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Breaking Tolerance to Thyroid Antigens: Changing Concepts in Thyroid Autoimmunity

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Abstract

Thyroid autoimmunity involves loss of tolerance to thyroid proteins in genetically susceptible individuals in association with environmental factors. In central tolerance, intrathymic autoantigen presentation deletes immature T cells with high affinity for autoantigen-derived peptides. Regulatory T cells provide an alternative mechanism to silence autoimmune T cells in the periphery. The TSH receptor (TSHR), thyroid peroxidase (TPO), and thyroglobulin (Tg) have unusual properties ("immunogenicity") that contribute to breaking tolerance, including size, abundance, membrane association, glycosylation, and polymorphisms. Insight into loss of tolerance to thyroid proteins comes from spontaneous and induced animal models: 1) intrathymic expression controls self-tolerance to the TSHR, not TPO or Tg; 2) regulatory T cells are not involved in TSHR self-tolerance and instead control the balance between Graves' disease and thyroiditis; 3) breaking TSHR tolerance involves contributions from major histocompatibility complex molecules (humans and induced mouse models), TSHR polymorphism(s) (humans), and alternative splicing (mice); 4) loss of tolerance to Tg before TPO indicates that greater Tg immunogenicity vs TPO dominates central tolerance expectations; 5) tolerance is induced by thyroid autoantigen administration before autoimmunity is established; 6) interferon-α therapy for hepatitis C infection enhances thyroid autoimmunity in patients with intact immunity; Graves' disease developing after T-cell depletion reflects reconstitution autoimmunity; and 7) most environmental factors (including excess iodine) "reveal," but do not induce, thyroid autoimmunity. Micro-organisms likely exert their effects via bystander stimulation. Finally, no single mechanism explains the loss of tolerance to thyroid proteins. The goal of inducing self-tolerance to prevent autoimmune thyroid disease will require accurate prediction of at-risk individuals together with an antigen-specific, not blanket, therapeutic approach.

Introduction

The thyroid gland plays a pivotal role in metabolic homeostasis. Graves' disease and Hashimoto's thyroiditis taken together have a prevalence of 2% (1), making autoimmunity to the thyroid gland the most common autoimmune disease affecting humans. These diseases arise because of the loss of tolerance to thyroid antigens in genetically susceptible individuals in association with environmental factors (2). Considerable progress has been made in determining the genes responsible for thyroid autoimmune disease. Moreover, the processes involved in the breakdown in tolerance to “self” thyroid antigens are gradually being revealed. The immunological principles underlying tolerance were originally established for nominal “autoantigens,” such as hen egg lysozyme, in transgenic mice. More recently, these principles have been applied to insulin, one of the autoantigens in type 1 diabetes.

There is presently no evidence that spontaneously arising Graves' disease occurs in species other than humans, whereas autoimmune thyroiditis does occur spontaneously in a number of mammals and birds. Understanding tolerance to thyroid autoantigens and the breakdown leading to thyroid autoimmunity can come from examining the following questions in both spontaneous disease and disease induced in experimental animals: 1) Which autoantigens are targeted in thyroid autoimmunity that develops

spontaneously in humans and other animals? 2) What approaches can be used to induce thyroid autoimmunity in nonhuman mammals? 3) Why does thyroid autoimmunity develop in some humans treated for other diseases? 4) Can induced thyroid autoimmunity be blocked experimentally?

These questions must be considered not only in the context of the immunological basis for self-tolerance but also taking into account information about the characteristics of the thyroid autoantigens. It should be appreciated that, in the spectrum of autoimmune diseases, thyroid autoimmunity is one of the few conditions for which the autoantigens have been unequivocally identified and are known to play a role in disease pathogenesis. Most importantly, the TSH receptor (TSHR), thyroid peroxidase (TPO), and thyroglobulin (Tg) carry within themselves unusual and sometimes unique characteristics that play critical roles in the breakdown in self-tolerance leading to thyroid autoimmunity.

II. Thyroid Autoantigens

A. Three major thyroid autoantigens

Tg, TPO, and the TSHR are complex glycosylated molecules (Figure 1). All three proteins undergo post-translational modifications that are required for their roles in thyroid function and/or greatly impact their ability to stimulate the immune system.

1. Thyroglobulin

Tg is the largest and most abundant thyroid autoantigen in the thyroid (Figure 1A) and, as will be shown later, in the thymus. It is a soluble molecule comprised of two 330-kDa monomers that undergo iodination. Iodination is critical for the function of Tg as a prohormone of T_4 and T_3. However, the presence of iodine is not required for recognition by human autoantibodies (3). Similarly, Tg autoantibodies that arise spontaneously in obese strain (OS) chickens are largely unaffected by high dietary iodine intake (4). In contrast, some induced responses including T-cell clones (5), and mouse monoclonal antibodies (6) are specific for iodinated Tg.

2. Thyroid peroxidase

TPO is the primary enzyme involved in thyroid hormone synthesis. It is a membrane-bound homodimer of two 107-kDa subunits (Figure 1A) with a heme prosthetic group. The heme is essential for enzymatic activity but is not involved in TPO autoantibody recognition (7, 8). Another TPO post-translational modification is the removal by cleavage of the N-terminal approximately 100 amino acids (9). Human T cells and autoantibodies specific for TPO interact with epitopes downstream of amino acid 109 (reviewed in Ref. 10).

3. TSH receptor

The TSHR is a member of the rhodopsin-like family of G protein-coupled receptors with seven transmembrane-spanning domains. The extracellular portion comprises a leucine-rich repeat domain linked by a hinge region to the membrane-spanning domain (Figure 1B). After trafficking to the thyrocyte surface, the holoreceptor undergoes intramolecular cleavage at two or more sites within the hinge region, resulting in the loss of a C-peptide component. This post-translational modification results in an extracellular A-subunit linked by disulfide bonds to a B-subunit comprising the remaining portion of the hinge region linked to the transmembrane-spanning domain. Breaking the disulfide bonds leads to shedding of the TSHR A-subunit (reviewed in Ref. 11). The other closely related members of the glycoprotein hormone receptor family, the LH and FSH receptors, 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. Accumulating observations for human TSHR autoantibodies indicate that the shed

TSHR A-subunit (Figure 1A), rather than the membrane-bound holo receptor (Figure 1B), is the autoantigen in Graves' disease in humans (reviewed in Ref. 11). The TSHR A-subunit is a heavily glycosylated soluble protein with a molecular mass of approximately 60 kDa. Moreover, the A-subunit is the smallest and the least abundant of the three thyroid autoantigens (Figure 1C).

B. Does autoimmunity arise to other thyroid autoantigens?

A possible role for the sodium-iodide symporter (NIS) as an autoantigen was investigated after its cloning in 1996 (12). Unlike TPO and the TSHR, which have large ectodomains, NIS has only a small ectodomain attached to 13 membrane-spanning segments. Some early studies detected autoantibodies in serum from patients with thyroid autoimmune disease that bound to NIS and inhibited its iodide transport function (13, 14). In contrast, other data provided no support for NIS as a major autoantigen (15, 16). Autoimmunity to NIS and to pendrin, another thyroidal iodide transporter, continue to raise interest (17), but a recent study has excluded pendrin from being a major thyroid autoantigen (18).

A role has been suggested for antibodies against the IGF-1 receptor in Graves' ophthalmopathy (19, 20), at least in some patients (21). In contrast, other studies do not support such a relationship (22). Therefore, whether autoimmunity to the IGF-1 receptor is involved in Graves' ophthalmopathy remains controversial. However, there is no doubt about a role for TSHR autoantibodies in causing Graves' disease. Maternally transferred TSHR autoantibodies cause transient Graves' disease in the neonate (23) and injecting a monoclonal stimulatory TSHR antibody is sufficient to induce hyperthyroidism in mice (24). Our focus is, therefore, on the three proteins that have been unequivocally identified as playing a role in thyroid autoimmune disease.

C. Properties of Tg, TPO, and the TSHR A-subunit that confer “immunogenicity”

Several characteristics of the three thyroid autoantigens are favorable for inducing immune responses (asterisks in Figure 1A denote arbitrary scores).

- The number of peptides processed and available for major histocompatibility complex (MHC) binding and presentation to T cells depends, in part, on the size and availability of the protein. Clearly, many more peptides can be generated from the abundant, large Tg protein, the major thyroid component (Figure 1C), than from TPO, which is intermediate in size and amount. Substantially fewer peptides are likely to be available from the TSHR A-subunit, which is not only smaller than Tg and TPO but is present at very low levels in the thyroid (Figure 1C) (25). Support for these differences is provided by the identification of Tg peptides (but not other thyroid-specific peptides) eluted from MHC class II protein purified from human thyroids (26).
- Greater efficacy has been observed for immunization with antigen-presenting cells (APCs) expressing membrane-bound vs providing the same cells with soluble nonself-proteins (27, 28). In this respect, membrane-bound TPO may score higher on an “immunogenicity scale” than soluble Tg or the TSHR A-subunit.
- Glycosylation is important for antigen binding to cell surface mannose receptors on APCs and their subsequent internalization (29), a process that markedly enhances the efficacy of T-cell responses (30). Tg and TPO are less glycosylated (12 and 10%, respectively) than the TSHR A-subunit (~40%). Both Tg and the heavily glycosylated TSHR A-subunit, but not TPO, bind to the mannose receptor (31).
- Polymorphisms in Tg (32) and the TSHR (33), but not TPO, confer susceptibility to thyroid autoimmunity (*Section VI.A.1.*).

Combining these characteristics, Tg has a higher “immunogenicity score” than either TPO or the TSHR A-subunit (Figure 1A).

III. Spontaneous Thyroid Autoimmunity

A. Thyroid autoimmunity in humans (Table 1)

Thyroid-stimulating antibodies (TSAbs) that activate the TSHR are the direct cause of Graves' hyperthyroidism (34) (reviewed in Ref. 11). In a small number of individuals, hypothyroidism and thyroid atrophy are caused by TSH-blocking antibodies (TBAbs) that are competitive inhibitors for ligand activation of the TSHR (35, 36). Neutral, neither stimulating nor blocking, TSHR antibodies may contribute to Graves' hyperthyroidism as well as to Graves' ophthalmopathy (37). TSHR antibodies and probably also TSHR-specific T cells and cytokines play a role in Graves' ophthalmopathy and dermopathy (reviewed in Ref. 38).

At the opposite end of the spectrum, autoimmunity to TPO and/or Tg is associated with lymphocytic infiltration of the thyroid and sometimes with hypothyroidism (reviewed in Ref. 39). Humoral autoimmune responses were first observed for autoantibodies to Tg (40) and the “thyroid microsomal autoantigen” (41) before the latter was identified as TPO immunologically (42, 43) and by molecular cloning (44, 45). Subsequently, T cells and cytokine responses specific for TPO and Tg were demonstrated (46–51).

Many adults, particularly women, have autoantibodies (IgG class) to TPO and/or Tg but do not progress to hypothyroidism (52). Autopsy studies have demonstrated the presence of thyroid lymphocytic infiltration associated with the presence in serum of thyroid autoantibodies in the absence of clinical disease (53). The important implication of this association is that TPO and Tg autoantibodies in euthyroid individuals are not simply assay background noise or pathophysiologically insignificant. Rather, the detection of these autoantibodies reflects subclinical disease in a gland with a large functional reserve, as recently demonstrated in vivo by ultrasound (54). Tolerance to TPO and/or Tg is clearly broken in individuals with subclinical autoimmune thyroid disease (52). Consequently, the term “Hashimoto's thyroiditis” refers to individuals with thyroid autoantibodies regardless of their thyroid function status. It should also be appreciated that many Graves' patients have autoantibodies to TPO and sometimes also to Tg (55), indicating overlap in the breakdown in self-tolerance to more than one thyroid autoantigen.

B. Spontaneous thyroiditis in other animals

Without immunization, thyroiditis develops spontaneously in Biobreeding rats (56), nonobese diabetic (NOD) (57) and NOD.H2h4 (58–60) mice, OS chickens (61, 62), some breeds of dogs (63, 64), and some (65) but not all (66) colonies of marmosets (Table 1). It is striking that unlike the occurrence of thyroiditis in many nonhuman species, neither TSHR antibodies nor Graves' hyperthyroidism develop spontaneously in animals.

C. Cellular interactions leading to immune responses

Autoimmune responses, like those to exogenous proteins, are complex (Figure 2). Briefly, proteins taken up by “professional” APCs, namely macrophages, dendritic cells (DCs), and sometimes B cells, are processed into peptides that bind to MHC molecules for presentation to the T-cell receptor. In addition to recognition by T-cell receptors of MHC-bound peptides, T-cell activation requires a process of “costimulation” involving several other critical molecules:

- APCs constitutively express CD40, and T cells constitutively express CD28 on their surface.
- The interaction between the T-cell receptor and MHC peptide leads to the induction on T cells of

CD40 ligand (italics in Figure 2).

- Binding of CD40 to CD40 ligand induces B7–1/2 expression on the APC (italics in Figure 2).
- B71/2 binding to CD28 on T cells completes costimulation and initiates T-cell activation. This process also induces expression of the inhibitory molecule CTLA4.

Activated T helper cells provide “help” for B cells that recognize the same antigen to replicate and subsequently differentiate into plasma cells that secrete antibody. Helper T cells can also develop (for example) into thyroid-infiltrating cells or provide help for the development of cytotoxic T cells. It should be emphasized that B cells differ from other professional APCs by virtue of their membrane-associated Ig receptors. Thus, unlike capturing and internalizing many antigens in their environment, B-cell surface Ig function as receptors for specific antigens, even if present in minute amounts. These specific antigens (or autoantigens) are then internalized, processed, and presented to T cells (67). The importance of B cells as APCs is illustrated by the inability of NOD mice lacking B cells to develop type 1 diabetes and by the absence of thyroiditis in B-cell knockout NOD.H2h4 mice (68, 69). Moreover, the ability of B cells to control T-cell activity (70) is illustrated by the efficacy of a therapeutic antibody (rituximab) to target CD20 expressed on B cells in ameliorating Graves' ophthalmopathy in humans (71) as well as hyperthyroidism in an animal model of Graves' disease (72).

The processes of antigen presentation, T-cell help, and B-cell differentiation are subject to regulation. As described in *Sections VI.A.1.* and *VI.B.2.*, genetic susceptibility to thyroid autoimmunity involves polymorphisms in the TSHR and Tg as well as in molecules responsible for regulating these responses. In contrast, some features enhance immunity, like the TSHR itself (Figure 1).

IV. Immunological Basis for Self-Tolerance

Development of tolerance is a complex process that includes central and peripheral mechanisms acting together to eliminate or suppress self-reactive lymphocytes.

A. Central tolerance

Immature T cells generated in the bone marrow enter the thymus where they undergo processes of negative and positive selection and finally exit as CD4+ or CD8+ T cells depleted of high-affinity binding to self-peptides.

The process of acquiring functional T-cell receptors and CD4 or CD8 occurs in the thymic cortex (73). Briefly, the T-cell receptor comprises two chains (α and β), each derived from subunits that are separated in the germline and need to be productively rearranged. In the thymus, cells with productively rearranged T-cell β-chains are paired with the pre-α-chain to form the pre-T-cell receptor. Pre-T cells, which lack expression of the characteristic T-cell markers, CD3, CD4, and CD8, expand and give rise to CD4+ CD8+ double positive cells. In the next steps, pre-α-chains are replaced by productively rearranged α-chains, and the mature T cells become committed to either the CD4 or the CD8 lineage.

Central tolerance is based on negative selection of autoreactive T cells in the thymic medulla (74). As illustrated in Figure 3A, stromal thymic medullary epithelial cells “ectopically” express a spectrum of peptides from self-proteins (75) and, in cooperation with DCs, present them to immature T cells (reviewed in Ref. 76). T cells that recognize self-peptides with high affinity are deleted from the repertoire (74). 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. A naturally occurring example of central tolerance is provided by the autoantigen insulin. A type 1 diabetes susceptibility locus in humans maps to a variable number of tandem repeats (VNTR) upstream of the insulin gene (77, 78). This VNTR

locus controls the level of intrathymic insulin expression and, by maintaining tolerance to insulin, is protective of disease.

The relationship between the concentration of autoantigen in the thymus and the degree of self-tolerance has been dissected in detail in the following elegant experiments. Transgenic mice were generated for hen egg lysozyme (HEL) expressed systemically or targeted to thyrocytes or pancreatic islet cells (79). Single transgenics were crossed with mice transgenic for a HEL-specific T-cell receptor. HEL expression was confirmed in the specifically targeted tissues (thyrocytes or islet cells) but was only detected in thymic tissues of the systemic HEL transgenics. The reduction in peripheral T cells expressing the HEL-specific T-cell receptor correlated with the extent of thymic HEL expression and was directly related to the degree of self-tolerance to HEL immunization. There was greater HEL self-tolerance in systemic HEL transgenics, less in HEL-thyroid transgenics, and least in HEL-islet cell transgenics (79).

Of interest, differences have been observed for the magnitude of T-cell tolerance in relation to transgenic expression of membrane-bound vs soluble autoantigen. In one study, T-cell tolerance was greater for membrane-bound than for soluble HEL expressed systemically using the MHC class I promoter (79). However, a second study of HEL transgenics generated using the α-crystallin promoter demonstrated greater T-cell tolerance for the soluble vs membrane-bound HEL (80). These findings emphasize that the general “rules” for central tolerance are not universally applicable and depend on the manner of expression. In these HEL models, the antigen is the same. However, as will be illustrated later, the rules for central tolerance are not necessarily the same for individual-specific autoantigens.

B. Autoimmune regulator (Aire)

Insight into the factor(s) controlling thymic self-protein expression came from investigations of autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy (APECED), also known as autoimmune polyendocrine syndrome-type 1. This condition is linked to defects in the autoimmune regulator (“Aire”) gene (81, 82). Aire is a transcription factor that regulates the expression of numerous self-proteins in medullary thymic epithelial cells. Mice genetically engineered to be Aire deficient have decreased levels of some self-proteins in thymic medullary epithelial cells (83, 84) and display characteristics of APECED patients including self-reactive T cells and autoantibodies (Figure 3B).

Intrathymic expression of a number of autoantigens is controlled by Aire, and autoimmunity develops spontaneously in its absence (reviewed in Ref. 85). In mice lacking one or both Aire alleles, thymic expression of insulin is reduced or absent (86, 87), but other autoantigens including glutamic acid decarboxylase 65 and α-fodrin are unaffected by the absence of Aire (83, 88). It is striking that the spectrum of autoimmunity was modified by the genetic background. Aire-deficient NOD and SJL mice developed thyroiditis and pancreatitis, whereas gastritis was observed in BALB/c mice with minimal autoreactivity in C57BL/6 mice (89).

C. Regulatory T cells

Depletion of autoreactive T cells by central tolerance may not eliminate all self-reactive cells. Another potent mechanism for self-tolerance involves regulatory T cells (Treg) (Figure 3C). Treg may be “natural” (constitutive) or inducible (involved in the adaptive immune response). Natural Treg develop in the thymus (90). Both natural and inducible Treg are characterized by the expression of CD4, CD25 (the IL-2 receptor a chain), and the transcription factor Foxp3 (forkhead box P3 protein) (reviewed in Ref. 91). Deletion studies showed that natural CD4+ CD25+ Treg regulate (for example) the development of autoimmune gastritis in BALB/c mice (92). Another subset of Treg that express CD8 and CD122 (IL-2 receptor b chain) also controls autoreactive effector T cells in the periphery (93, 94).

Cytokines are in part involved in the effector mechanisms of Treg. For example, TNF or antibody to TNF

regulates CD4+CD25+ T cells in NOD mice (95). In addition, CD8+ CD122+ Treg generate IL-10, which suppresses production of interferon (IFN)-γ as well as the proliferation of CD8+ T cells (96).

D. B-cell tolerance

B cells with affinity receptors for self-antigens are tolerized by a number of mechanisms including clonal deletion, anergy (functional inactivation), receptor editing, and perhaps competition for growth factors. Some autoantigens are abundant (like Tg). However, other autoantigens, such as insulin at physiological concentrations, may be too low for B-cell tolerance induction (97).

Insight into the processes involved in B-cell tolerance comes from transgenic mice expressing both the Ig genes for autoantibodies to a specific autoantigen and the autoantigen. In transgenic mice expressing soluble HEL and the genes for a high-affinity HEL antibody, B-cell tolerance did not involve clonal deletion (98). In contrast, B cells expressing the genes for an antibody against MHC, a ubiquitous membrane-bound autoantigen, were eliminated (99). On the other hand, when small amounts of membrane-bound HEL were expressed on thyroid cells, HEL-specific B cells were neither deleted nor inactivated (100). Genetic background also has an impact on B-cell tolerance. In NOD mice expressing both soluble HEL and HEL antibody transgenes, HEL-antibody-specific B cells were not deleted or efficiently anergized, unlike the same transgenes expressed in mice (C57BL/6) not susceptible to autoimmunity (101).

