but also further downstream in the hinge region [66, 84]. On the basis of the foregoing information, we propose that TSAb activation of the TSHR occurs because of partial steric hindrance to its primary binding site at other regions of the receptor protomer or multimer.

Switching from Hyper- to Hypothyroidism (or Vice Versa)

Switching between predominant TBAb to TSAb activities (or vice-versa) occurs in unusual patients after thyroxine (L-T4) therapy for hypothyroidism or anti-thyroid drug treatment for Graves' disease. TBAb-induced hypothyroidism must be distinguished from Hashimoto's thyroiditis (even more common than Graves' disease) in which massive lymphocytic infiltration and fibrosis overwhelms the TSH-driven regenerative capacity of the thyroid gland for example [85, 86].

Examination of case reports has provided insight into the basis for "switching" (reviewed in [87]) (summarized in Table 2.3). TSHR Ab switching involves differences in TSAb versus TBAb concentrations, affinities and/or potencies in individual patients. Thus, anti-thyroid drugs or suppression/hemodilution in pregnancy reduce initially low TSAb levels even further and lead to TBAb dominance. In contrast, emergence of TSAb following L-T4 administration may be sufficient to counteract

Table 2.3 Insight into the basis for "switching" between TSAb and TBAb (or vice versa) based on case reports (reviewed in [87])

	Factors that may impact the shift
(a)	L-T4 treatment, usually associated with decreased thyroid autoantibodies, occasionally induces or enhances thyroid autoantibody levels
(b)	Antithyroid drug treatment decreases thyroid autoantibody levels
(c)	Hyperthyroidism polarizes antigen presenting cells leading to impaired development of regulatory T cells
(d)	Immune suppression/hemodilution reduces thyroid autoantibodies during pregnancy with a rebound postpartum
(e)	Maternally transferred IgG transiently impacts thyroid function in neonates

TBAb inhibition, if present. Of interest, a model of Graves' disease involving immunizing TSHR "knockout mice" with mouse TSHR-adenovirus and transfer of TSHR antibody secreting splenocytes to athymic mice demonstrates the TSAb to TBAb shift, paralleling the outcome of maternally transferred "term limited" TSHR antibodies in neonates.

Why Measure TSHR Antibodies?

The occurrence of "switching" from TBAb To TSAb (or vice versa) emphasizes the need for careful patient monitoring and management, including measuring TSHR antibodies in selected individuals. In addition, there are other situations where measurement of TSHR antibodies is important:

Pregnancy. TSHR antibodies cross the placenta. In women with Graves' disease previously treated with ^{131}I, especially with a history of ophthalmopathy or dermopathy, TSHR antibody levels can be high and can persist through parturition, though generally declining during the third trimester. The fetal thyroid is fully functional at the end of the first trimester. Therefore, performing a TBI or TSAb test during the first trimester is of value. Even though the mother may be euthyroid or hypothyroid on thyroid hormone replacement, high TSAb levels may cause fetal hyperthyroidism.

If antithyroid drugs are required to treat maternal hyperthyroidism during pregnancy, both methimazole and propylthiouracil cross the placenta and can counteract the effect of TSAb on the fetal thyroid. However, after parturition, neonatal clearance of these drugs is much more rapid than that of TSAb, which can persist for weeks. Therefore, in women with Graves' disease being treated with anti-thyroid drugs, it is of value to determine maternal TSAb in late pregnancy.

High TSHR antibody levels transferred to the fetus can persist unopposed for many weeks and the baby can develop hyperthyroidism even after uneventful delivery and initial home care [8].

Prediction of remission: TSHR Ab levels are higher in Graves' patients that relapse after anti-thyroid drugs than in those that remain in remission (for example [88]). The predictive value is not absolute but provides some insight for physician and patient as to chances of remission if anti-thyroid drug therapy is contemplated. Unlike in Europe and in Asia, this prediction may not be a major issue in the USA where radio-iodine therapy is most commonly the primary choice [89]. However, the situation may be changing with the decrease of the use of radio-iodine therapy in the USA [89] and the very large increase in the number of prescriptions dispensed for methimazole between 1991 and 2008 [90].

Guidance for management of Graves' ophthalmopthy and dermopathy: As will be discussed later (Chap. 13), there is a strong relationship between TSHR autoantibodies and the extra-thyroidal manifestation of Graves' disease. In brief, the TSH

receptor is expressed in orbital tissue and the prevalence of ophthalmopathy in untreated Graves' patients is positively correlated with the levels of TSH receptor autoantibodies [91, 92]. The aggravating effect of radio-iodine therapy (unlike surgery and anti-thyroid drugs) on Graves' ophthalmopathy [93] is associated with an increase in TSHR antibody levels [94]. TBI levels decreased to baseline within one and a half years after medication or surgery but persist above base line levels 5 years after radioiodine [94]. Patients treated with medication or surgery have high numbers of individuals that become TBI negative. In contrast, three years after radio-iodine, only 50 % of patients are TBI negative [94]. In summary, the close relationship between TSHR autoantibodies and Graves' ophthalmopathy provides important insight into the optimal means of treating this distressing condition.

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