An important check point involves B-cell receptor editing. Ig molecules expressed on the B-cell surface function as antigen receptors. If the rearranged Ig variable (V) region genes have specificity for an autoantigen, B cells can “edit” and replace their receptors with different antibody gene arrangements (102). Although perhaps not involved in breaking tolerance, regulatory B cells may secrete IL-10 (reviewed in 103), and regulating antibody responses may involve, for example, Fcγ RIIB (104) and complement (105). Finally, B-cell tolerance may also be controlled by the limited availability of growth factors such B lymphocyte stimulator, a B-cell survival factor that acts as a peripheral selection checkpoint (106).

Overall, 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 APCs, illustrated by the title “B-cells—Masters of the Immunoverse” (70), emphasizes the importance of silencing B-cell autoreactivity even when the major players are T cells.

E. Tolerogenic dendritic cells

Plasmacytoid DCs (pDCs) generate type 1 IFN in response to viral RNA or DNA. Their activities are complex and not easy to dissect. On the one hand, pDCs are immunogenic because they have the ability to present antigens and induce naive T cells to differentiate. On the other hand, pDCs can be tolerogenic by inducing deletion of CD8+ cells and effector CD4+ T cells. These cells contribute to both innate and adaptive immunity and should be considered as likely contributors to autoimmunity (reviewed in Ref. 107).

V. Induced Thyroid Autoimmunity

The experimental approaches used to induce thyroid autoimmunity, detailed in Tables 2 to 4, are summarized in this section.

A. Conventional approach to induce thyroiditis

Immunization using protein and adjuvant has been used for many years to induce thyroiditis and continues to be an effective approach (Table 2). The following types of thyroid antigen have been used: 1) crude thyroid extracts in rabbits, guinea pigs, dogs (108, 109), and monkeys (110, 111); 2) purified Tg in rabbits

(109, 110) and mice (112–114); 3) purified porcine TPO (115, 116), recombinant mouse TPO ectodomain (117), and selected TPO peptides (118, 119) in mice; and 4) Purified TSHR ectodomain protein in mice (120, 121).

B. Novel approaches to induce thyroiditis

A number of alternative and often novel approaches have been explored to induce thyroiditis in mice (Table 3):

- Neonatal thymectomy and irradiation induce thyroiditis in rats that can be enhanced by administration of the polyaromatic hydrocarbon 3-methylcholanthrene (3-MCA) (122–124).
- Injecting lymphocytes sensitized to Tg together with Tg antibody (TgAb) (125), in vitro activated lymphocytes (126, 127), or lymphocytes sensitized on monolayers of thyrocytes (128).
- Injecting DCs pulsed with Tg (129) or injecting fibroblasts that coexpress TPO and MHC class II (130).
- Implanting a thyroid gland from another mouse and subsequently injecting lipopolysaccharide (LPS) (131).
- Expressing TPO or Tg in vivo using plasmid (132, 133) or adenoviral vectors (134, 135).
- Transgenic expression of a chemokine or a cytokine or deletion of a chemokine receptor. Thyroiditis develops in transgenic mice that express the chemokine CCL21 (136) or the cytokine IFN-α (137) in the thyroid. In addition, NOD mice lacking the chemokine receptor CCR7 develop thyroiditis and primary hypothyroidism (138).

Surprisingly, transgenic intrathyroidal IFN-γ expression caused hypothyroidism without thyroid lymphocytic infiltration (139). Although the explanation for hypothyroidism has been disputed (140), the absence of lymphocytic infiltration was unexpected for the following reasons: previously observed MHC class II expression on thyrocytes from patients with autoimmune thyroid disease combined with the ability of IFN-γ to induce class II expression (141) are the basis for the Bottazzo hypothesis (142), namely that thyrocyte IFN-γ expression plays a critical role in thyroid autoimmunity.

- Transgenic mice (TAZ10) that express a human T-cell receptor specific for TPO (and lack other functional T cells and B cells) develop extensive thyroiditis that leads to hypothyroidism (143, 144). The absence of functional B cells in these mice precludes the development of antibodies.
- A protocol to induce or amplify tumor regression in mice combined with immunization using mouse Tg plus adjuvant led to thyroiditis in a normally resistant strain (145, 146).
- Unexpectedly, thyroiditis developed after depleting Treg before A-subunit adenovirus immunization of transgenic mice expressing the human TSHR A-subunit targeted to the thyroid and expressed at low levels (147).

C. Principles for successful experimentally induced thyroiditis

Most conventional and novel approaches induce antibodies to the immunogen. However, the successful induction of thyroiditis is usually more restricted.

- Syngeneic antigen, that is antigen derived from the species to be immunized, is usually required to induce thyroiditis. For example, thyroiditis was induced in rabbits using rabbit thyroid extract emulsified in Complete Freund's adjuvant (CFA), but not using bovine or human thyroid extract plus

the same adjuvant (CFA) (108). On the other hand, because they are closely related, both monkey and human thyroid extract induced thyroiditis in monkeys (110, 111).

- Genetic background is important, even using syngeneic antigen. When different strains of mice were immunized with adjuvant plus purified murine Tg, thyroiditis developed in “high responder” but not in “low responder” strains. The difference between these responses depended on MHC antigens of class II (I-A and sometimes I-E). Mice positive for I-Ak are high responders to murine Tg, whereas mice positive for I-Ad are low responders (reviewed in Ref. 148).
- TPO-induced thyroiditis is also genetically restricted, but the MHC class II association differs from that for Tg: I-Ab for TPO responders vs I-Ak for TPO nonresponders (118).
- Exceptions to the requirement for syngeneic antigen include the use of human Tg or human TPO in transgenic mice that express the human MHC class II antigen DR3 (114, 132, 134). The use of an unusual, extremely potent adjuvant may explain why wild-type BALB/c mice immunized with human TSHR protein develop thyroiditis.

The explanation for the ability of porcine TPO to induce thyroiditis in C57BL/6 mice (115) is not known. Possible insight is provided by observations of thyroiditis in H2-k mice transgenic for the human T-cell receptor specific for a human TPO peptide (143): binding to IA-k of the human TPO536–547 peptide and the autologous mouse peptide produced similar antigenic surfaces (149).

- As discussed in *Section VII*, thyroiditis and TPOAb sometimes develop despite the “wrong” MHC (BALB/c, I-Ad, and NOD I-Ak).

D. Inducing TSAb and Graves' hyperthyroidism using the human TSHR

The criteria for the successful induction of Graves' disease or its markers are the presence of biologically active antibodies (TSAb) and/or hyperthyroidism. Neither TSAb nor hyperthyroidism was induced by conventional immunization with TSHR protein (reviewed in Ref. 150). In brief, injecting many different mouse strains with recombinant TSHR protein together with a variety of adjuvants induces antibodies that bind TSHR protein in ELISA. However, these antibodies lack the ability to stimulate cAMP release from TSHR-expressing cells (“bioactivity”), and the mice remain euthyroid.

Approaches to induce TSAb and hyperthyroidism are summarized in Table 4.

- Transgenic mice were generated that express the heavy and light chain Ig variable region genes for a human TSAb, B6B7, as human IgM (151). Transgene expression was monitored by measuring human IgM in serum and expressed on B cells. The total number of B cells was lower in the transgenics than in wild-type controls, suggesting clonal deletion of self-reactive B cells. Among 74 TSAb transgenic mice, 50 animals had elevated free T_4, and 24 had undetectable TSH. Other manifestations of hyperthyroidism included thyroid hyperplasia, increased thyroid technetium uptake, and increased basal body temperature. It should be noted that an extremely high concentration (30 000 ng/mL) of B6B7 IgG, obtained after Epstein Barr virus infection of Graves' peripheral blood lymphocytes, was required for maximum stimulation of cAMP levels (152) compared with 2000 and 3000 ng/mL for mouse monoclonals TSmAb1 (153) and IRI-SAb1 (154), 5000 ng/mL for hamster monoclonal MS-1 (155), and 10 ng/mL for M22, the most potent human TSAb monoclonal (156). Expression of B6B7 as an IgM pentamer in the transgenic mice rather than as monomeric IgG may have enhanced the thyroid-stimulating ability of B6B7.
- Antibodies that stimulate the thyroid gland (TSAb), with or without subsequent hyperthyroidism, are induced by injecting mice with intact cells expressing the TSHR (157–161). In one approach rarely

used, mice are immunized with TSHR-expressing B cells plus TSHR protein and adjuvant (159).

- TSAb and/or hyperthyroidism are effectively induced by in vivo expression of the TSHR receptor or its A-subunit using plasmid or adenoviral vectors or plasmid DNA plus in vivo electroporation (162–167). Immunizing mice with the A-subunit is more effective for inducing hyperthyroidism than immunization with a TSHR modified so as not to cleave into two subunits or the full-length TSH holoreceptor (160, 165, 166, 168). In addition, overexpressing CD40 in the thyroid gland increases the severity of murine Graves' disease induced by human A-subunit-adenovirus immunization (169). As will be described in *Section VIII*, CD40 is one of the genes associated with autoimmune thyroid disease (170).

E. Implications and consequences of human TSHR immunization

The use of the human TSHR (or its A-subunit) to induce TSAb and/or hyperthyroidism as described above has several important implications:

- A cross-reacting antigen, rather than the autoantigen, successfully breaks tolerance in mice and in hamsters.
- Testing for TSAb activity in sera from immunized mice is readily performed using Chinese hamster ovary (CHO) cells expressing the human TSHR.
- However, for the mice to become hyperthyroid, TSAb induced using the human TSHR or the human A-subunit must cross-react with the mouse TSHR. Measuring TSAb activity with CHO cells expressing the mouse TSHR demonstrated cross-reactivity in some mouse strains (such as BALB/c, C3H/He) but not in others (such as C57BL/6) (171). These findings explain in part the inability of some strains to develop hyperthyroidism.

F. Immunization with the mouse TSHR

The desirability of using the mouse TSHR for immunization is clear from the above paragraph. However, neither C57BL/6 nor BALB/c mice immunized with the mouse TSHR A-subunit generated TSHR antibodies, although thyroid lymphocytic infiltrates developed in some C57BL/6 mice (172). Two approaches have been adopted to induce Graves' disease by immunization with the mouse TSHR (Table 4, v and vi).

In the first approach, in order to overcome self-tolerance, it was necessary to use TSHR knockout mice (173) to induce TSHR antibodies using mouse A-subunit adenovirus (172). TSHR null mice lacking the TSHR cannot, obviously, respond to TSAb. Therefore, splenocytes were adoptively transferred from mouse A-subunit-Ad immunized TSHR knockout mice into athymic TSHR-expressing mice of the same genetic background (174). Serum TSAb activity determined in an ex vivo assay was present 4–8 weeks after immunizing TSHR knockout mice at the same time interval in athymic recipients of primed splenocytes (174). Consistent with the presence of TSAb activity in serum, some athymic recipients developed hyperthyroidism. However, after 6 months, TSAb activity was replaced by TBAb activity, and athymic recipients of primed splenocytes became hypothyroid (174). In contrast, neither TBAb nor hypothyroidism developed in mice of the same genetic background immunized with human A-subunit in a plasmid (167) or adenovirus vector (175).

The second approach is based on the recent induction of Graves'-like disease in mice by immunization with a mouse TSHR variant, TSHR 739, cloned from thyroid tissue (176). This variant lacks a central part of the leucine-rich domain of the mouse TSHR and does not bind TSH. As for other “noncanonical” spliced variants, it may include untolerized epitopes (177). Moreover, because of its low level of expression in the

thyroid, it is likely to be virtually absent from the thymus. It was suggested to be a candidate autoantigen in autoimmune thyroid disease (176). Although this variant does not appear to correspond to any of the TSHR variants described for human disease, it may be an invaluable tool for the induction of Graves' disease in mice.

G. Novel concepts from experimentally induced thyroiditis and Graves' disease

Several important issues arise from studies of induced thyroid disease in mice.

- The successful outcome of responses in animals immunized with Tg or TPO (Table 2) is different from the well-known hallmarks of thyroid autoimmunity in humans (Table 1). As described in *Section III*, the presence of spontaneously arising IgG class autoantibodies to TPO and/or Tg in many adult humans (52) reflects the presence of thyroid lymphocytic infiltration (53, 54). In contrast, as shown in Tables 2 and 3, virtually all immunized mice develop antibodies to Tg or TPO, but not all strains develop thyroiditis. Induced thyroiditis involves immunization at a site distal from the thyroid, whereas spontaneous thyroiditis likely arises in the target organ (or in the draining lymph nodes). However, this difference does not explain the absence of thyroiditis because thyroid inflammation develops in strains with the appropriate MHC.

The induction of antibodies without thyroiditis indicates that (at least in immunized animals) breaking tolerance at the B-cell level, although requiring T-cell help, occurs more readily than breaking T-cell tolerance leading to thyroid lymphocytic infiltration, which may include cytotoxic T cells. Indeed, if T helper cell tolerance is broken to part of an autoantigen, autoreactive B cells can be activated (178).

- The immunization approach has a major influence on the properties of induced antibodies. The critical difference between biologically active and inactive antibodies induced using TSHR expressed on intact cells or in vivo vs antibodies that develop after conventional immunization has been described. Similarly, it has long been recognized that there are marked differences in the epitopic range of antibodies induced by conventional immunization with Tg and adjuvant (and later with TPO) compared with antibodies that develop in humans. In particular, Tg antibodies or TPO antibodies that arise spontaneously in humans interact with a restricted “immunodominant region” on their respective autoantigens Tg or TPO (179–182).

The first breakthrough to induce Graves' disease in mice involved injecting fibroblasts expressing the TSHR (157, 183). Likewise, mice injected with TPO-expressing fibroblasts, but not human TPO plus adjuvant, develop antibodies closely resembling human TPO autoantibodies in terms of their high affinity and restricted epitopic recognition (184).

- The MHC restriction for thyroiditis induced conventionally, namely I-Ak using Tg and I-Ab using TPO, does not always apply:

First, thyroiditis-prone NOD.H2h4 mice (I-Ak) spontaneously develop autoantibodies to both Tg and TPO (135). Second, thyroiditis develops in BALB/c mice (I-Ad) injected with Her2 tumor cells, then with plasmid DNA encoding Her2 to promote tumor regression, and subsequently immunized with mouse Tg plus adjuvant (145). Third, BALB/c mice (I-Ad) transgenic for the human TSHR A-subunit (low expressors) depleted of Treg and immunized with A-subunit adenovirus develop thyroiditis and autoantibodies to both Tg and TPO (147, 185).

Thyroiditis accompanied by antibody “spreading” to Tg in all three examples, and to TPO in two instances, resembles the situation in many humans who have autoantibodies to both thyroid autoantigens. Importantly, for human A-subunit Lo-expressor transgenics and for NOD.H2h4 mice, the “self” antigens are located in

the thyroid gland.

VI. Genetic Control of Thyroid Autoimmunity in Humans and Animals

Susceptibility to human autoimmune thyroid disease includes genes encoding molecules that play a role in immune function as well as thyroid-specific genes. Excellent overviews of susceptibility genes for thyroid autoimmunity are available (186–188). The goal of the present review is to focus on the genes that exert their effects early in the autoimmune response and thereby control the breakdown in self-tolerance leading to thyroid autoimmunity (Figure 4).

A. Genes that impact tolerance in the thymus

1. TSH receptor

Polymorphisms in the TSHR gene contribute to susceptibility to Graves' disease in humans (189, 190). A single nucleotide polymorphism (SNP) in intron 1 of the TSHR gene is associated with RNA splice variants, ST4 and ST5. The ratio of RNA for the full-length TSHR to the variant ST4 was low for individuals with the TSHR-susceptible genotype and high for the nonassociated genotype (33). These polymorphic variants could contribute to thyroid autoimmunity by increasing the “level of potentially autoantigenic A-subunits” (33).

A different approach provides direct evidence of a link between central tolerance and TSHR SNP rs179247 that predisposes to Graves' disease. Individuals homozygous or heterozygous for this SNP have significantly fewer thymic TSHR mRNA transcripts than individuals homozygous for the protective allele (191). As already mentioned, lower intrathymic insulin expression in individuals with a particular VNTR for the insulin gene is associated with decreased central tolerance to insulin that can lead to autoimmunity to insulin and ultimately to type 1 diabetes. In the same way, decreased intrathymic expression of the TSHR is likely to decrease central tolerance and increase the risk of autoimmunity developing to the TSHR.

2. Aire

Mutations in Aire (like those in APECED patients) are not by themselves susceptibility genes for autoimmune thyroid disease (192, 193). However, 50% of APECED patients in southern Italy had antibodies to Tg, and particularly to TPO, as well as hypothyroidism in some patients (194).

A variety of single nucleotide substitutions, small insertions, and deletions in the coding sequence of the Aire gene are responsible for the malfunction of Aire in APECED patients. In Finnish and northern Italian patients, the most common mutation leads to a premature stop codon (R257X) and a predicted truncated Aire protein (195). Other changes include a 13- base pair (1094–1106) deletion (196). In a family with APECED, a novel Aire mutation (G228W) closely cosegregated with autoimmune thyroiditis and hypothyroidism (197). Mice engineered to express this naturally occurring Aire mutation (G228W) had partial inhibition of intrathymic expression of some self-antigens (198). Moreover, these G228W knock-in mice developed autoimmune syndromes that were dependent on the genetic background and differed from those in Aire knockout mice. In particular, thyroiditis in G228W heterozygotes on the NOD background was more severe than in NOD mice heterozygous for the absence of Aire (198).

B. Genes involved in antigen presentation that impact central or peripheral tolerance

Susceptibility genes for molecules involved in presenting antigens are likely to play a role in central tolerance as well as in the ability of peripheral immune cells to respond to thyroid autoantigens.

1. MHC class II

MHC molecules are critical for binding and presenting peptides from thyroid autoantigens. The association of MHC genes with thyroid autoimmunity was first recognized in association studies (199) and later confirmed in genome-wide associations (200). Detailed analysis of the MHC class II molecule HLA-DR3 demonstrated the critical nature of replacing alanine or glutamine with arginine at position 74 in the MHC binding pocket (201).

The importance of MHC class II has already been mentioned for induced thyroiditis using Tg (reviewed in Ref. 148) and TPO (88, 89) (Tables 2 and 3). Transgenic mice expressing human HLA molecules (HLA-DRB1 and DQ) have been used to demonstrate the importance of interactions between MHC and human- or murine-Tg (114) (reviewed in Ref. 202). In a mouse model of Graves' disease, MHC region genes are linked to the induction of TSHR antibodies (measured by inhibition of TSH binding) but do not play a role in the development of hyperthyroidism (158, 203–205). In this context, it is of interest that a genome-wide association study identified strong associations of MHC class II variants with Graves' patients persistently positive for TSHR antibodies (206).

2. Susceptible Tg haplotype

A polymorphism in Tg is associated with thyroid autoimmunity in humans as well as in experimentally induced thyroiditis (207). The susceptible Tg haplotype alone and combined with a particular MHC class II enhances antigen presentation (133, 208, 209). Whether the susceptible Tg haplotype (alone and combined with MHC) contributes to the loss of central or peripheral self-tolerance is not clear. Associations between TPO polymorphisms and thyroid autoimmunity have been reported but not confirmed (reviewed in Ref. 186).

C. Genes that regulate immune responses

In addition to MHC class I and class II, a number of susceptibility genes for autoimmune thyroid disease including CTLA4, CD40, PTPN22, and CD25 (FoxP3) are likely to regulate peripheral responses in humans (reviewed in Refs. 186–188). Intriguing functional studies have been performed explore the mechanism(s) by which these genes influence autoimmune responses. We will consider immune regulatory genes that may impact central tolerance as well as controlling responses to thyroid autoantigens.

1. IL-2 receptor α (CD25) and FoxP3

Both of these genes encode markers for Treg, and therefore they could play a role in central tolerance and/or in peripheral immune responses. CD25 is a marker for the IL-2 receptor a chain present predominantly on CD25-positive T cells (and on some effector cells), and it is a susceptibility locus for Graves' disease (210). FoxP3, expressed intracellularly, is the definitive marker of Treg. Polymorphisms in FoxP3 do not contribute to the susceptibility to Graves' disease, at least in the UK population (211), but are associated with juvenile Graves' disease (212). Moreover, some APECED patients have Treg defects (213).

2. Cytotoxic T lymphocyte-associated factor 4 (CTLA4)

CTLA4 was the first non-HLA association identified for Graves' disease (214). It is one of 10 confirmed Graves' disease loci (186, 187) and is also associated with other autoimmune diseases (reviewed in Ref. 187). Because CTLA4 functions by blocking or reducing interactions between T cells and APCs, it is likely to exert its effects both intrathymically and in the periphery.

3. PTPN22

The protein phosphatase-22 is associated with thyroid autoimmunity including Graves' disease (reviewed in

Refs. 186 and 187). In autoimmune diseases in general, functional studies of PTPN variants provide contradictory data. In one study, substitution (PTPN22 620W) was associated with increased levels of acetylcholine receptor autoantibodies in patients with myasthenia gravis (reviewed in Ref. 187). On the other hand, both thymic and peripheral regulatory cells were increased in PTPN22 knockout mice on the B6 background (215). These observations were consistent with increased experimental encephalomyelitis in PTPN22 knockout mice, a model of multiple sclerosis, a disease that is not associated with PTPN polymorphisms. Currently, it is not known how PTPN22 polymorphisms contribute to the breakdown in tolerance in thyroid autoimmunity.

4. CD40

This immune regulatory gene, a member of the TNF superfamily, is also expressed on nonimmune cells such as thyroid follicular cells. The CD40 genotype associated with susceptibility to Graves' disease increases expression of CD40 protein (170). In an induced model of Graves' disease, transgenic mice expressing CD40 in the thyroid had enhanced TSHR antibody responses and more severe hyperthyroidism than controls. Moreover, fewer CD40 knockout mice reconstituted with wild-type bone marrow developed TSHR antibodies than comparable controls (169). These studies confirm an important role for intrathyroidal CD40 expression in immune responses leading to Graves' disease. However, it is not known whether CD40 expression is involved in (for example) reducing the efficacy of deleting self-reactive T cells in the thymus.

5. Unidentified immune response genes in animals

In OS chickens that develop spontaneous thyroiditis, one to two dominant but unidentified genes are responsible for abnormal reactivity of the immune system (216). In a totally different approach, thyroiditis, insulitis, and insulin-dependent diabetes mellitus type 1 were studied in mice that express the MHC class II molecule I-Ag7 or I-Ak on the NOD background. Both I-Ag7 and NOD-non-MHC genes were necessary for overt diabetes, whereas only the relevant non-MHC genes appeared to be “permissive” for thyroiditis (60).

D. Other genes and mechanisms

Pregnancy is well known to influence thyroid autoimmunity before as well as after delivery of an infant (reviewed in Refs. 217 and 218). However, pregnancy cannot be a critical factor in breaking self-tolerance to thyroid autoantigens because thyroid autoimmunity is present in many women before pregnancy, as well in women who do not become pregnant and in men. As is well known, thyroid autoimmunity is more common in women than in men (1). However, female gender alone cannot be a primary determinant in breaking tolerance because thyroid autoimmunity occurs in men and women.

Other mechanisms that may contribute to the genetic basis for thyroid autoimmune disease include skewed X chromosome inactivation and fetal microchimerism (reviewed in Ref. 187). The presence in the mother of a small cell population derived from the fetus is well established for human thyroid autoimmunity (187) and is supported by a study of murine experimental thyroiditis (161, 219). If microchimerism contributes to the development of autoimmune thyroid disease, the latter should be more prevalent in parous vs nulliparous women. The data addressing this issue are contradictory, with some studies being against (220–222) and some in support (223, 224) of this possible mechanism. Although possibly involved in thyroid autoimmunity arising during pregnancy, fetal microchimerism is not considered to be a general risk factor (187). Likewise, whether skewed X chromosome inactivation plays a role in thyroid autoimmunity has yet to be determined.

Some susceptibility genes determine the outcome of thyroid autoimmune responses. For example, an

unidentified recessive gene controls autoimmune thyroid attack in spontaneous thyroiditis in OS chickens (216). Similarly, induced hyperthyroidism in mice is linked to several chromosomal loci that are not related to those responsible for induced TSHR antibodies (203–205). None of these susceptibility genes are involved in self-tolerance.

VII. Insight Into Central Tolerance to Thyroid Autoantigens

A. Thymic expression of thyroid autoantigens

Intrathymic expression in wild-type mice, assessed by real-time PCR, is higher for Tg than for both TPO and the TSHR (Figure 5A). In mice expressing a transgene during the fetal period, the transgenic protein is "self" and (as described earlier for the model antigen HEL) plays a crucial role in central tolerance. Hi-expressor and Lo-expressor human TSHR A-subunit transgenics differ in the amount of transgenic protein in the thyroid gland and, importantly, in mRNA transcripts for the human A-subunit in the thymus. In Hi-expressor mice, consistent with high intrathyroidal TSHR A-subunit expression, thymic expression of this autoantigen is extremely high (Figure 5B), exceeding the very well expressed insulin gene and dwarfing the intrathymic levels of the mouse TSHR and the human A-subunit in the Lo-expressor transgenics (225). Wild-type mice lack transcripts for human TSHR A-subunit mRNA. However, mouse TSHR A-subunit mRNA transcripts (Figure 5A) are present at low levels in all three mouse strains (225).

If central tolerance is the most critical of the mechanisms leading to self-tolerance, these observations suggest that breaking tolerance would be most difficult for the human TSHR A-subunit in Hi-expressor transgenic mice, followed by Tg, and least difficult for the lesser expressed thyroid autoantigens, namely TPO, the mouse TSHR in wild-type mice, and the human A-subunit in the Lo-expressor transgenics (Figure 5C).

B. Central tolerance controls responses to the transgenic human TSHR

The extent of intrathymic TSHR expression was sometimes, but not always, predictive of self-tolerance. Intrathymic levels of the human TSHR A-subunit were very high in Hi-expressor transgenics, intermediate in Lo-expressors, and absent in wild-type mice. Assuming central tolerance to be of paramount importance, the expectation for breaking tolerance against the human A-subunit would be least difficult in wild-type mice, intermediate in Lo-expressor transgenics, and most difficult in Hi-expressor transgenics. Indeed, these expectations were confirmed. Immunization with adenovirus (a mild adjuvant) expressing the human A-subunit readily induced TSHR antibodies in wild-type mice, lower antibody concentrations in Lo-expressors, but no TSHR antibodies in Hi-expressor transgenics (Figure 6A).

Breaking tolerance in Hi-expressor transgenics required the use of much more potent immunization protocols: injecting microgram amounts of purified human TSHR A-subunit protein emulsified in Complete Freund's adjuvant, followed either by the same protein in Incomplete Freund's adjuvant (226) or by immunization with human A-subunit adenovirus combined with Treg depletion (185).

C. Factors involved in controlling responses to the endogenous mouse TSHR

Similar levels of mouse TPO and mouse TSHR mRNA are present within the thymus (Figure 5A). Self-tolerance to TPO, measured by antibody production, was readily broken using adenovirus encoding mouse TPO (Figure 6C) (135). However, despite a similar intrathymic level of mouse TSHR, self-tolerance to the mouse TSHR could not be broken by adenovirus encoding the mouse TSHR A-subunit (172) (Figure 6B). Instead, as described (Table 4), generating antibody responses using mouse A-subunit adenovirus required immunizing TSHR knockout mice, which lack tolerance to the mouse TSHR (172). Of interest, the induced TSHR antibody response was stronger in TSHR knockout mice immunized with the human TSHR

A-subunit adenovirus than with the mouse TSHR A-subunit adenovirus (Figure 6B). Moreover, self-tolerance was broken in Lo-expressor transgenics using human A-subunit adenovirus (147, 226), despite similar intrathymic expression of the transgenic human A-subunit (Figure 6, A vs B).

Incidentally, the intrathymic level of a novel TSHR transcript (lacking exon 5) has not been determined. Because its expression in the thyroid is one tenth that of the wild-type TSHR (176), low intrathymic levels of this TSHR transcript would be expected. A lack of tolerance to this TSHR transcript could explain (as described in *Section V* and Table 4) the induction of Graves' disease by immunization using cDNA for the TSHR transcript, but not with the full-length TSHR (176).

Returning to the intriguing difference between the inability of adenovirus expressing the mouse TSHR A-subunit to break tolerance in mice vs the ability of the human TSHR A-subunit to break tolerance to the transgene, a potential explanation may relate to the presence of one less N-linked glycosylation site in the mouse TSHR A-subunit than in the human TSHR A-subunit (172). The importance of glycosylation has already been mentioned in relation to “immunogenicity” of thyroid autoantigens by enhancing antigen uptake. Perhaps even more important, the presence of carbohydrate moieties can modify the spectrum of peptides processed from proteins (227, 228). Glycosylation differs in the thymus and the periphery, as indicated by studies of carcinoembryonic antigen (229). Consequently, the length and nature of the peptides processed and available for thymic tolerance may be different from those presented in the periphery. In the studies using TSHR knockout mice (Figure 6, A and B), tolerance to the TSHR is absent. Nevertheless, the absence of one N-glycan could influence T-cell and, ultimately, antibody responses.

D. Lessons from NOD.H2h4 mice

The relative contributions of central tolerance vs “immunogenicity” of thyroid autoantigens was revealed by studies in NOD.H2h4 mice. This mouse strain spontaneously develops thyroiditis in association with autoantibodies to Tg (58–60). One study suggested the presence of autoantibodies to an unknown membrane-associated thyroid autoantigen in NOD mice (57). However, the presence of TPO autoantibodies was only recently sought (and detected) in NOD.H2h4 mice (135).

On the basis of the relative intrathymic expressions of Tg and TPO in BALB/c and C57BL/6 mice (25), summarized in Figure 5A, it would be anticipated that central tolerance is less for TPO than for Tg. If correct, the spontaneous development of TPO autoantibodies would precede Tg autoantibodies. Moreover, Tg (230), but not TPO (231), is detectable in serum, and therefore B-cell tolerance to Tg might be greater than to TPO. However, this issue is not clear-cut because contradictory results were obtained from two studies of T-cell tolerance to transgenically expressed soluble vs membrane-bound HEL (79, 80) (discussed in *Section IV.A*). On the other hand, the “immunogenicity index,” based on characteristics of thyroid autoantigens (Figure 1) is greater for Tg than for TPO, which would favor the earlier appearance of autoantibodies to Tg. For spontaneously arising autoantibodies to Tg and TPO, expectations based on intrathymic expression (central tolerance) were not confirmed (135). TgAb were present in NOD.H2h4 mice about 8 weeks after exposure to iodine, in agreement with other findings (58, 59), but TPOAb were only detected in mice aged 7 months or older (135). These findings for NOD.H2h4 mice (Figure 7A) were paralleled by observations made in relatives of patients with juvenile Hashimoto's thyroiditis; seven of eight siblings had TgAb, and only one had TPOAb (Figure 7B).

How can these observations be explained? Peptides processed from Tg, the most abundant thyroid protein, can be expected to dominate the binding sites of MHC molecules for presentation to T cells. Indeed, Tg peptides were the only identifiable thyroid-specific peptides eluted from MHC class II protein purified from human thyroids (26). Moreover, a particular Tg haplotype, together with HLA-DR, confers susceptibility to the development of autoimmune thyroid disease (209) (reviewed in Ref. 232). Overall, as summarized in Figure 7C, for Tg and TPO, thymic tolerance seems less important for the development of

thyroid autoimmunity than thyroid autoantigen immunogenicity combined with genetic polymorphisms in the latter.

E. Aire deficiency and thyroid autoimmunity in mice

In Aire knockout mice, intrathymic expression is reduced to varying degrees for different thyroid autoantigens (225). The smallest reduction is observed for the TSHR, followed by Tg. Surprisingly, there is a much greater (20- to 30-fold) reduction in TPO expression (Figure 8A). Indeed, the greatly reduced intrathymic TPO expression in Aire knockouts is comparable to that for insulin, which is highly expressed intrathymically in wild-type mice but absent in Aire knockouts (86). Based on these observations, the absence of Aire might be expected to greatly reduce self-tolerance to insulin. Tolerance to TPO and Tg would be reduced to an intermediate extent, and tolerance to the TSHR would be little affected (Figure 8B).

The absence of Aire leads to spontaneous thyroiditis in NOD mice, but not in mice on other genetic backgrounds (89). Based on the strong influence of Aire on TPO thymic expression, it is tempting to speculate that TPO might be the key autoantigen in the development of this disease. However, the specific thyroid autoantigen involved in the Aire-dependent form of thyroiditis has not been identified.

As already described, intrathymic expression of the TSHR in wild-type mice is also Aire dependent, although to a lesser degree than TPO (Figure 8A). Unexpectedly, unlike the endogenous mouse TSHR, thymic expression of the transgenic human TSHR A-subunit in both Hi- and Low-expressors was unchanged in Aire knockout mice (225). In these transgenic mice, the bovine Tg promoter was used to target the human A-subunit to the thyroid (226). In contrast to the TSHR A-subunit transgenics, Aire dependence was demonstrated for the model antigen HEL expressed using the rat Tg promoter (87). An Aire-binding motif is present in the mouse Tg promoter, and therefore likely present in the rat Tg promoter (233). The present concept, albeit controversial, is that the bovine Tg promoter lacks a consensus Aire-binding site (85). If correct, the absence of this motif in the bovine Tg promoter may explain the Aire independence of the human TSHR A-subunit in the transgenic mice.

F. Aire defects in human thyroid autoimmunity and Down's syndrome

As mentioned above, Aire gene mutations do not appear to contribute to susceptibility to autoimmune thyroid disease in humans (192, 193). Nevertheless, a novel Aire mutation (G228W) in a family with APECED closely cosegregated with autoimmune thyroiditis and hypothyroidism (197). Moreover, a high prevalence (50%) of APECED patients in southern Italy had antibodies to Tg, and particularly to TPO, as well as hypothyroidism in some individuals (194). These observations in humans are consistent with the marked reduction in intrathymic TPO expression in Aire knockout mice (Figure 8A). It is, therefore, tempting to speculate that genetic variants in the promoter region may affect TPO intrathymic expression in humans and, in concert with other genes, contribute to the predisposition to developing Hashimoto's thyroiditis.

There are no reported cases of Graves' disease among APECED patients, and an Italian APECED cohort lacked detectable TSHR antibodies (194). Even if APECED patients have lower intrathymic TSHR levels, the magnitude of the decrease may not be sufficient to permit breakdown of tolerance to this autoantigen. Down's syndrome involves trisomy of chromosome 21, the chromosome on which Aire is located. Aire expression and thymic function are decreased in Down's syndrome (234). Although increased autoimmunity (including thyroid autoimmunity) occurs in some patients with APECED and Down's syndrome, the mechanism by which AIRE contributes to these disorders is different (234). Also, because the onset of hypothyroidism in Down's patients preceded thyroid autoimmunity in children aged 8 years or younger (235), thyroiditis cannot be the explanation for thyroid dysfunction in these patients.

VIII. Insight Into Peripheral Tolerance to Thyroid Autoantigens

T-cell deletion by central tolerance may not eliminate all self-reactive cells. Another potent mechanism involves Treg, such as naturally occurring CD25+ CD4+ cells or CD8+ CD122+ T cells. These Treg control autoreactive effector T cells in the periphery (93, 94), in some but not in all autoimmune responses.

A. Depleting regulatory T cells does not break TSHR tolerance in mice

Treg do not maintain self-tolerance to the TSHR, at least in mice, as shown by the following studies using anti-CD25 to deplete Treg:

- In transgenic mice that express high intrathymic levels of human TSHR A-subunit mRNA transcripts (Hi-expressors; Figure 6A), immunization with high doses of adenovirus encoding the human A-subunit did not induce TSHR antibodies. Importantly, even Treg depletion before immunization by this means failed to induce antibodies to the human A-subunit, which is was unable to break tolerance (147, 236). On the other hand, as previously described in *Section VII*, much more powerful immunization using CFA plus A-subunit protein, or a variant of this approach, was required to induce antibodies (albeit nonfunctional) to the TSHR (185, 226).
- Despite low intrathymic expression of the endogenous mouse TSHR, antibodies could not be induced in either BALB/c or C57BL/6 mice by immunization with mouse A-subunit adenovirus even after Treg depletion (172). However, using this approach, small lymphocytic infiltrates developed in some C57BL/6 mice (172).

Overall, self-tolerance could not be broken to the endogenous mouse TSHR or the transgenic human TSHR A-subunit (expressed at high levels) by Treg depletion before immunization with adenovirus encoding the autoantigen (mouse TSHR A-subunit or human TSHR A-subunit, respectively). Turning to humans, there is evidence in some populations of genetic susceptibility to thyroid autoimmunity related to polymorphisms in Treg molecules, namely the IL-2 receptor α (CD25) and FoxP3 (*Section VI*). However, Treg could play a role in central tolerance and/or in peripheral immune responses. Indeed, the importance of Treg in peripheral responses is described in *Section VIII.B*.

B. The magnitude of induced TSHR responses is controlled by regulatory T cells

Although depleting Treg had no effect on breaking self-tolerance, Treg act as a powerful brake on some immune responses. For example, depleting Treg that express CD4+ CD25 or CD8 and CD122 enhanced Graves' disease in C57BL/6 and BALB/c mice induced by immunization with adenovirus expressing the human TSHR A-subunit (237, 238). Similarly, the incidence of TSAb activity was increased in C57BL/6 mice by Treg depletion before TSHR plasmid immunization (239).

Surprisingly, despite similar intrathymic expression of the TSHR and TPO (Figure 5A), self-tolerance to mouse TPO was readily broken using mouse TPO adenovirus (Figure 6C), and the levels of induced TPO antibodies were unchanged by prior Treg depletion (135). This apparent discrepancy likely relates to other immunogenic factors including the larger size of TPO vs the TSHR A-subunit, the higher intrathyroidal concentration, and consequently a greater number of processed peptides from TPO than from the A-subunit (discussed in Ref. 240).

C. Regulatory T cells control development of thyroiditis and epitope spreading

Treg play a major role in controlling the development of thyroiditis and, where investigated, in epitope spreading as illustrated by the following investigations:

- Pioneering studies demonstrated that thyroiditis develops in neonatally thymectomized mice or rats

(123, 241). Although the specific mechanisms were not addressed, a role for Treg (rather than central tolerance) in neonatal thymectomy was suggested by Kong et al (242). Also, Treg depletion facilitates thyroid lymphocytic infiltration in thyroiditis-resistant strains (243).

- Depletion of CD25+ Treg accelerates spontaneous thyroiditis associated with increased iodide intake in NOD.H2h4 mice (244) and in HLA-DR3 transgenic mice (245). Moreover, Treg may be involved in the suppressive effects of MHC class genes that regulate disease induction with mouse Tg vs human Tg (246).
- B-cell-deficient NOD.H2h4 mice, unlike their wild-type counterparts, fail to develop thyroiditis. However, depleting Treg in such B-cell-deficient mice permits thyroiditis to develop (69).
- Depleting CD4+ CD25+ Treg permits development of thyroiditis in IL-17 knockout NOD.H2h4 mice that do not develop thyroiditis (247). On the other hand, in IFN-γ knockout mice of the same strain, Treg depletion did not overcome the inability to develop thyroiditis (247).
- In the thyroiditis "resistant" BALB/c strain, inducing regression of implanted tumors simultaneous with Treg depletion and conventional immunization (mouse Tg plus adjuvant) leads to thyroiditis and antibodies to mouse Tg (145).
- In transgenic mice expressing low levels of the human TSHR A-subunit, extensive thyroiditis developed after Treg depletion (using anti-CD25) before immunization with the human A-subunit (147, 236). In addition to low serum T_4 levels (presumably reflecting thyroid damage), these mice also developed autoantibodies to murine Tg and TPO. Likewise, depleting Treg before immunization with CFA plus human A-subunit protein, followed by Treg depletion before each of two immunizations with human A-subunit-adenovirus, broke self-tolerance to Tg and TPO (as expected) as well as to the mouse TSHR measured by antibody binding to TSHR peptides (185).

D. Treg in human thyroid autoimmunity

In human thyroid autoimmunity, the number and function of Treg are still unclear, depending on the Treg markers and assays employed, as well as the disease variant studied. In one study, abundant Treg were found infiltrating the thyroid gland of Graves' patients, but the suppressor function of peripheral Treg was decreased (248). In another study, intrathyroidal Treg were reduced compared with the peripheral blood, possibly because of increased apoptosis (249). Of particular interest, reduced expression of CD25 was observed in first- and second-degree female relatives of autoimmune thyroid disease patients, suggesting a "sign of a poor capability to preserve tolerance" (250). Despite the limited number of studies (and in some cases the limited number of patients investigated), these data are consistent with the early studies of Volpe et al (251) concerning a "suppressor T-cell defect" and for the association between thyroiditis and Treg in mice (147, 236).

E. Autoantigen cross-reactivity and autoantigen spreading

T cells recognize linear peptides processed from proteins and bound to MHC class I (for CD8+ cells) and MHC class II (for CD4+ T cells). Although some antibodies recognize linear epitopes, most thyroid autoantibodies in humans interact with conformational epitopes that involve discontinuous portions of the amino acid sequence that come together in the folded protein. Because of these differences, cross-reactivity between thyroid autoantigens is more likely to occur for T-cell epitopes than for B-cell epitopes.

1. "TgPO" antibodies

Many patients have autoantibodies to both Tg and TPO. Similarly, NOD.H2h4 mice develop thyroiditis in association with autoantibodies to Tg and subsequently to TPO (135). Because autoreactivity to Tg and

TPO often occurs in the same individual, it was postulated that these thyroid autoantigens share “TgPO” epitopes at the T- and/or B-cell level.

Several studies suggested the presence of bispecific “TgPO” autoantibodies: TgAb affinity purified from the sera of patients with chronic autoimmune thyroiditis bound TPO, and this binding was inhibited by Tg (252). Similarly, “TgPOAb” were isolated from patients' IgG by affinity chromatography on Tg followed by TPO, and the eluted antibodies bound with high affinity to both Tg and TPO (253). Moreover, bispecific “TgPOAb” were reported to distinguish between patients with hypothyroidism due to Hashimoto's thyroiditis and thyroid autoantibody-positive euthyroid individuals, as well as between patients with thyroid autoimmunity and nonautoimmune thyroid diseases (254–256). A multicenter study involving 3122 individuals established a prevalence of TgPOAb in 35–41% of patients with thyroid autoimmunity (257). In the light of these studies, a molecular approach was used to search for monoclonal “TgPO antibodies.” A phage display Ig gene combinatorial, constructed from thyroid-infiltrating B cells of a TgPO antibody-positive patient, was sequentially “panned” for binding to purified Tg, followed by panning to TPO. In contrast to expectations, enrichment for Tg- and TPO-binding phage was attributable to multiple antibodies specific for either Tg or TPO. These findings provide powerful evidence against shared, cross-reactive antibody epitopes on Tg and TPO (258).

2. TgPO T-cell epitopes

An alternative explanation for the development of antibodies to both TPO and Tg in many patients invokes cross-reactive T-cell epitopes. Indeed, an eight-amino acid region in human TPO (residues 119–126) includes six identical and two conserved residues when compared with the human Tg sequence (residues 2763–2770) (Figure 9A) (259), and this region conformed to an algorithm for a T-cell epitope (260). Support for this hypothetical TgPO T-cell epitope was obtained by immunizing mice (261) but was not provided by responses in vitro by lymphocytes from patients with autoimmune thyroid disease (reviewed in Ref. 10). Such studies generally test the responses of CD4+ T cells. However, very recently, recombinant tetramers of HLA-A2 molecules combined with predicted Tg or TPO peptide epitopes were used to examine epitope recognition by CD8+ cells (likely cytotoxic T cells) in patients with Hashimoto's thyroiditis. This approach demonstrated recognition of several Tg and TPO peptides in HLA-A2-positive patients, including the TgPO epitope (262).

Despite the evidence in favor of the existence of TgPO autoantibodies, our assessment of available information, supported by experimental studies in mice (*Section V.G*), is that autoantibodies to Tg and TPO in the same individual arise consequent to breaking tolerance to each autoantigen separately.

3. Species cross-reactivity between T-cell epitopes for TPO

Most studies demonstrated the need to use syngeneic antigen to induce thyroiditis (Table 2). However, some notable examples of cross-species reactivity should be addressed.

As described in *Section V*, transgenic mice expressing a human T-cell receptor specific for human TPO have been generated (143). Molecular modeling demonstrated that binding to MHC class II (IA-k) by the human TPO 536–547 peptide and the corresponding mouse peptide involved similar antigenic surfaces (149). Moreover, the transgenic T cells were functional in vivo and induced severe thyroiditis leading to hypothyroidism in H2-k mice (143). Likewise, similar antigenic peptide conformation, despite differences in amino acid residues, may account for the ability of porcine TPO, or a porcine TPO peptide, to induce thyroiditis in C57BL/6 (mice) (115, 118).

4. Lack of cross-reactivity between human and murine TSHR T-cell epitopes

T cells from mice immunized with the human TSHR A-subunit do not appear to cross-react with mouse

TSHR peptides, as exemplified by the lack of thyroiditis in most wild-type mice immunized with the human TSHR even after Treg depletion (147, 236). In other words, tolerance to the mouse TSHR is not broken by human TSHR immunization. These findings are surprising because in silico binding affinities are similar for the mouse MHC class II TSHR peptides (likely helper T-cell epitopes) corresponding to the human peptides recognized by immunized mice (Figure 9B).

IX. Immune Intervention Inadvertently Leading to Thyroid Autoimmunity

Some immunological treatments for other diseases induce or enhance thyroid autoimmunity. The difference in the magnitude and duration of the autoimmune responses in Hashimoto's thyroiditis vs Graves' disease is relevant for the outcome of inadvertent immune intervention (263, 264). In Hashimoto's thyroiditis, autoimmunity to Tg and/or TPO rises gradually over many years as reflected in generally high autoantibody levels. Because of the tropic effects of TSH, thyroid reserve is maintained, typically for many years, until it is ultimately overcome by massive lymphocytic infiltration, fibrosis, and thyroid follicle damage (Figure 10A). In contrast, thyroid-stimulating TSHR antibodies (TSAbs), which are potent at low concentrations (265–267), induce hyperthyroidism at an early stage in the autoimmune response (Figure 10 B).

The outcome of immune intervention usually depends on the patient's immune status at the time of immune therapy or as a consequence of the treatment (Figure 10C). If the immune system is essentially “normal” (for example, in terms of lymphoid cell numbers), an immune stimulus (like IFN-α) enhances (and sometimes induces) pre-existing autoimmune responses. In contrast, in lymphopenic individuals, Graves' disease has been observed to develop during the immune reconstitution phase. These contrasting issues are described in more detail below.

A. Interferon-α therapy for hepatitis

Many studies have reported the presence of autoantibodies to Tg and/or TPO in patients treated with IFN-α for hepatitis C infection (reviewed in Refs. 268 and 269). In most cases, IFN-α treatment is associated with autoimmune thyroiditis and hypothyroidism, although Graves' disease has also been described (270–272) (reviewed in Ref. 268). The characteristics of IFN-α-associated thyroid autoimmunity include:

- An increase in the level of pre-existing autoantibodies to Tg and/or TPO (273), as well as evidence in some patients for de novo induction of autoantibodies to Tg and/or TPO (271, 274, 275).
- Reversibility of thyroid autoimmune disease after stopping IFN-α therapy in some (276), but not all (271, 277), cases.
- Activation by IFN-α of genes leading to enhanced MHC class I expression and secretion of potent proinflammatory cytokines, namely IFN-γ and IL-2 (reviewed in Ref. 269).
- Direct effects of IFN-α on thyrocytes, including changes in the expression of TSHR, Tg, and TPO, as well as thyroid cell death by necrosis that may lead to thyroid inflammation (137, 269). These data are consistent with observations that IFN-α therapy can induce hypothyroidism in the absence of thyroid autoimmunity (278).

Patients with IFN-α-associated thyroid autoimmunity share susceptibility genes with other thyroiditis patients (269). Consequently, it is likely that IFN-α usually acts as an enhancer, rather than as a primary inducing agent of thyroid autoimmunity.

B. T-cell depletion to treat multiple sclerosis (and other conditions)

Early attempts to induce thyroiditis in rats involved thymectomy and whole-body irradiation, procedures

that lead to lymphopenia (123). As described in *Section III*, NOD.H2h4 mice spontaneously develop thyroiditis, which is enhanced on exposure to iodide-supplemented drinking water (58–60). In one study, moderate lymphopenia was observed in untreated NOD.H2h4 mice vs two nonautoimmune-prone strains (244). However, increasing peripheral lymphocyte numbers by splenocyte transfer or treatment with CFA did not affect thyroiditis development. In contrast, thyroiditis was enhanced by depleting CD4+ CD25+ naturally occurring Treg (244). More recently, development of Graves' disease and sometimes Hashimoto's thyroiditis has been reported in lymphopenic individuals undergoing immune reconstitution (reviewed by Weetman in Ref. 279).

Lymphopenia after therapy for acute or chronic myeloid leukemia may be treated by transplanting bone marrow or hematopoietic stem cells. Transplanting bone marrow or stem cells from a relative with established thyroid autoimmunity can lead to Graves' disease in adults and children and less commonly to Hashimoto's thyroiditis (280–283). These findings are consistent with previously described studies (*Section V.B.*) in which thyroiditis was induced in naive guinea pigs or mice by injecting lymphocytes sensitized to Tg or thyroid antigens (125–128).

More unexpected is the development of Graves' disease (and occasionally thyroiditis) during the spontaneous immune reconstitution phase that follows lymphopenia:

- After antibody-mediated lymphocyte depletion to treat aplastic anemia (284, 285), multiple sclerosis (286), or immunosuppression for islet cell transplantation in type 1 diabetes patients with pre-existing TPO autoantibodies (287).
- In severely immunocompromised patients suffering from HIV-1 infection (288–292). It should be emphasized that, assuming the emergence of TSHR-specific T cells after immune reconstitution, only very low levels of TSAb secreted by B cells are required to stimulate the thyroid gland and cause Graves' hyperthyroidism (Figure 10B).

C. Mechanisms responsible for “reconstitution autoimmunity”

To compensate for severe lymphopenia, the remaining T cells undergo extensive cycles of T-cell proliferation, a process called “homeostasis.” Several different mechanisms may be involved as indicated by studies in mice and in humans:

- Spontaneous differentiation of natural Treg into pathogenic helper T cells under conditions of lymphopenia (Figure 11A). Natural Treg transferred to lymphopenic mice lacking recombination activating genes (RAG deficient) lose Foxp3 expression and their regulatory function. These changes can be prevented by providing IL-2. In the absence of IL-2, Treg that lose Foxp3 expression develop into pathogenic T cells that infiltrate the pancreas, lung, and liver (293).
- One cytokine involved in homeostasis and autoimmunity in NOD mice is IL-21 (294). Higher IL-21 levels in NOD mice are associated with an increase in effector cytokines and development of pathogenic T cells (295).
- Similarly, genetically determined pretreatment levels of IL-21 drive T-cell cycling and apoptosis leading to human autoimmunity (Figure 11B). Multiple sclerosis patients develop lymphopenia after treatment with alemtuzumab (formerly called Campath-1H), an antibody against CD52, an antigen of unknown function on lymphocytes and monocytes. Fresh or stored pretreatment lymphocytes or sera were investigated from patients that did, or did not, develop Graves' disease (286) or idiopathic thrombocytopenic purpura (296). Autoimmunity developed in patients with increased T-cell cycling and T-cell apoptosis and was driven by IL-21. This cytokine enhances proliferation of Th17 cells (297), B-cell differentiation, and antibody production (298) (299) and reduces the inhibitory effect of

Treg (300).

Importantly, patients with low concentrations of IL-21 before alemtuzumab treatment did not develop autoimmunity. In contrast, patients with high pretreatment IL-21 levels had an increased risk for developing self-reactive T cells (298). Despite prolonged T-cell lymphopenia (greater than 3 months), there was a rapid recovery of B cells by 3 months, with increased levels of B-cell activating factor that remain elevated for at least 12 months (301).

Systemic levels of IL-21 (high vs low) are genetically determined (298). Thus, IL-21 is one susceptibility gene for the development of Graves' disease after alemtuzumab treatment for multiple sclerosis. In contrast, unlike Graves' disease that develops in nonlymphopenic individuals, neither HLA nor TNF-a nor IL-10 promoter polymorphisms are involved in alemtuzumab-induced Graves' disease (286). It would be particularly interesting to know whether the TSHR-SNP responsible for reduced intrathymic TSHR expression (191) characterizes patients with Graves' disease arising in the immune reconstitution phase. However, it is possible that one or more unique genetic susceptibilities are involved in Graves' disease associated with immune reconstitution.

X. Induced Tolerance in Experimental Thyroid Autoimmunity

A. Immune permissive or preventive factors not involving tolerance

1. Permissive factors

A number of studies have addressed the cytokine requirements for the induction or expansion of induced thyroid autoimmunity. For example, in a transfer model of granulomatous thyroiditis (127), IL-12 is needed for in vitro restimulation of Tg-sensitized splenocytes, but endogenous IL-12 is not required for effector cell sensitization or activation (302). Graves' disease induced by immunization with TSHR adenovirus is attenuated in the absence of either IL-4 or IFN-γ, demonstrating that both T helper 1 (Th1) and Th2 cytokines are required for optimal disease induction (303). Lymphotoxin-b receptor signaling is necessary for the development of thyroiditis in transgenic mice expressing CCL21 in the thyroid (304).

2. Preventive measures

An interesting approach involves treating DCs with TNF-a ex vivo to induce them to become “tolerogenic.” Such cells have been used to suppress the development of experimentally induced thyroiditis (305).

Protocols for inducing thyroiditis usually involve relatively short time intervals; for example, two immunizations with Tg (or TPO), 1 week apart, and euthanasia a month after the initial challenge. Some thyroiditis lesions resolve or become fibrotic within 35 days (127, 306). In contrast, the time scale for induced Graves' disease is very different: adenovirus immunization is performed three times at 3-week intervals, and plasmid DNA immunization on six occasions at 2-week intervals. TSHR antibodies and sometimes hyperthyroidism induced by these protocols persist for up to 6 months (167, 174, 185). Because of its chronic nature, immune approaches have been explored to prevent or treat induced Graves' disease. For example, cytokine deviation away from type 1 T helper cell (IFN-γ) toward Th2 type responses (IL-4 and IL-10) (307, 308) reduces the proportion of mice that become hyperthyroid. In addition, mice infected with *Schistosoma* or treated with α-galactosylceramide before TSHR immunization are “protected” from developing Graves' disease (309). Another example of prophylaxis is that one injection of anti-CD20 eliminates B cells for 3 weeks and reduces the incidence of Graves' disease. The antibody needs to be administered before or within 2 weeks after the first TSHR immunization in order to reduce the development of hyperthyroidism (72). (In humans, anti-CD20 monoclonal antibody [rituximab] is being

used to treat patients with Graves' hyperthyroidism or ophthalmopathy. The clinical amelioration frequently observed is likely caused by interrupting antigen presentation to T cells [71, 310, 311].)

None of the above approaches could treat established Graves' disease in mice. In contrast, TSHR antibodies or hyperthyroidism was reduced in some hyperthyroid mice using decoy molecules of the TNF family ligand inhibitors (B-cell activating factor and a proliferation-inducing ligand, APRIL) to target proliferation or survival of B cells (312).

3. Nonspecific “antigenic interference”

Immunizing guinea pigs with thyroid extract and CFA induced antithyroid antibodies and thyroiditis. However, when the immunization was performed with bovine gamma globulin (BGG) plus thyroid extract and CFA, thyroiditis was abolished and antibody responses switched away from thyroid proteins to BGG (313). The extent of reduction in the thyroid-specific responses depended on the relative doses of thyroid extract and BGG. Moreover, the reduction was also observed using bovine serum albumin or hemocyanin instead of BGG. This phenomenon is described as “antigenic interference” or antigenic competition. Unexpectedly, responses to thyroid extract were not prevented if immunization was performed in one hind quarter with the inciting antigen (thyroid extract) and immunization with the competing antigen (BGG) in the other hind quarter (313).

Guinea pigs that received repeated injections of rabbit sera containing high titers of antibodies to guinea pig Tg were protected for up to 165 days against thyroiditis induced by immunization with guinea pig Tg and adjuvant (314). Transient protection was also observed after injecting rabbit sera against keyhole limpet hemocyanin, possibly because of antigenic competition. However, the mechanism involved using hyperimmune serum to Tg is not fully understood (314). As noted earlier, injecting serum containing high titers of TgAb together with thyroid-sensitized lymph node cells potentiated thyroiditis (125).

4. Summary

The studies described above provide important “clues” into the requirements for ongoing immune responses to the TSHR and Tg. However, none are antigen-specific: they are all directed at different “arms” of the general immune response. Consequently, they may have unexpected and undesirable effects if applied to humans.

The goals of the protocols to be described next involve the use of specific thyroid antigens to induce tolerance (Table 5).

B. Increasing circulating autoantigen levels

1. Injecting crude thyroid extracts

Thyroiditis develops spontaneously in Buffalo rats, and the process is enhanced by administration of the polyaromatic hydrocarbon 3-MCA or thymectomy (122, 124). Injecting Buffalo rats with thyroid extract (but not liver extract) suppressed thyroiditis in 3-MCA-treated animals (315). Similarly, thyroiditis develops in PVG rats subjected to thymectomy and subsequent irradiation to deplete T cells (123). Injecting rat thyroid extract, but not liver extract, during the course of irradiation prevented the development of thyroid lesions and autoantibodies to Tg (316). For both rat strains, injecting thyroid extract had no effect on established thyroiditis (315, 316).

2. Increasing systemic Tg levels

Subsequent protocols utilized purified Tg, the major component of thyroid extracts. Higher Tg levels can be implemented by injecting mouse Tg or by implanting osmotic mini-pups containing TRH or TSH

(reviewed in Ref. 242). Mice pretreated to increase higher Tg levels have a reduced capacity for, or are resistant to, thyroiditis induced by immunization with mouse Tg and the adjuvant LPS (317). Induced tolerance is related to the length of time for which Tg is elevated (318). Moreover, although tolerance could be induced using this approach in CBA/J, SJL, and some B10 congenic strains, tolerance could only be transferred using tolerized splenocytes to naive CBA/J recipients (319).

The following rationale for increasing the systemic levels of Tg invokes Treg: Treg maintain self-tolerance; their efficacy can be enhanced by increasing systemic levels of autoantigen, for example Tg (320) (reviewed in Ref. 242). Indeed, naturally occurring Treg (expressing CD25) are required for induced tolerance to experimental autoimmune thyroiditis (243).

3. Immune diversion away from functional TSHR antibodies

The TSHR A-subunit protein is heavily glycosylated (as mentioned in *Section II*) and binds to the mannose receptor present on macrophages and DCs. Immune responses are initiated by “mature” DCs that express MHC class II and costimulatory molecules. However, without maturation signals, “immature” DCs induce antigen-specific peripheral T-cell tolerance (321).

Experiments were performed to test the hypothesis that preadministering TSHR A-subunit protein without activating the innate immune system would induce tolerance and thereby attenuate the induction of hyperthyroidism by subsequent A-subunit adenovirus immunization. Indeed, amelioration of hyperthyroidism was achieved by injecting A-subunit protein (in the absence of an immune stimulus). The attenuation was antigen-specific because the effect was not achieved using Tg or TPO, and it required eukaryotic, not bacterial, protein (possibly because the bacterial protein was not correctly folded). However, the reduction in hyperthyroidism did not involve the anticipated mechanism. Instead of inducing tolerance, TSHR protein pretreatment diverted the antibody response away from functional TSAbs toward production of nonstimulatory TSHR antibodies (322). Moreover, injecting A-subunit protein into previously immunized mice had no effect on established hyperthyroidism.

An interesting question is whether TSHR A-subunit deviation toward nonfunctional antibodies could be applied therapeutically in humans. In principle, it would be possible to vaccinate against pathogenic antibodies in the relatives of Graves' patients, some of whom would be at risk for developing Graves' hyperthyroidism. However, assuming that the practical hurdles of generating sufficient A-subunit protein could be overcome, redirecting antibody epitopes may not preclude activating TSHR-specific T cells. Consequently, before the A-subunit protein vaccination approach could be considered for use in humans, it would be essential to ensure that injecting it did not activate TSHR-specific T cells with the potential to home to the orbit and precipitate or enhance Graves' ophthalmopathy.

C. Oral tolerance

1. Background

It is well known that individuals do not usually generate immune responses to the food they eat. In the gut mucosa, the most extensive immune component of the body, tolerance is the “default” immune response (reviewed by Weiner et al [323]). A range of regulatory mechanisms play a role in tolerance, including anergy or deletion, induction of Treg, and the regulatory cytokines TGF-β and IL-10. Such observations gave rise to the concept of “oral tolerance” as a possible means of preventing autoimmune disease development and perhaps even treating established disease. In common with increasing autoantigen levels systemically, but unlike approaches that involve generalized immune treatment, oral tolerance involves the use of specific autoantigens.

2. Experimentally induced thyroiditis

Orally administered porcine Tg (324) and human Tg (325) decreased subsequent approaches to immunization with Tg. In particular, oral intake of human Tg decreased thyroiditis, partially inhibited the levels of induced TgAb, and reduced the proliferation of lymph node lymphocytes to human Tg (325). Oral tolerance was dependent on the Tg dose, and administration of bovine serum albumin instead of human Tg had no effect on subsequent responses to immunization with human Tg (325). Turning to the adoptive transfer protocol, feeding porcine Tg before immunization with mouse Tg and LPS had no affect on immune responses in the donor mice. However, recipients of splenocytes from porcine Tg-fed donors had reduced levels of TgAb, and the magnitude of induced granulomatous thyroiditis was decreased (324).

The suppressive effects of feeding human Tg reduced production of IL-2 and IFN-γ and increased IL-4 and TGF-β generation by lymph node lymphocytes restimulated with Tg (326). These changes reflect a shift away from Th1 to Th2 cytokines. It was suggested (327) that because Tg-mediated oral tolerance leads to suppressive cytokines, this regimen might suppress responses to other autoantigens in the same organ, including TPO and the TSHR. If correct, oral tolerance involving Tg could possibly be used to treat Graves' disease. Most TSHR antibodies in Graves' patients are IgG1 (328) (Th1 type in humans). However, TSHR autoantibodies affinity-purified using recombinant antigen from two of three high-titer Graves' sera contained IgG4, remarkably restricted to this subclass (Th2 type) in one patient (329). Consequently, as indicated by the cytokine requirements for induced murine hyperthyroidism (303), Th1 to Th2 deviation may not be effective for treating Graves' disease in humans (327).

3. Oral tolerance for human autoimmune thyroid disease

As for other human autoimmune conditions (reviewed in Ref. 323), specific antigen (Tg) administration to humans with ongoing thyroid autoimmunity was much less effective, with relatively minor effects on T-cell responses and unchanged thyroid autoantibody levels (330). Translating oral tolerance to humans may require establishing biomarkers to indicate particular human populations that are likely to respond to oral tolerance (323). In addition, if oral tolerance is used to treat individuals before thyroid autoimmunity develops, it will be essential to be able to predict with certainty which individuals are at high risk for developing a particular autoimmune disease.

D. Neonatal tolerance to the TSHR

Early exposure to a novel antigen renders immune cells unresponsive to subsequent challenge with that antigen. For example, iv injection of α-fodrin 24 hours after birth blocked the development of Sjögren's syndrome-like lesions (331).

The effect of neonatally induced tolerance was applied to the TSHR-adenovirus model of murine Graves' disease. One injection of a high dose of TSHR-Ad into mice 24 hours after birth established tolerance and prevented subsequent induction of TSAb and Graves' hyperthyroidism. A lower TSHR-adenovirus dose was less effective, consistent with the presence of fewer Treg in splenocytes than in fully tolerized mice (332).

XI. Environmental Factors That May Contribute to Breaking Self-tolerance

Thyroid autoimmunity involves both genetic and environmental factors (reviewed in Refs. 2 and 333). Environmental factors or agents may alter thyroid function by direct effects on thyroid cells or indirectly by affecting thyroid autoimmunity (reviewed in Refs. 334 and 335). The question has also been raised (but not yet answered) as to whether “the gut microbiota trigger Hashimoto thyroiditis (336). An important future issue will be the role played by environmental factors in epigenetic modulation (337). The purpose of this section is to distinguish, where possible, between environmental factors that “trigger” autoimmune

responses to thyroid autoantigens vs factors that enhance or perpetuate ongoing thyroid autoimmune responses.

A. Dietary iodine and selenium

1. Iodide

In humans, increased iodide intake has long been recognized to cause hypothyroidism (iodide myxedema) or hyperthyroidism (Jod-Basedow). More than 40 years ago, these consequences were demonstrated to result from the failure of the iodide autoregulatory mechanism in susceptible individuals with underlying autoimmune thyroid disease (338–340). Hypothyroidism occurs because of a failure to escape from the Wolff-Chaikoff block (341) in Hashimoto's thyroiditis or in treated Graves' disease. A well-documented example of the Jod-Basedow phenomenon is the outbreak of hyperthyroidism after the introduction of iodized salt in Tasmania (34). It is the authors' experience with present medical trainees and clinicians that iodide autoregulation is either an unknown or a forgotten phenomenon. The unfortunate consequence of this lack of information is the frequent assumption that iodide induces de novo thyroid autoimmunity rather than leading to thyroid dysfunction in individuals with pre-existing thyroid autoimmunity (in some cases subclinical or unrecognized). Indeed, this distinction is overlooked in some contemporary reviews on thyroid autoimmunity in humans and in animals (335).

Of course, it remains possible that the level of iodide consumption in humans may, in addition, increase the incidence of de novo thyroid autoimmunity. Epidemiological studies addressing this issue are important; however, it is difficult for this conclusion to be made because it is necessary to demonstrate the conversion from thyroid autoantibody negativity to positivity (342). It should also be recognized that the quantity of daily iodide intake is an important factor to be considered. Failure to escape from inhibition of iodide organification (the Wolff-Chaikoff block) generally occurs at very high levels of iodide intake, as occurs with ingestion of some medications such as amiodarone. In contrast, epidemiological studies typically examine populations with different, but low to moderate, levels of iodide intake.

The distinction between de novo induction vs exacerbation of thyroid autoimmunity can be more easily made in laboratory animals. In animal models, increased (generally very high) iodide intake enhances development of thyroid autoantibodies and thyroiditis in OS chickens (343), BB rats (56), and mice of the strains NOD and NOD.H2h4 (58–60). However, in mouse strains that do not develop thyroid autoimmunity spontaneously, increased iodide intake did not lead to thyroiditis. Instead, goitrous hypothyroidism without thyroid autoimmunity developed in SJL mice, but no thyroid function changes were observed in CBA/J mice (344), a strain commonly used to study Tg-induced thyroiditis.

Mechanisms do exist whereby iodide could induce de novo thyroid autoimmunity. Iodide excess can exert its effects by causing thyroid damage as well as by contributing to the antigenicity of the prohormone Tg (reviewed in Ref. 345). Thyroid injury, in the absence of infection, can contribute to thyroid autoimmunity. For example, DCs exposed to necrotic thyrocytes became “mature” and can facilitate induction of experimental thyroiditis (346). In addition, genomic DNA released from damaged rat FRTL5 cells can activate the innate immune system and could, in principle, trigger thyroid autoimmunity (347). However, in humans, massive thyroid destruction occurs in subacute thyroiditis with, in almost all instances, spontaneous recovery without autoimmune sequelae. Moreover, although acute high-level iodide ingestion by animals can induce thyroid necrosis, such an effect has not been documented in humans.

Turning to the effects of iodide on the immune system, the absence of iodide from human Tg or chemically iodinated human Tg affects recognition by some mouse monoclonal antibodies (6). However, iodinated Tg is not required for recognition by human autoantibodies (reviewed in Ref. 348). In contrast, the importance of iodinated Tg is well established for T cells in mouse models of experimental thyroiditis (5, 349) and has

also been demonstrated for the ability of human T cells to respond to Tg (51).

Spontaneous thyroiditis does occur in NOD.H2h4 mice and is unrelated to increased iodide organification or differences in T-cell or antibody epitopes on Tg (350). Moreover, the development of autoantibodies to Tg or TPO in this mouse strain does not require increased exposure to iodide (135). These findings, taken together with the absence of an iodide effect on nonautoimmune strains, suggest that although increased iodide intake greatly enhances ongoing autoimmune responses, it is not, in itself, a trigger of thyroid autoimmunity.

2. Selenium and goitrogens

Other dietary components include selenium and goitrogens. Selenocysteines are present in all three deiodinases as well as in the families of glutathione peroxidases and thioredoxin reductases, proteins that protect thyrocytes from oxidative damage (reviewed in Ref. 351). Selenium levels are reported to be low in the sera of patients with newly diagnosed autoimmune thyroid conditions, particularly Graves' disease (352). Supplementation with selenium is associated with decreased thyroid autoantibody levels, albeit to variable extents in different studies and different patient groups (reviewed in Ref. 353). These studies do support a role for this trace element in thyroid autoimmunity. However, further studies are necessary to clearly establish such a relationship, and there is no definitive evidence that selenium is involved in the induction of autoimmune responses to thyroid autoantigens.

Goitrogens are present in foods such as cassava, a major dietary component in some tropical and subtropical regions. The goitrogens in cabbage cause thyroid hyperplasia in rabbits (354), and ingestion of excessive amounts of raw bok choy (Chinese white cabbage) induced myxedema coma in a woman (355). Breakdown products in these foods include thiocyanates that inhibit thyroid function. It is unlikely that goitrogens play a triggering role in thyroid autoimmunity.

B. Radiation, smoking, drugs, and environmental toxins

1. Radiation

Studies of the effects of radioactive fallout after atomic bombs dropped on Hiroshima and Nagasaki and the nuclear accident at Chernobyl, as well as earlier studies on low levels of radiation administered for diagnostic or therapeutic purposes, provided unequivocal evidence for increases in thyroid cancer. A highly controversial issue is whether these same levels of thyroid irradiation can induce thyroid autoimmunity, particularly thyroiditis and hypothyroidism. Data in post-Chernobyl follow-up studies indicated that, in addition to thyroid neoplasia, there was an increased incidence of thyroid autoantibodies in individuals exposed to low level thyroid irradiation compared with control populations (reviewed in Refs. 356 and 357). The question was whether these thyroid autoantibodies reflected bona fide Hashimoto's thyroiditis with the subsequent risk of hypothyroidism or, alternatively, a normal immunosurveillance response to neoantigens on irradiated thyroid cells that would subside with deletion of the abnormal cells (in individuals not progressing to overt malignancy) without the development of long-term hypothyroidism. Recent evidence supports the latter conclusion: more than 50 years after radiation exposure in Nagasaki atomic bomb survivors, there was no relationship between radiation dose and the development of autoimmune thyroid diseases (358). Moreover, follow-up studies 13–15 years after radiation exposure from the Chernobyl accident showed that the increased prevalence of thyroid autoantibodies observed at the 6- to 8-year time point had disappeared, and thyroid function remained unaffected (359). These data suggested that “radioactive fallout elicited a transient response without triggering full-blown autoimmune disease.”

Some patients with Graves' disease and thyroid cancer are treated using 131-I. Moreover, neck radiation was performed for Hodgkin's disease in the past. These treatments involve much higher doses of radiation

than experienced by most individuals after radioactive fallout; for example, 5000 cGy for Graves's disease, and more than 4500 cGy for Hodgkin's disease. Nearly 80% of Graves' patients, but not patients with toxic nodular goiter, had transient elevations in TSHR autoantibodies in the 3-month period after 131-I treatment (360). Another study reported hyperthyroidism after high-dose radiation (3500 cGy) for Hodgkin's disease (361). Using a sensitive assay for TSHR antibodies to exclude patients with pre-existing Graves' disease, the incidence of hyperthyroidism was unaltered. However, these patients did have pre-existing "thyroid immunopathy" as reflected by the presence of autoantibodies to TPO (362).

The mechanism involved in the transient rise in thyroid autoantibodies after radiation for Graves' disease has been investigated experimentally. Lymphocytes from Graves' patients irradiated in vitro added to nonirradiated autologous lymphocytes enhanced thyroid autoantibody synthesis, mimicking the increases observed in vivo after 131-I therapy (363). A likely explanation for these findings is that thyroid irradiation destroys "suppressor T cells" (now called Treg) and B cells without affecting T helper cell function. Thyroid-autoantigen-specific B cells invade the thyroid remnant and are provided with T-cell "help" in the absence of T-cell suppression. Thyroid antibodies, like most IgG molecules, have long half-lives. The loss of thyroid antigens removes the stimulus to the immune response and, many years after radiation therapy, all thyroid autoantibodies disappear (364).

In vivo studies have been carried out in NOD.H2h4 mice that parallel the effects of radioactive fallout. After a single low dose of radiation (0.5 Gray), iodide-induced thyroiditis and antibodies to mouse Tg were increased to a greater extent than in nonirradiated littermates, both after 8 weeks (365) and after 15 months (366). Overall, clinical studies in humans and experimental studies in mice suggest that radiation is an enhancer, rather than an inducer, of thyroid autoimmunity.

2. Smoking, amiodarone, and lithium

Smoking has direct effects on thyroid function and is a risk factor for Graves' hyperthyroidism and ophthalmopathy (reviewed in Ref. 367). Perhaps unexpectedly, discontinuing smoking increases the risk of developing antibodies to Tg and TPO (368). These findings are consistent with the anti-inflammatory effects of nicotine in experimental autoimmune encephalitis, an animal model of multiple sclerosis (369). It is unlikely that amiodarone induces thyroid autoimmunity. Amiodarone, with a very high iodine content, is stored for very long periods in adipose tissue and serves as a depot for the long-term release of large quantities of iodide. This iodide can induce hypothyroidism or thyrotoxicosis in individuals with pre-existing subclinical thyroid disease, typically women with thyroid autoantibodies (reviewed in Ref. 333). Lithium treatment for bipolar disorder has direct clinical effects on the thyroid (commonly goiter and hypothyroidism) but may also exacerbate ongoing thyroid autoimmunity (reviewed in Ref. 370).

3. Environmental toxins

Polyaromatic hydrocarbons, such as 3-MCA, enhance thyroiditis and antibodies to mouse Tg in Buffalo rats (371). Similarly, development of thyroiditis was five times greater in NOD.H2h4 mice exposed to 3-MCA than in untreated animals (335). However, as for iodide, the effect of 3-MCA was observed in genetically predisposed rodents and was rare in rats of a hybrid strain not genetically predisposed to develop spontaneous thyroiditis, namely offspring of Buffalo rats crossed to Lewis rats (371).

C. Infections and thyroid autoimmunity

1. Effects of micro-organisms in general

Viral and bacterial infections are important environmental factors in human thyroid autoimmunity (reviewed in Refs. 333, 335, and 372). Infections also play a major role in the outcome of other

autoimmune diseases in animals. For example, the marked variability in diabetes in NOD mouse colonies around the world, despite similar breeding protocols, reflects environmental factors including conventional vs pathogen-free housing conditions (373).

A role for micro-organisms is observed for some models of thyroid autoimmunity. First, spontaneous thyroiditis was significantly increased in NOD.H2h4 mice housed in conventional vs pathogen-free conditions (335). Second, plasmid DNA immunization (without electroporation) induced TSHR antibodies in conventionally housed mice (162, 163). However, the adjuvant properties of plasmid DNA alone were insufficient to induce TSHR in mice housed in pathogen-free facilities (164, 374). Using TSHR-expressing adenovirus, a more potent "adjuvant" than plasmid DNA, Graves' disease was not enhanced in mice housed conventionally vs in pathogen-free conditions (375). Overall, these findings indicate that exposure to infectious organisms has an additive effect on thyroid autoimmunity that develops spontaneously or is induced using a mild adjuvant (like DNA) but not a more potent adjuvant (like adenovirus). The important issue of a role for specific organisms is considered below.

2. Yersinia enterocolitica

Binding of TSH to *Yersinia enterocolitica* (376) was an intriguing observation supporting previous clinical associations between antibodies to this micro-organism and autoimmune thyroid disease (377, 378). As described below, these in vitro data spurred numerous clinical and basic studies into the possible role of this organism in the pathogenesis of Graves' disease. An important criticism of this in vitro finding, as well as to the purported existence of the TSHR on other micro-organisms, is the very low affinity of this binding, comparable to the binding of TSH to plastic (379).

Several experimental investigations support a role for *Yersinia* infection in triggering Graves' disease. Mice immunized with *Y. enterocolitica* proteins develop antibodies that bind to the human TSHR (380, 381). Conversely, epitopes on *Y. enterocolitica* lipoprotein are cross-reactive with antibody epitopes on the human TSHR (382), and *Y. enterocolitica* proteins have B-cell mitogenic activity (383). Moreover, a recombinant *Y. enterocolitica* lipoprotein generated antibody that cross-reacted with the TSHR and up-regulated the costimulatory molecules B7–1 and B7.2 on APCs (384). More recently, mass spectrometry and protein structure modeling has been used to support cross-reactivity between the epitope of a TSHR antibody and an epitope on the *Y. enterocolitica* ompF protein (385). Bioinformatic studies (386) suggest that the outer membrane proteins of *Y. enterocolitica* contain cross-reactive T-cell epitopes (see *Section XI.C.4.*).

Clinical studies provide evidence for (378, 387) as well as against (388) a role for this bacterium in Graves' disease or suggest that it is "too early to dismiss *Y. enterocolitica* infection in the etiology of Graves' disease" (389). Very recently, binding to *Y. enterocolitica* proteins was demonstrated for the germline heavy and light chain gene precursors of two stimulating TSHR antibodies (390). Therefore, it remains possible that *Y. enterocolitica* plays a role in the etiology of Graves' disease, at least in some patients. If correct, this concept implies that other triggering factors are involved in breaking tolerance to the TSHR in the many other individuals who subsequently develop Graves' disease without *Y. enterocolitica* infection.

3. Hepatitis C and other viruses

Viruses have been implicated in thyroid autoimmunity, for example, Coxsackie B virus in Graves' disease (391) and enteroviruses in Hashimoto's thyroiditis (392). However, the strongest evidence of a role for viruses in thyroid autoimmunity is for hepatitis C virus (HCV). Autoimmune, as well as nonautoimmune, hypothyroidism is significantly higher in untreated children with HCV infection than in non-HCV controls (278). According to Tomer (269), HCV is "the only infectious agent that is clearly associated with an increased risk for autoimmune thyroiditis." It is well recognized that treatment with IFN-α enhances

ongoing thyroid autoimmunity (described in *Section IX*). Prummel and Laurberg (268) suggest that IFN-α is "one of the environmental factors capable of triggering the onset of AITD in genetically susceptible individuals."

Can HCV infection induce thyroid autoimmune responses in genetically susceptible individuals? In other words, does HCV infect and damage thyroid cells, leading to the release of proinflammatory cytokines? HCV virions have been detected in thyroid tissue from patients with chronic HCV infection (reviewed in Ref. 269). In addition, HCV has recently been shown to infect human thyroid cells in vitro, leading to production of the proinflammatory cytokine IL-8 (393), a contributor to bystander activation (*Section XI.B.5.*). These findings, together with the observation that thyroid autoantibodies are present in significantly more children with HCV than in controls, suggest that HCV may indeed play a role in the autoimmune process.

4. Molecular mimicry T-cell epitope hypothesis

The studies described above invoking a role for infection with *Y. enterocolitica* in Graves' disease were based primarily on cross-reactivity between antibody epitopes on *Y. enterocolitica* proteins and the TSHR. However, a more likely hypothesis for the contribution of micro-organisms to breaking tolerance is the existence of T-cell cross-reactive epitopes. T cells recognize short linear peptides, approximately 20 amino acid residues long for CD4+ T cells and 8–10 residues for CD8+ T cells. Some amino acids in these peptides are of particular importance for binding to MHC molecules, and "algorithms" have been developed to predict amino acid sequences likely to confer high-affinity binding (260). The Rothbard algorithm was used to search for a potential shared Tg-TPO epitope (259) (Figure 9A), which was later found to be recognized by CD8+ T cells from patients with Hashimoto's thyroiditis (262).

Other approaches include searching for: 1) homologies between the amino acid sequences of thyroid autoantigens and proteins of micro-organisms possibly associated with thyroid autoimmunity; or 2) peptide sequences in micro-organisms capable of binding to HLA-DR molecule motifs associated with thyroid autoimmunity. Searches of this type revealed homologies between the TSHR and *Borrelia burgdorferi* and the outer protein of *Y. enterocolitica* (386, 394–397), as well as between DR3/DR7 binding motifs and *Clostridium* neurotoxin (398).

T-cell epitopic mimicry is intriguing and has generated much enthusiasm, but evidence that autoimmunity is provoked by infection has been questioned: "For decades there have been tantalizing associations between infections agents and autoimmunity … many of the associations appear less than convincing and even for those that seem to be solid footing, there is no real understanding of the underlying mechanism(s)" (399). Incidentally, indirect evidence against cross-reactive TSHR T-cell epitopes comes from an animal model of Graves' disease: wild-type mice, unlike their littermates that expressed the human TSHR A-subunit in the thyroid, do not develop thyroiditis after Treg depletion and immunization with human TSHR A-subunit adenovirus (147). These wild-type animals respond to human TSHR peptides but, despite predicted high-affinity binding to the appropriate MHC class II, do not respond to the corresponding mouse TSHR peptides (236). The lack of cross-reactivity, despite relatively high homology between human and mouse TSHR amino acid sequences (Figure 9B) (discussed in *Section VIII*), suggests that it is unlikely that T cells would be triggered by micro-organism protein sequences with much lower homology.

An alternative, equally intriguing hypothesis has been put forward by David and colleagues (400). The haplotypes commonly associated with autoimmunity (HLA-DR2DQ6, DR4DQ8, and DR3DQ2) persisted in evolution because of their ability to present pathogenic peptides to activate T cells and clear infections. Unfortunately, these haplotypes also present self-peptides to activate autoimmune cells.

5. Bystander activation vs interference by micro-organisms

The enhancement of autoimmune responses by micro-organisms likely occurs as a result of bystander activity (401) involving the cytokines IL-2 and IL-7 (402) and possibly also consequent to tissue damage (403). The mechanisms of central and peripheral tolerance delete the most aggressive CD8+ T cells but not T cells that respond weakly to tissue-restricted self-antigens. During infection, the threshold for activation of CD8+ T cells is lower than that required for negative selection (deletion) of cells that respond weakly to tissue-restricted self-antigens and may lead to organ damage (404).

The power of infectious organisms has long been used in the form of adjuvants to overcome self-tolerance and induce autoimmunity to self-proteins. However, micro-organisms can also interfere with ongoing immune responses. The ability of *Schistosoma* infection or α-galactosylceramide to reduce the efficacy of TSHR adenovirus immunization for inducing Graves' disease has already been mentioned (309). In addition, in experiments directed at breaking tolerance to the human TSHR A-subunit in high expressor transgenics, injecting CFA in the absence of A-subunit protein markedly reduced the efficacy of subsequent immunization with A-subunit adenovirus (175).

It is also useful to mention the “hygiene hypothesis,” which postulates that human populations regularly exposed to infectious organisms develop less autoimmunity and allergy than individuals living in “clean” environments (405, 406). A likely basis for this observation is the failure of individuals living under hygienic conditions to routinely activate immune regulatory mechanisms, leading to “a lazy immune system.”

In 1989, an epidemiological approach was used to answer the question “Does infection initiate Graves' disease?” The conclusion that infectious epidemics do not have a significant causative role in triggering Graves' disease (407) may be explained, at least in part, by the contrasting effects of micro-organisms described above (185, 309), together with the hygiene hypothesis (405, 406).

XII. Summary and Conclusions

A. The breakdown in self-tolerance leading to thyroid autoimmunity requires a susceptible genetic background, together with the unusual characteristics of thyroid autoantigens. Tg, TPO, and the TSHR have differing “immunogenicity” profiles depending on their size, extent of glycosylation, concentration in the thyroid, whether membrane-bound, soluble or “shed” (the TSHR A-subunit), ability to bind to the mannose receptor on APCs, as well as the existence of polymorphic variations.

B. Spontaneous thyroiditis occurs in a number of mammals, but Graves' disease only develops in humans. Thyroiditis is readily induced in rodents by conventional as well as novel immunization approaches. However, Graves' disease is only induced by injecting TSHR-expressing cells or by expressing the TSHR in vivo using plasmid or adenovirus vectors.

C. Central tolerance, determined by intrathymic expression, is high for Tg and lower for TPO and the TSHR. Despite similar expression levels of TPO and TSHR in the thymus, immunization of mice with mouse TPO adenovirus, but not mouse TSHR adenovirus, can break tolerance and induce specific antibodies.

D. Treg are not involved in controlling self-tolerance to the TSHR or to TPO, at least in mice. However, Treg depletion enhances both spontaneous and induced thyroiditis once self-tolerance is broken. Moreover, in an animal model, Treg control the balance between Graves' hyperthyroidism and Hashimoto's thyroiditis.

E. In NOD.H2h4 mice, as in juvenile Hashimoto thyroiditis patients, the spontaneous breakdown in self-tolerance occurs first for Tg and much later for TPO. This pattern is unexpected because intrathymic expression of Tg is higher than for TPO and, instead of being controlled by central

tolerance, may involve the greater immunogenicity of Tg than TPO.

F. Breaking tolerance to the TSHR A-subunit involves contributions from MHC molecules (humans and induced mouse models), TSHR polymorphism(s) (humans), and alternative splicing (mice).

G. Thyroid autoimmunity, usually Graves' disease, develops inadvertently in the “immune reconstitution” phase of patients treated with antibodies to deplete T cells. Hashimoto's thyroiditis develops after treatment with IFN-α for HCV infection.

H. Antigen-specific tolerance can be induced experimentally in mice by increasing systemic Tg levels, by feeding Tg (oral tolerance), and by neonatal immunization with TSHR adenovirus (the latter reduces the efficacy of inducing Graves' disease). In adult mice, im pretreatment with TSHR A-subunit protein “deviates” the subsequent immune response to TSHR A-subunit adenovirus immunization toward biologically inactive antibodies. Oral tolerance applied to humans suggests changes in T-cell (but not antibody) responses. However, in line with the difficulties experienced for other autoimmune diseases, the future application of any approaches to induce antigen-specific tolerance will require accurate prediction of genetically at-risk individuals.

I. A multiplicity of environmental factors influence thyroid autoimmunity, including iodine uptake, radiation, smoking, selenium, drugs, environmental toxins, and micro-organisms. Most of these factors, including iodine, enhance ongoing thyroid autoimmunity. However, there is evidence that infection with HCV plays a primary role in inducing autoimmune thyroiditis.

J. Micro-organisms probably exert their effects via bystander stimulation rather than by T-cell “epitopic mimicry.” It is also important to note that micro-organisms can have inhibitory rather than stimulatory effects either directly or, as suggested by the “hygiene hypothesis,” indirectly by maintaining healthy regulatory responses in individuals with more frequent exposure to low-level infections.

In conclusion, there appears to be no single mechanism that can explain the loss of tolerance to thyroid proteins leading to thyroid autoimmunity. Central tolerance (intrathymic expression of thyroid proteins), Treg, and the unusual characteristics of the thyroid autoantigens themselves, together with genetic susceptibility, all play a role. Despite their importance, virtually all environmental factors “reveal” or enhance, but do not induce, thyroid autoimmunity. Working toward induced self-tolerance, an admirable goal, emphasizes the need for accurate prediction of at-risk individuals. Above all, it is crucial to recognize that antigen-specific, not blanket, approaches will be required for the successful application of future protocols to induce self-tolerance. In this respect, thyroid autoimmunity has an advantage over many other autoimmune diseases in that the specific antigens directly involved in the disease are clearly identified.

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Footnotes

Abbreviations:

AIRE autoimmune regulator

APC antigen-presenting cell

APECED autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy

BGG bovine gamma globulin
CFA Complete Freund's adjuvant
CHO Chinese hamster ovary
CTLA4 cytotoxic T lymphocyte-associated factor 4
DC dendritic cell
Foxp3 forkhead box P3 protein
HCV hepatitis C virus
HEL hen egg lysozyme
IFN interferon
LPS lipopolysaccharide
3-MCA 3-methylcholanthrene
MHC major histocompatibility complex
NIS sodium-iodide symporter
NOD nonobese diabetic
OS obese strain
pDC plasmacytoid DC
SNP single nucleotide polymorphism
TBAb TSH-blocking antibody
Tg thyroglobulin
TgAb Tg antibody
Th1 T helper 1
TPO thyroid peroxidase
TPOAb TPO antibody
Treg regulatory T cells
TSAb thyroid-stimulating antibody
TSHR TSH receptor
VNTR variable number of tandem repeats.

References

1. Hollowell JG, Staehling NW, Flanders WD, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002;87:489–499 [PubMed: 11836274]

2. Tomer Y, Huber A. The etiology of autoimmune thyroid disease: a story of genes and environment. J Autoimmun. 2009;32:231–239 [PMCID: PMC3561494] [PubMed: 19307103]

3. Nye L, Pontes de Carvalho LC, Roitt IM. Restrictions in the response to autologous thyroglobulin in the human. Clin Exp Immunol. 1980;41:252–263 [PMCID: PMC1537003] [PubMed: 6160004]

4. Sundick RS, Herdegen DM, Brown TR, Bagchi N. The incorporation of dietary iodine into thyroglobulin increases its immunogenicity. Endocrinology. 1987;120:2078–2084 [PubMed: 3569125]

5. Champion BR, Page KR, Parish N, et al. Identification of a thyroxine-containing self-epitope of thyroglobulin which triggers thyroid autoreactive T cells. J Exp Med. 1991;174:363–370 [PMCID: PMC2118919] [PubMed: 1713250]

6. Saboori AM, Rose NR, Bresler HS, Vladut-Talor M, Burek CL. Iodination of human thyroglobulin (Tg) alters its immunoreactivity. I. Iodination alters multiple epitopes of human Tg. Clin Exp Immunol. 1998;113:297–302 [PMCID: PMC1905042] [PubMed: 9717981]

7. Grennan Jones F, Wolstenholme A, Fowler S, et al. High-level expression of recombinant

immunoreactive thyroid peroxidase in the High Five insect cell line. J Mol Endocrinol. 1996;17:165–174 [PubMed: 8938592]

8. Guo J, McLachlan SM, Hutchison S, Rapoport B. The greater glycan content of recombinant human thyroid peroxidase of mammalian than of insect cell origin facilitates purification to homogeneity of enzymatically protein remaining soluble at high concentration. Endocrinology. 1998;139:999–1005 [PubMed: 9492031]

9. Le Fourn V, Ferrand M, Franc JL. Endoproteolytic cleavage of human thyroperoxidase: role of the propeptide in the protein folding process. J Biol Chem. 2005;280:4568–4577 [PubMed: 15590661]

10. McLachlan SM, Rapoport B. Thyroid peroxidase as an autoantigen. Thyroid. 2007;17:939–948 [PubMed: 17822378]

11. Rapoport B, McLachlan SM. The thyrotropin receptor in Graves' disease. Thyroid. 2007;17:911–922 [PubMed: 17822379]

12. Dai G, Levy O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. Nature. 1996;379:458–460 [PubMed: 8559252]

13. Raspé E, Costagliola S, Ruf J, Mariotti S, Dumont JE, Ludgate M. Identification of the thyroid Na+/I− cotransporter as a potential autoantigen in thyroid autoimmune disease. Eur J Endocrinol. 1995;132:399–405 [PubMed: 7711875]

14. Ajjan RA, Kemp EH, Waterman EA, et al. Detection of binding and blocking autoantibodies to the human sodium-iodide symporter in patients with autoimmune thyroid disease. J Clin Endocrinol Metab. 2000;85:2020–2027 [PubMed: 10843191]

15. Seissler J, Wagner S, Schott M, et al. Low frequency of autoantibodies to the human Na(+)/I(−) symporter in patients with autoimmune thyroid disease. J Clin Endocrinol Metab. 2000;85:4630–4634 [PubMed: 11134119]

16. Heufelder AE, Joba W, Morgenthaler NG. Autoimmunity involving the human sodium/iodide symporter: fact or fiction? Exp Clin Endocrinol Diabetes. 2001;109:35–40 [PubMed: 11573137]

17. Czarnocka B. Thyroperoxidase, thyroglobulin, Na(+)/I(−) symporter, pendrin in thyroid autoimmunity. Front Biosci (Landmark Ed). 2011;16:783–802 [PubMed: 21196203]

18. Kemp EH, Sandhu HK, Watson PF, Weetman AP. Low frequency of pendrin autoantibodies detected using a radioligand binding assay in patients with autoimmune thyroid disease. J Clin Endocrinol Metab. 2013;98:E309–E313 [PubMed: 23322815]

19. Pritchard J, Han R, Horst N, Cruikshank WW, Smith TJ. Immunoglobulin activation of T cell chemoattractant expression in fibroblasts from patients with Graves' disease is mediated through the insulin-like growth factor I receptor pathway. J Immunol. 2003;170:6348–6354 [PubMed: 12794168]

20. Smith TJ. Is IGF-I receptor a target for autoantibody generation in Graves' disease? J Clin Endocrinol Metab. 2013;98:515–518 [PMCID: PMC3565121] [PubMed: 23390263]

21. Varewijck AJ, Boelen A, Lamberts SW, et al. Circulating IgGs may modulate IGF-I receptor stimulating activity in a subset of patients with Graves' ophthalmopathy. J Clin Endocrinol Metab. 2013;98:769–776 [PubMed: 23295466]

22. Minich WB, Dehina N, Welsink T, et al. Autoantibodies to the IGF1 receptor in Graves' orbitopathy. J Clin Endocrinol Metab. 2013;98:752–760 [PubMed: 23264397]

23. Zakarija M, McKenzie JM, Eidson MS. Transient neonatal hypothyroidism: characterization of maternal antibodies to the thyrotropin receptor. J Clin Endocrinol Metab. 1990;70:1239–1246 [PubMed: 1970827]

24. Flynn JC, Gilbert JA, Meroueh C, et al. Chronic exposure in vivo to thyrotropin receptor stimulating monoclonal antibodies sustains high thyroxine levels and thyroid hyperplasia in thyroid autoimmunity-prone HLA-DRB1*0301 transgenic mice. Immunology. 2007;122:261–267 [PMCID: PMC2265995] [PubMed: 17535305]

25. Misharin AV, Rapoport B, McLachlan SM. Thyroid antigens, not central tolerance, control responses to immunization in BALB/c versus C57BL/6 mice. Thyroid. 2009;19:503–509 [PMCID: PMC2857445] [PubMed: 19348579]

26. Muixí L, Carrascal M, Alvarez I, et al. Thyroglobulin peptides associate in vivo to HLA-DR in autoimmune thyroid glands. J Immunol. 2008;181:795–807 [PubMed: 18566446]

27. Unanue ER, Cerottini JC. The immunogenicity of antigen bound to the plasma membrane of macrophages. J Exp Med. 1970;131:711–725 [PMCID: PMC2138775] [PubMed: 5430785]

28. Bergers JJ, Den Otter W, De Groot JW, et al. Reconstituted membranes of tumour cells (proteoliposomes) induce specific protection to murine lymphoma cells. Cancer Immunol Immunother. 1992;34:233–240 [PubMed: 1537056]

29. Stahl P, Gordon S. Expression of a mannosyl-fucosyl receptor for endocytosis on cultured primary macrophages and their hybrids. J Cell Biol. 1982;93:49–56 [PMCID: PMC2112098] [PubMed: 6279673]

30. Engering AJ, Cella M, Fluitsma D, et al. The mannose receptor functions as a high capacity and broad specificity antigen receptor in human dendritic cells. Eur J Immunol. 1997;27:2417–2425 [PubMed: 9341788]

31. Chazenbalk GD, Pichurin PN, Guo J, Rapoport B, McLachlan SM. Interactions between the mannose receptor and thyroid autoantigens. Clin Exp Immunol. 2005;139:216–224 [PMCID: PMC1809290] [PubMed: 15654820]

32. Tomer Y, Greenberg DA, Concepcion E, Ban Y, Davies TF. Thyroglobulin is a thyroid specific gene for the familial autoimmune thyroid diseases. J Clin Endocrinol Metab. 2002;87:404–407 [PubMed: 11788684]

33. Brand OJ, Barrett JC, Simmonds MJ, et al. Association of the thyroid stimulating hormone receptor gene (TSHR) with Graves' disease. Hum Mol Genet. 2009;18:1704–1713 [PubMed: 19244275]

34. Adams DD, Kennedy TH, Stewart JC, Utiger RD, Vidor GI. Hyperthyroidism in Tasmania following iodide supplementation: measurements of thyroid-stimulating autoantibodies and thyrotropin. J Clin Endocrinol Metab. 1975;41:221–228 [PubMed: 51028]

35. Orgiazzi J, Williams DE, Chopra IJ, Solomon DH. Human thyroid adenyl cyclase-stimulating activity in immunoglobulin G of patients with Graves' disease. J Clin Endocrinol Metab. 1976;42:341–354 [PubMed: 946604]

36. Endo K, Kasagi K, Konishi J, et al. Detection and properties of TSH-binding inhibitor immunoglobulins in patients with Graves' disease and Hashimoto's thyroiditis. J Clin Endocrinol Metab. 1978;46:734–739 [PubMed: 45421]

37. Morshed SA, Ando T, Latif R, Davies TF. Neutral antibodies to the TSH receptor are present in Graves' disease and regulate selective signaling cascades. Endocrinology. 2010;151:5537–5549

[PMCID: PMC2954721] [PubMed: 20844004]

38. Khoo TK, Bahn RS. Pathogenesis of Graves' ophthalmopathy: the role of autoantibodies. Thyroid. 2007;17:1013–1018 [PMCID: PMC3902014] [PubMed: 17935483]

39. Rapoport B, McLachlan SM. Thyroid autoimmunity. J Clin Invest. 2001;108:1253–1259 [PMCID: PMC209450] [PubMed: 11696565]

40. Campbell PN, Doniach D, Hudson RV, Roitt IM. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). Lancet. 1956;271:820–821 [PubMed: 13368530]

41. Belyavin G, Trotter WR. Investigations of thyroid antigens reacting with Hashimoto sera; evidence for an antigen other than thyroglobulin. Lancet. 1959;1:648–652 [PubMed: 13642835]

42. Czarnocka B, Ruf J, Ferrand M, Carayon P, Lissitzky S. Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases. FEBS Lett. 1985;190:147–152 [PubMed: 2995127]

43. Portmann L, Hamada N, Heinrich G, DeGroot LJ. Anti-thyroid peroxidase antibody in patients with autoimmune thyroid disease: possible identity with anti-microsomal antibody. J Clin Endocrinol Metab. 1985;61:1001–1003 [PubMed: 2995429]

44. Seto P, Hirayu H, Magnusson RP, et al. Isolation of a complementary DNA clone for thyroid microsomal antigen. Homology with the gene for thyroid peroxidase. J Clin Invest. 1987;80:1205–1208 [PMCID: PMC442367] [PubMed: 3654979]

45. Libert F, Ruel J, Ludgate M, et al. Thyroperoxidase, an auto-antigen with a mosaic structure made of nuclear and mitochondrial gene modules. EMBO J. 1987;6:4193–4196 [PMCID: PMC553903] [PubMed: 3443105]

46. Blomgren H, Lundell G. Proliferation of peripheral lymphoid cells from euthyroid and hyperthyroid individuals cultured with human thyroglobulin. Acta Endocrinol (Copenh). 1979;90:227–232 [PubMed: 311130]

47. MacKenzie WA, Schwartz AE, Friedman EW, Davies TF. Intrathyroidal T cell clones from patients with autoimmune thyroid disease. J Clin Endocrinol Metab. 1987;64:818–824 [PubMed: 2880865]

48. Fukuma N, McLachlan SM, Rapoport B, et al. Thyroid autoantigens and human T cell responses. Clin Exp Immunol. 1990;82:275–283 [PMCID: PMC1535115] [PubMed: 2242608]

49. Fisfalen ME, Soliman M, Okamoto Y, Soltani K, DeGroot LJ. Proliferative responses of T-cells to thyroid antigens and synthetic thyroid peroxidase peptides in autoimmune thyroid disease. J Clin Endocrinol Metab. 1995;80:1597–1604 [PubMed: 7745006]

50. Fisfalen ME, Palmer EM, Van Seventer GA, et al. Thyrotropin-receptor and thyroid peroxidase-specific T cell clones and their cytokine profile in autoimmune thyroid disease. J Clin Endocrinol Metab. 1997;82:3655–3663 [PubMed: 9360522]

51. Rasooly L, Rose NR, Saboori AM, Ladenson PW, Burek CL. Iodine is essential for human T cell recognition of human thyroglobulin. Autoimmunity. 1998;27:213–219 [PubMed: 9623499]

52. Prentice LM, Phillips DI, Sarsero D, Beever K, McLachlan SM, Smith BR. Geographical distribution of subclinical autoimmune thyroid disease in Britain: a study using highly sensitive direct assays for autoantibodies to thyroglobulin and thyroid peroxidase. Acta Endocrinol (Copenh). 1990;123:493–498 [PubMed: 2256432]

53. Yoshida H, Amino N, Yagawa K, et al. Association of serum antithyroid antibodies with lymphocytic infiltration of the thyroid gland: Studies of seventy autopsied cases. J Clin Endocrinol Metab. 1978;46:859–862 [PubMed: 263468]

54. Acar T, Ozbek SS, Erdogan M, Ozgen AG, Demirel SO. US findings in euthyroid patients with positive antithyroid autoantibody tests compared to normal and hypothyroid cases. Diagn Interv Radiol. 2013;19:265–270 [PubMed: 23302287]

55. Marcocci C, Chiovato L, Mariotti S, Pinchera A. Changes of circulating thyroid autoantibody levels during and after the therapy with methimazole in patients with Graves' disease. J Endocrinol Invest. 1982;5:13–19 [PubMed: 6896520]

56. Allen EM, Appel MC, Braverman LE. The effect of iodide ingestion on the development of spontaneous lymphocytic thyroiditis in the diabetes-prone BB/W rat. Endocrinology. 1986;118:1977–1981 [PubMed: 3754509]

57. Bernard NF, Ertug F, Margolese H. High incidence of thyroiditis and anti-thyroid autoantibodies in NOD mice. Diabetes. 1992;41:40–46 [PubMed: 1727738]

58. Rasooly L, Burek CL, Rose NR. Iodine-induced autoimmune thyroiditis in NOD-H-2h4 mice. Clin Immunol Immunopathol. 1996;81:287–292 [PubMed: 8938107]

59. Braley-Mullen H, Sharp GC, Medling B, Tang H. Spontaneous autoimmune thyroiditis in NOD.H-2h4 mice. J Autoimmun. 1999;12:157–165 [PubMed: 10222025]

60. Hutchings PR, Verma S, Phillips JM, Harach SZ, Howlett S, Cooke A. Both CD4(+) T cells and CD8(+) T cells are required for iodine accelerated thyroiditis in NOD mice. Cell Immunol. 1999;192:113–121 [PubMed: 10087179]

61. Wick G, Most J, Schauenstein K, et al. Spontaneous autoimmune thyroiditis-a bird's eye view. Immunol Today. 1985;6:359–365 [PubMed: 25291225]

62. Aichinger G, Kofler H, Diaz-Merida O, Wick G. Nonthyroid autoantibodies in obese strain (OS) chickens. Clin Immunol Immunopathol. 1984;32:57–69 [PubMed: 6428787]

63. Tucker WE., Jr Thyroiditis in a group of laboratory dogs. A study of 167 beagles. Am J Clin Pathol. 1962;38:70–74 [PubMed: 13923002]

64. Thacker EL, Davis JM, Refsal KR, Bull RW. Isolation of thyroid peroxidase and lack of autoantibodies to the enzyme in dogs with autoimmune thyroid disease. Am J Vet Res. 1995;56:34–38 [PubMed: 7695146]

65. Levy BM, Hampton S, Dreizen S, Hampton JK. Thyroiditis in the marmoset (Callithrix spp. and Saguinus spp.). J Comp Pathol. 1972;82:99–103 [PubMed: 4622910]

66. David JM, Dick EJ, Jr, Hubbard GB. Spontaneous pathology of the common marmoset (*Callithrix jacchus*) and tamarins (*Saguinus oedipus, Saguinus mystax).* J Med Primatol. 2009;38:347–359 [PMCID: PMC2740810] [PubMed: 19522731]

67. Simitsek PD, Campbell DG, Lanzavecchia A, Fairweather N, Watts C. Modulation of antigen processing by bound antibodies can boost or suppress class II major histocompatibility complex presentation of different T cell determinants. J Exp Med. 1995;181:1957–1963 [PMCID: PMC2192058] [PubMed: 7539034]

68. Serreze DV, Fleming SA, Chapman HD, Richard SD, Leiter EH, Tisch RM. B lymphocytes are critical antigen-presenting cells for the initiation of T cell-mediated autoimmune diabetes in nonobese diabetic

mice. J Immunol. 1998;161:3912–3918 [PubMed: 9780157]

69. Yu S, Maiti PK, Dyson M, Jain R, Braley-Mullen H. B Cell-deficient NOD.H-2h4 mice have CD4+CD25+ T regulatory cells that inhibit the development of spontaneous autoimmune thyroiditis. J Exp Med. 2006;203:349–358 [PMCID: PMC2118195] [PubMed: 16446379]

70. Vaughan AT, Roghanian A, Cragg MS. B cells–masters of the immunoverse. Int J Biochem Cell Biol. 2011;43:280–285 [PubMed: 21147251]

71. Salvi M, Vannucchi G, Campi I, et al. Treatment of Graves' disease and associated ophthalmopathy with the anti-CD20 monoclonal antibody rituximab: an open study. Eur J Endocrinol. 2007;156:33–40 [PubMed: 17218723]

72. Ueki I, Abiru N, Kobayashi M, et al. B cell-targeted therapy with anti-CD20 monoclonal antibody in a mouse model of Graves' hyperthyroidism. Clin Exp Immunol. 2011;163:309–317 [PMCID: PMC3048613] [PubMed: 21235532]

73. Yassai M, Ammon K, Goverman J, Marrack P, Naumov Y, Gorski J. A molecular marker for thymocyte-positive selection: selection of CD4 single-positive thymocytes with shorter TCRB CDR3 during T cell development. J Immunol. 2002;168:3801–3807 [PubMed: 11937532]

74. Kappler JW, Roehm N, Marrack P. T cell tolerance by clonal elimination in the thymus. Cell. 1987;49:273–280 [PubMed: 3494522]

75. Derbinski J, Schulte A, Kyewski B, Klein L. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. Nat Immunol. 2001;2:1032–1039 [PubMed: 11600886]

76. Ferguson BJ, Cooke A, Peterson P, Rich T. Death in the AIRE. Trends Immunol. 2008;29:306–312 [PubMed: 18515183]

77. Vafiadis P, Bennett ST, Todd JA, et al. Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. Nat Genet. 1997;15:289–292 [PubMed: 9054944]

78. Pugliese A, Zeller M, Fernandez A, Jr, et al. The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. Nat Genet. 1997;15:293–297 [PubMed: 9054945]

79. Akkaraju S, Ho WY, Leong D, Canaan K, Davis MM, Goodnow CC. A range of CD4 T cell tolerance: partial inactivation to organ-specific antigen allows nondestructive thyroiditis or insulitis. Immunity. 1997;7:255–271 [PubMed: 9285410]

80. Zhang M, Vacchio MS, Vistica BP, et al. T cell tolerance to a neo-self antigen expressed by thymic epithelial cells: the soluble form is more effective than the membrane-bound form. J Immunol. 2003;170:3954–3962 [PubMed: 12682222]

81. Finnish-German APECED Consortium An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. Nat Genet. 1997;17:399–403 [PubMed: 9398840]

82. Nagamine K, Peterson P, Scott HS, et al. Positional cloning of the APECED gene. Nat Genet. 1997;17:393–398 [PubMed: 9398839]

83. Anderson MS, Venanzi ES, Klein L, et al. Projection of an immunological self shadow within the thymus by the aire protein. Science. 2002;298:1395–1401 [PubMed: 12376594]

84. Ramsey C, Winqvist O, Puhakka L, et al. Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. Hum Mol Genet. 2002;11:397–409 [PubMed: 11854172]

85. Mathis D, Benoist C. A decade of AIRE. Nat Rev Immunol. 2007;7:645–650 [PubMed: 17641664]

86. Kont V, Laan M, Kisand K, Merits A, Scott HS, Peterson P. Modulation of Aire regulates the expression of tissue-restricted antigens. Mol Immunol. 2008;45:25–33 [PMCID: PMC1994210] [PubMed: 17599412]

87. Liston A, Gray DH, Lesage S, et al. Gene dosage–limiting role of Aire in thymic expression, clonal deletion, and organ-specific autoimmunity. J Exp Med. 2004;200:1015–1026 [PMCID: PMC2211852] [PubMed: 15492124]

88. Niki S, Oshikawa K, Mouri Y, et al. Alteration of intra-pancreatic target-organ specificity by abrogation of Aire in NOD mice. J Clin Invest. 2006;116:1292–1301 [PMCID: PMC1440703] [PubMed: 16628255]

89. Jiang W, Anderson MS, Bronson R, Mathis D, Benoist C. Modifier loci condition autoimmunity provoked by Aire deficiency. J Exp Med. 2005;202:805–815 [PMCID: PMC2212943] [PubMed: 16172259]

90. Mouchess ML, Anderson M. Central tolerance induction [published online ahead of print May 10, 2013]. Curr Top Microbiol Immunol. doi:10.1007/82_2013_321

91. Lourenço EV, La Cava A. Natural regulatory T cells in autoimmunity. Autoimmunity. 2011;44:33–42 [PMCID: PMC3057884] [PubMed: 21091291]

92. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155:1151–1164 [PubMed: 7636184]

93. Rifa'I M, Kawamoto Y, Nakashima I, Suzuki H. Essential roles of CD8+CD122+ regulatory T cells in the maintenance of T cell homeostasis. J Exp Med. 2004;200:1123–1134 [PMCID: PMC2211869] [PubMed: 15520244]

94. Miyara M, Sakaguchi S. Natural regulatory T cells: mechanisms of suppression. Trends Mol Med. 2007;13:108–116 [PubMed: 17257897]

95. Wu AJ, Hua H, Munson SH, McDevitt HO. Tumor necrosis factor-α regulation of CD4+CD25+ T cell levels in NOD mice. Proc Natl Acad Sci U S A. 2002;99:12287–12292 [PMCID: PMC129437] [PubMed: 12221281]

96. Endharti AT, Rifa'I M, Shi Z, et al. Cutting edge: CD8+CD122+ regulatory T cells produce IL-10 to suppress IFN-γ production and proliferation of CD8+ T cells. J Immunol. 2005;175:7093–7097 [PubMed: 16301610]

97. Thomas JW. Antigen-specific responses in autoimmunity and tolerance. Immunol Res. 2001;23:235–244 [PubMed: 11444388]

98. Goodnow CC, Crosbie J, Adelstein S, et al. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature. 1988;334:676–682 [PubMed: 3261841]

99. Hartley SB, Crosbie J, Brink R, Kantor AB, Basten A, Goodnow CC. Elimination from peripheral lymphoid tissues of self-reactive B lymphocytes recognizing membrane-bound antigens. Nature. 1991;353:765–769 [PubMed: 1944535]

100. Akkaraju S, Canaan K, Goodnow CC. Self-reactive B cells are not eliminated or inactivated by autoantigen expressed on thyroid epithelial cells. J Exp Med. 1997;186:2005–2012 [PMCID: PMC2199176] [PubMed: 9396769]

101. Silveira PA, Dombrowsky J, Johnson E, Chapman HD, Nemazee D, Serreze DV. B cell selection defects underlie the development of diabetogenic APCs in nonobese diabetic mice. J Immunol. 2004;172:5086–5094 [PMCID: PMC3792717] [PubMed: 15067092]

102. Luning Prak ET, Monestier M, Eisenberg RA. B cell receptor editing in tolerance and autoimmunity. Ann N Y Acad Sci. 2011;1217:96–121 [PMCID: PMC3077556] [PubMed: 21251012]

103. Mauri C, Bosma A. Immune regulatory function of B cells. Annu Rev Immunol. 2012;30:221–241 [PubMed: 22224776]

104. Lehmann B, Schwab I, Böhm S, Lux A, Biburger M, Nimmerjahn F. FcγRIIB: a modulator of cell activation and humoral tolerance. Expert Rev Clin Immunol. 2012;8:243–254 [PubMed: 22390489]

105. Carroll MC, Isenman DE. Regulation of humoral immunity by complement. Immunity. 2012;37:199–207 [PMCID: PMC5784422] [PubMed: 22921118]

106. Scholz JL, Cancro MP. Resolve, revise, and relax: the 3 Rs of B cell repertoire adjustment. Immunol Lett. 2012;143:2–8 [PMCID: PMC3677571] [PubMed: 22330846]

107. Guéry L, Hugues S. Tolerogenic and activatory plasmacytoid dendritic cells in autoimmunity. Front Immunol. 2013;4:59. [PMCID: PMC3589693] [PubMed: 23508732]

108. Terplan KL, Witebsky E, Rose NR, Paine JR, Egan RW. Experimental thyroiditis in rabbits, guinea pigs and dogs, following immunization with thyroid extracts of their own and of heterologous species. Am J Pathol. 1960;36:213–239 [PMCID: PMC1942197] [PubMed: 13837481]

109. Witebsky E, Rose NR, Terplan K, Paine JR, Egan RW. Chronic thyroiditis and autoimmunization. J Am Med Assoc. 1957;164:1439–1447 [PubMed: 13448890]

110. Rose NR, Kite JH, Jr, Doebbler TK, Spier R, Skelton FR, Witebsky E. Studies on experimental thyroiditis. Ann NY Acad Sci. 1965;124:201–230 [PubMed: 4954835]

111. Pudifin DJ, Duursma J, Brain P. Experimental autoimmune thyroiditis in the vervet monkey. Clin Exp Immunol. 1977;29:256–260 [PMCID: PMC1541107] [PubMed: 409579]

112. Kong YM. Experimental models for autoimmune thyroid disease: recent developments. In: Volpé R, editor. , ed. Autoimmune Endocrinopathies (Contemporary Endocrinology). Totawa, NJ: Humana Press; 2004:91–111

113. Esquivel PS, Rose NR, Kong YC. Induction of autoimmunity in good and poor responder mice with mouse thyroglobulin and lipopolysaccharide. J Exp Med. 1977;145:1250–1263 [PMCID: PMC2180674] [PubMed: 323406]

114. Kong YC, Lomo LC, Motte RW, et al. HLA-DRB1 polymorphism determines susceptibility to autoimmune thyroiditis in transgenic mice: definitive association with HLA-DRB1*0301 (DR3) gene. J Exp Med. 1996;184:1167–1172 [PMCID: PMC2192804] [PubMed: 9064334]

115. Kotani T, Umeki K, Hirai K, Ohtaki S. Experimental murine thyroiditis induced by porcine thyroid peroxidase and its transfer by the antigen-specific T cell line. Clin Exp Immunol. 1990;80:11–18 [PMCID: PMC1535231] [PubMed: 1691060]

116. McLachlan SM, Atherton MC, Nakajima Y, et al. Thyroid peroxidase and the induction of autoimmune thyroid disease. Clin Exp Immunol. 1990;79:182–188 [PMCID: PMC1534760] [PubMed: 2311297]

117. Ng HP, Banga JP, Kung AW. Development of a murine model of autoimmune thyroiditis induced with

homologous mouse thyroid peroxidase. Endocrinology. 2004;145:809–816 [PubMed: 14592961]

118. Kotani T, Umeki K, Yagihashi S, Hirai K, Ohtaki S. Identification of thyroiditogenic epitope on porcine thyroid peroxidase for C57BL/6 mice. J Immunol. 1992;148:2084–2089 [PubMed: 1372023]

119. Ng HP, Kung AW. Induction of autoimmune thyroiditis and hypothyroidism by immunization of immunoactive T cell epitope of thyroid peroxidase. Endocrinology. 2006;147:3085–3092 [PubMed: 16527849]

120. Costagliola S, Many MC, Stalmans-Falys M, Tonacchera M, Vassart G, Ludgate M. Recombinant thyrotropin receptor and the induction of autoimmune thyroid disease in BALB/c mice: a new animal model. Endocrinology. 1994;135:2150–2159 [PubMed: 7956939]

121. Costagliola S, Many MC, Stalmans-Falys M, Vassart G, Ludgate M. The autoimmune response induced by immunising female mice with recombinant human thyrotropin receptor varies with the genetic background. Molec Cell Endocrinol. 1995;115:199–206 [PubMed: 8824895]

122. Silverman DA, Rose NR. Autoimmunity in methylcholanthrene-induced and spontaneous thyroiditis in Buffalo strain rats. Proc Soc Exp Biol Med. 1971;138:579–584 [PubMed: 5000784]

123. Penhale WJ, Farmer A, McKenna RP, Irvine WJ. Spontaneous thyroiditis in thymectomized and irradiated Wistar rats. Clin Exp Immunol. 1973;15:225–236 [PMCID: PMC1553892] [PubMed: 4543427]

124. Silverman DA, Rose NR. Neonatal thymectomy increases the incidence of spontaneous and methylcholanthrene-enhanced thyroiditis in rats. Science. 1974;184:162–163 [PubMed: 4815720]

125. Sharp GC, Mullen H, Kyriakos M. Production of augmented experimental autoimmune thyroiditis lesions by combined transfer of antiserum and lymph node cells. J Immunol. 1974;112:478–487 [PubMed: 4130688]

126. Braley-Mullen H, Johnson M, Sharp GC, Kyriakos M. Induction of experimental autoimmune thyroiditis in mice with in vitro activated splenic T cells. Cell Immunol. 1985;93:132–143 [PubMed: 3873286]

127. Braley-Mullen H, Sharp GC. Adoptive transfer murine model of granulomatous experimental autoimmune thyroiditis. Int Rev Immunol. 2000;19:535–555 [PubMed: 11129114]

128. Charreire J, Michel-Bechet M. Syngeneic sensitization of mouse lymphocytes on monolayers of thyroid epithelial cells. III. Induction of thyroiditis by thyroid-sensitized T lymphoblasts. Eur J Immunol. 1982;12:421–425 [PubMed: 6980128]

129. Knight SC, Farrant J, Chan J, Bryant A, Bedford PA, Bateman C. Induction of autoimmunity with dendritic cells: studies on thyroiditis in mice. Clin Immunol Immunopathol. 1988;48:277–289 [PubMed: 3402103]

130. Jaume JC, Guo J, Wang Y, Rapoport B, McLachlan SM. Cellular thyroid peroxidase (TPO), unlike purified TPO and adjuvant, induces antibodies in mice that resemble autoantibodies in human autoimmune thyroid disease. J Clin Endocrinol Metab. 1999;84:1651–1657 [PubMed: 10323395]

131. Okayasu I, Hatakeyama S. The difference of susceptibility of target thyroid gland to autoimmune thyroiditis induced by a combination of implantation of thyroid gland and injection of lipopolysaccharide. Clin Immunol Immunopathol. 1983;29:51–57 [PubMed: 6883813]

132. Flynn JC, Gardas A, Wan Q, et al. Superiority of thyroid peroxidase DNA over protein immunization in replicating human thyroid autoimmunity in HLA-DRB1*0301 (DR3) transgenic mice. Clin Exp Immunol. 2004;137:503–512 [PMCID: PMC1809151] [PubMed: 15320899]

133. Jacobson EM, Concepcion E, Ho K, Kopp P, Vono Toniolo J, Tomer Y. cDNA immunization of mice with human thyroglobulin generates both humoral and T cell responses: a novel model of thyroid autoimmunity. PLoS One. 2011;6:e19200. [PMCID: PMC3084781] [PubMed: 21559421]

134. Guo J, McLachlan SM, Pichurin PN, et al. Relationship between thyroid peroxidase T cell epitope restriction and antibody recognition of the autoantibody immunodominant region in human leukocyte antigen DR3 transgenic mice. Endocrinology. 2005;146:4961–4967 [PubMed: 16081633]

135. Chen CR, Hamidi S, Braley-Mullen H, et al. Antibodies to thyroid peroxidase arise spontaneously with age in NOD.H-2h4 mice and appear after thyroglobulin antibodies. Endocrinology. 2010;151:4583–4593 [PMCID: PMC2940509] [PubMed: 20573721]

136. Martin AP, Coronel EC, Sano G, et al. A novel model for lymphocytic infiltration of the thyroid gland generated by transgenic expression of the CC chemokine CCL21. J Immunol. 2004;173:4791–4798 [PubMed: 15470018]

137. Akeno N, Smith EP, Stefan M, et al. IFN-α mediates the development of autoimmunity both by direct tissue toxicity and through immune cell recruitment mechanisms. J Immunol. 2011;186:4693–4706 [PMCID: PMC3106338] [PubMed: 21402899]

138. Martin AP, Marinkovic T, Canasto-Chibuque C, et al. CCR7 deficiency in NOD mice leads to thyroiditis and primary hypothyroidism. J Immunol. 2009;183:3073–3080 [PubMed: 19675158]

139. Barin JG, Afanasyeva M, Talor MV, Rose NR, Burek CL, Caturegli P. Thyroid-specific expression of IFN-γ limits experimental autoimmune thyroiditis by suppressing lymphocyte activation in cervical lymph nodes. J Immunol. 2003;170:5523–5529 [PubMed: 12759429]

140. Nagayama Y. Observations on the proposed “nonclassical” model of autoimmune hypothyroidism. Thyroid. 2010;20:665–666; author reply 666–667 [PubMed: 20470211]

141. Todd I, Pujol-Borrell R, Hammond LJ, Bottazzo GF, Feldmann M. Interferon-γ induces HLA-DR expression by thyroid epithelium. Clin Exp Immunol. 1985;61:265–273 [PMCID: PMC1577305] [PubMed: 3930110]

142. 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 [PubMed: 6138647]

143. Quaratino S, Badami E, Pang YY, et al. Degenerate self-reactive human T-cell receptor causes spontaneous autoimmune disease in mice. Nat Med. 2004;10:920–926 [PubMed: 15311276]

144. Badami E, Maiuri L, Quaratino S. High incidence of spontaneous autoimmune thyroiditis in immunocompetent self-reactive human T cell receptor transgenic mice. J Autoimmun. 2005;24:85–91 [PubMed: 15829400]

145. Wei WZ, Jacob JB, Zielinski JF, et al. Concurrent induction of antitumor immunity and autoimmune thyroiditis in CD4+ CD25+ regulatory T cell-depleted mice. Cancer Res. 2005;65:8471–8478 [PubMed: 16166327]

146. Jacob JB, Kong YC, Nalbantoglu I, Snower DP, Wei WZ. Tumor regression following DNA vaccination and regulatory T cell depletion in neu transgenic mice leads to an increased risk for autoimmunity. J Immunol. 2009;182:5873–5881 [PMCID: PMC3833444] [PubMed: 19380836]

147. McLachlan SM, Nagayama Y, Pichurin PN, et al. The link between Graves' disease and Hashimoto's thyroiditis: A role for regulatory T cells. Endocrinology. 2007;148:5724–5733 [PubMed: 17823263]

148. Kong YC. Experimental autoimmune thyroiditis in the mouse. Curr Protoc Immunol. 2007;15:15.7 [PubMed: 18432986]

149. Quaratino S, Thorpe CJ, Travers PJ, Londei M. Similar antigenic surfaces, rather than sequence homology, dictate T-cell epitope molecular mimicry. Proc Natl Acad Sci USA. 1995;92:10398–10402 [PMCID: PMC40804] [PubMed: 7479792]

150. Nagayama Y. Graves' animal models of Graves' hyperthyroidism. Thyroid. 2007;17:981–988 [PubMed: 17822377]

151. Kim-Saijo M, Akamizu T, Ikuta K, et al. Generation of a transgenic animal model of hyperthyroid Graves' disease. Eur J Immunol. 2003;33:2531–2538 [PubMed: 12938229]

152. Akamizu T, Matsuda F, Okuda J, et al. Molecular analysis of stimulatory anti-thyrotropin receptor antibodies (TSAbs) involved in Graves' disease. Isolation and reconstruction of antibody genes, and production of monoclonal TSAbs. J Immunol. 1996;157:3148–3152 [PubMed: 8816426]

153. Sanders J, Jeffreys J, Depraetere H, et al. Thyroid-stimulating monoclonal antibodies. Thyroid. 2002;12:1043–1050 [PubMed: 12593717]

154. Costagliola S, Franssen JD, Bonomi M, et al. Generation of a mouse monoclonal TSH receptor antibody with stimulating activity. Biochem Biophys Res Commun. 2002;299:891–896 [PubMed: 12470663]

155. Ando T, Latif R, Pritsker A, Moran T, Nagayama Y, Davies TF. A monoclonal thyroid-stimulating antibody. J Clin Invest. 2002;110:1667–1674 [PMCID: PMC151640] [PubMed: 12464672]

156. Sanders J, Evans M, Premawardhana LD, et al. Human monoclonal thyroid stimulating autoantibody. Lancet. 2003;362:126–128 [PubMed: 12867115]

157. Shimojo N, Kohno Y, Yamaguchi K, et al. Induction of Graves-like disease in mice by immunization with fibroblasts transfected with the thyrotropin receptor and a class II molecule. Proc Natl Acad Sci USA. 1996;93:11074–11079 [PMCID: PMC38286] [PubMed: 8855311]

158. Yamaguchi K, Shimojo N, Kikuoka S, et al. Genetic control of anti-thyrotropin receptor antibody generation in H-2k mice immunized with thyrotropin receptor-transfected fibroblasts. J Clin Endocrinol Metab. 1997;82:4266–4269 [PubMed: 9398751]

159. Kaithamana S, Fan J, Osuga Y, Liang SG, Prabhakar BS. Induction of experimental autoimmune Graves' disease in BALB/c mice. J Immunol. 1999;163:5157–5164 [PubMed: 10528222]

160. Kita-Furuyama M, Nagayama Y, Pichurin P, McLachlan SM, Rapoport B, Eguchi K. Dendritic cells infected with adenovirus expressing the thyrotrophin receptor induce Graves' hyperthyroidism in BALB/c mice. Clin Exp Immunol. 2003;131:234–240 [PMCID: PMC1808615] [PubMed: 12562382]

161. Ando T, Imaizumi M, Graves P, Unger P, Davies TF. Induction of thyroid-stimulating hormone receptor autoimmunity in hamsters. Endocrinology. 2003;144:671–680 [PubMed: 12538630]

162. Costagliola S, Rodien P, Many MC, Ludgate M, Vassart G. Genetic immunization against the human thyrotropin receptor causes thyroiditis and allows production of monoclonal antibodies recognizing the native receptor. J Immunol. 1998;160:1458–1465 [PubMed: 9570567]

163. Costagliola S, Many MC, Denef JF, Pohlenz J, Refetoff S, Vassart G. Genetic immunization of outbred mice with thyrotropin receptor cDNA provides a model of Graves' disease. J Clin Invest. 2000;105:803–811 [PMCID: PMC377458] [PubMed: 10727449]

164. Nagayama Y, Kita-Furuyama M, Ando T, et al. A novel murine model of Graves' hyperthyroidism with intramuscular injection of adenovirus expressing the thyrotropin receptor. J Immunol. 2002;168:2789–2794 [PubMed: 11884447]

165. Chen CR, Pichurin P, Nagayama Y, Latrofa F, Rapoport B, McLachlan SM. The thyrotropin receptor autoantigen in Graves disease is the culprit as well as the victim. J Clin Invest. 2003;111:1897–1904 [PMCID: PMC161420] [PubMed: 12813025]

166. Kaneda T, Honda A, Hakozaki A, Fuse T, Muto A, Yoshida T. An improved Graves' disease model established by using *in vivo* electroporation exhibited long-term immunity to hyperthyroidism in BALB/c mice. Endocrinology. 2007;148:2335–2344 [PubMed: 17255207]

167. Zhao SX, Tsui S, Cheung A, Douglas RS, Smith TJ, Banga JP. Orbital fibrosis in a mouse model of Graves' disease induced by genetic immunization of thyrotropin receptor cDNA. J Endocrinol. 2011;210:369–377 [PMCID: PMC3152291] [PubMed: 21715431]

168. Gilbert JA, Gianoukakis AG, Salehi S, et al. Monoclonal pathogenic antibodies to the thyroid-stimulating hormone receptor in Graves' disease with potent thyroid-stimulating activity but differential blocking activity activate multiple signaling pathways. J Immunol. 2006;176:5084–5092 [PubMed: 16585606]

169. Huber AK, Finkelman FD, Li CW, et al. Genetically driven target tissue overexpression of CD40: a novel mechanism in autoimmune disease. J Immunol. 2012;189:3043–3053 [PMCID: PMC3436983] [PubMed: 22888137]

170. Jacobson EM, Concepcion E, Oashi T, Tomer Y. A Graves' disease-associated Kozak sequence single-nucleotide polymorphism enhances the efficiency of CD40 gene translation: a case for translational pathophysiology. Endocrinology. 2005;146:2684–2691 [PubMed: 15731360]

171. Rapoport B, Williams RW, Chen CR, McLachlan SM. Immunoglobulin heavy chain variable region genes contribute to the induction of thyroid-stimulating antibodies in recombinant inbred mice. Genes Immun. 2010;11:254–263 [PMCID: PMC4108286] [PubMed: 20407472]

172. Nakahara M, Mitsutake N, Sakamoto H, et al. Enhanced response to mouse thyroid-stimulating hormone (TSH) receptor immunization in TSH receptor-knockout mice. Endocrinology. 2010;151:4047–4054 [PubMed: 20555026]

173. Marians RC, Ng L, Blair HC, Unger P, Graves PN, Davies TF. Defining thyrotropin-dependent and -independent steps of thyroid hormone synthesis by using thyrotropin receptor-null mice. Proc Natl Acad Sci USA. 2002;99:15776–15881 [PMCID: PMC137792] [PubMed: 12432094]

174. Nakahara M, Johnson K, Eckstein A, et al. Adoptive transfer of antithyrotropin receptor (TSHR) autoimmunity from TSHR knockout mice to athymic nude mice. Endocrinology. 2012;153:2034–2042 [PubMed: 22334716]

175. McLachlan SM, Aliesky HA, Chen CR, Rapoport B. Role of self-tolerance and chronic stimulation in the long-term persistence of adenovirus-induced thyrotropin receptor antibodies in wild-type and transgenic mice. Thyroid. 2012;22:931–937 [PMCID: PMC3429281] [PubMed: 22827528]

176. Endo T, Kobayashi T. Immunization of mice with a newly identified thyroid-stimulating hormone receptor splice variant induces Graves'-like disease. J Autoimmun. 2013;43:18–25 [PubMed: 23538203]

177. Ng B, Yang F, Huston DP, et al. Increased noncanonical splicing of autoantigen transcripts provides the structural basis for expression of untolerized epitopes. J Allergy Clin Immunol. 2004;114:1463–1470 [PMCID: PMC3902068] [PubMed: 15577853]

178. Melchers F, Rolink AR. B cell tolerance–how to make it and how to break it. Curr Top Microbiol Immunol. 2006;305:1–23 [PubMed: 16724798]

179. Roitt IM, Torrigiani G, Doniach D. Immunochemical studies on the thyroglobulin autoantibody system in human thyroiditis. Immunology. 1968;15:681–696 [PMCID: PMC1409529] [PubMed: 4972267]

180. Ruf J, Toubert ME, Czarnocka B, Durand-Gorde JM, Ferrand M, Carayon P. Relationship between immunological structure and biochemical properties of human thyroid peroxidase. Endocrinology. 1989;125:1211–1218 [PubMed: 2474433]

181. Chazenbalk GD, Portolano S, Russo D, Hutchison JS, Rapoport B, McLachlan S. 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. J Clin Invest. 1993;92:62–74 [PMCID: PMC293532] [PubMed: 7686925]

182. Latrofa F, Phillips M, Rapoport B, McLachlan SM. Human monoclonal thyroglobulin autoantibodies: epitopes and immunoglobulin genes. J Clin Endocrinol Metab. 2004;89:5116–5123 [PubMed: 15472215]

183. Shimojo N, Arima T, Yamaguchi K, Kikuoka S, Kohn LD, Kohno Y. A novel mouse model of Graves' disease: implications for a role of aberrant MHC class II expression in its pathogenesis. Int Rev Immunol. 2000;19:619–631 [PubMed: 11129118]

184. Jaume JC, Guo J, Wang Y, Rapoport B, McLachlan SM. Cellular thyroid peroxidase (TPO), unlike purified TPO and adjuvant, induces antibodies in mice that resemble autoantibodies in human autoimmune thyroid disease. J Clin Endocrinol Metab. 1999;84:1651–1657 [PubMed: 10323395]

185. McLachlan SM, Aliesky HA, Chen CR, Chong G, Rapoport B. Breaking tolerance in transgenic mice expressing the human TSH receptor A-subunit: thyroiditis, epitope spreading and adjuvant as a ‘double edged sword’. PLoS One. 2012;7:e43517. [PMCID: PMC3436763] [PubMed: 22970131]

186. Brand OJ, Gough SC. Immunogenetic mechanisms leading to thyroid autoimmunity: recent advances in identifying susceptibility genes and regions. Curr Genomics. 2011;12:526–541 [PMCID: PMC3271307] [PubMed: 22654554]

187. Płoski R, Szymański K, Bednarczuk T. The genetic basis of graves' disease. Curr Genomics. 2011;12:542–563 [PMCID: PMC3271308] [PubMed: 22654555]

188. Hasham A, Tomer Y. Genetic and epigenetic mechanisms in thyroid autoimmunity. Immunol Res. 2012;54:204–213 [PMCID: PMC3601048] [PubMed: 22457094]

189. Dechairo BM, Zabaneh D, Collins J, et al. Association of the TSHR gene with Graves' disease: the first disease specific locus. Eur J Hum Genet. 2005;13:1223–1230 [PubMed: 16106256]

190. Hiratani H, Bowden DW, Ikegami S, et al. Multiple SNPs in intron 7 of thyrotropin receptor are associated with Graves' disease. J Clin Endocrinol Metab. 2005;90:2898–2903 [PubMed: 15741259]

191. Colobran R, Armengol Mdel P, Faner R, et al. Association of an SNP with intrathymic transcription of TSHR and Graves' disease: a role for defective thymic tolerance. Hum Mol Genet. 2011;20:3415–3423 [PubMed: 21642385]

192. Nithiyananthan R, Heward JM, Allahabadia A, Barnett AH, Franklyn JA, Gough SC. A heterozygous deletion of the autoimmune regulator (AIRE1) gene, autoimmune thyroid disease, and type 1 diabetes: no evidence for association. J Clin Endocrinol Metab. 2000;85:1320–1322 [PubMed: 10720083]

193. Meyer G, Donner H, Herwig J, Böhles H, Usadel KH, Badenhoop K. Screening for an AIRE-1 mutation in patients with Addison's disease, type 1 diabetes, Graves' disease and Hashimoto's thyroiditis as

well as in APECED syndrome. Clin Endocrinol (Oxf). 2001;54:335–338 [PubMed: 11298085]

194. Perniola R, Filograna O, Greco G, Pellegrino V. High prevalence of thyroid autoimmunity in Apulian patients with autoimmune polyglandular syndrome type 1. Thyroid. 2008;18:1027–1029 [PubMed: 18713028]

195. Björses P, Halonen M, Palvimo JJ, et al. Mutations in the AIRE gene: effects on subcellular location and transactivation function of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protein. Am J Hum Genet. 2000;66:378–392 [PMCID: PMC1288090] [PubMed: 10677297]

196. Scott HS, Heino M, Peterson P, et al. Common mutations in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients of different origins. Mol Endocrinol. 1998;12:1112–1119 [PubMed: 9717837]

197. Cetani F, Barbesino G, Borsari S, et al. A novel mutation of the autoimmune regulator gene in an Italian kindred with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, acting in a dominant fashion and strongly cosegregating with hypothyroid autoimmune thyroiditis. J Clin Endocrinol Metab. 2001;86:4747–4752 [PubMed: 11600535]

198. Su MA, Giang K, Zumer K, et al. Mechanisms of an autoimmunity syndrome in mice caused by a dominant mutation in Aire. J Clin Invest. 2008;118:1712–1726 [PMCID: PMC2293336] [PubMed: 18414681]

199. Stenszky V, Kozma L, Balázs C, Rochlitz S, Bear JC, Farid NR. The genetics of Graves' disease: HLA and disease susceptibility. J Clin Endocrinol Metab. 1985;61:735–740 [PubMed: 3861611]

200. Burton PR, Clayton DG, Cardon LR, et al. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat Genet. 2007;39:1329–1337 [PMCID: PMC2680141] [PubMed: 17952073]

201. Ban Y, Davies TF, Greenberg DA, et al. Arginine at position 74 of the HLA-DR β1 chain is associated with Graves' disease. Genes Immun. 2004;5:203–208 [PubMed: 15029234]

202. Kong YC, Flynn JC, Banga JP, David CS. Application of HLA class II transgenic mice to study autoimmune regulation. Thyroid. 2007;17:995–1003 [PubMed: 17900224]

203. Aliesky HA, Pichurin PN, Chen CR, Williams RW, Rapoport B, McLachlan SM. Probing the genetic basis for thyrotropin receptor antibodies and hyperthyroidism in immunized CXB recombinant inbred mice. Endocrinology. 2006;147:2789–2800 [PubMed: 16543368]

204. McLachlan SM, Aliesky HA, Pichurin PN, Chen CR, Williams RW, Rapoport B. Shared and unique susceptibility genes in a mouse model of Graves' disease determined in BXH and CXB recombinant inbred mice. Endocrinology. 2008;149:2001–2009 [PMCID: PMC2276708] [PubMed: 18162518]

205. McLachlan SM, Aliesky H, Chen CR, Williams RW, Rapoport B. Exceptional hyperthyroidism and a role for both major histocompatibility class I and class II genes in a murine model of Graves' disease. PLoS One. 2011;6:e21378. [PMCID: PMC3124500] [PubMed: 21738647]

206. Chu X, Pan CM, Zhao SX, et al. A genome-wide association study identifies two new risk loci for Graves' disease. Nat Genet. 2011;43:897–901 [PubMed: 21841780]

207. Tomer Y, Greenberg DA, Concepcion E, Ban Y, Davies TF. Thyroglobulin is a thyroid specific gene for the familial autoimmune thyroid diseases. J Clin Endocrinol Metab. 2002;87:404–407 [PubMed: 11788684]

208. Ban Y, Greenberg DA, Concepcion E, Skrabanek L, Villanueva R, Tomer Y. Amino acid substitutions

in the thyroglobulin gene are associated with susceptibility to human and murine autoimmune thyroid disease. Proc Natl Acad Sci USA. 2003;100:15119–15124 [PMCID: PMC299918] [PubMed: 14657345]

209. Jacobson EM, Yang H, Menconi F, et al. Employing a recombinant HLA-DR3 expression system to dissect major histocompatibility complex II-thyroglobulin peptide dynamism: a genetic, biochemical, and reverse immunological perspective. J Biol Chem. 2009;284:34231–34243 [PMCID: PMC2797193] [PubMed: 19776016]

210. Brand OJ, Lowe CE, Heward JM, et al. Association of the interleukin-2 receptor α (IL-2Rα)/CD25 gene region with Graves' disease using a multilocus test and tag SNPs. Clin Endocrinol Oxf. 2007;66:508–512 [PubMed: 17371467]

211. Owen CJ, Eden JA, Jennings CE, Wilson V, Cheetham TD, Pearce SH. Genetic association studies of the FOXP3 gene in Graves' disease and autoimmune Addison's disease in the United Kingdom population. J Mol Endocrinol. 2006;37:97–104 [PubMed: 16901927]

212. Tomer Y, Menconi F, Davies TF, et al. Dissecting genetic heterogeneity in autoimmune thyroid diseases by subset analysis. J Autoimmun. 2007;29:69–77 [PubMed: 17644307]

213. Kekäläinen E, Tuovinen H, Joensuu J, et al. A defect of regulatory T cells in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J Immunol. 2007;178:1208–1215 [PubMed: 17202386]

214. Yanagawa T, Hidaka Y, Guimaraes V, Soliman M, DeGroot LJ. CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. J Clin Endocrinol Metab. 1995;80:41–45 [PubMed: 7829637]

215. Maine CJ, Hamilton-Williams EE, Cheung J, et al. PTPN22 alters the development of regulatory T cells in the thymus. J Immunol. 2012;188:5267–5275 [PMCID: PMC3358490] [PubMed: 22539785]

216. Vasícek D, Vasícková K, Kaiser P, Drozenová R, Cítek J, Hala K. Analysis of genetic regulation of chicken spontaneous autoimmune thyroiditis, an animal model of human Hashimoto's thyroiditis. Immunogenetics. 2001;53:776–785 [PubMed: 11862410]

217. Weetman AP. Immunity, thyroid function and pregnancy: molecular mechanisms. Nat Rev Endocrinol. 2010;6:311–318 [PubMed: 20421883]

218. Morshed SA, Latif R, Davies TF. Delineating the autoimmune mechanisms in Graves' disease. Immunol Res. 2012;54:191–203 [PMCID: PMC4504182] [PubMed: 22434518]

219. Imaizumi M, Pritsker A, Unger P, Davies TF. Intrathyroidal fetal microchimerism in pregnancy and postpartum. Endocrinology. 2002;143:247–253 [PubMed: 11751616]

220. Walsh JP, Bremner AP, Bulsara MK, et al. Parity and the risk of autoimmune thyroid disease: a community-based study. J Clin Endocrinol Metab. 2005;90:5309–5312 [PubMed: 15956077]

221. Bülow Pedersen I, Laurberg P, Knudsen N, et al. Lack of association between thyroid autoantibodies and parity in a population study argues against microchimerism as a trigger of thyroid autoimmunity. Eur J Endocrinol. 2006;154:39–45 [PubMed: 16381989]

222. Sgarbi JA, Kasamatsu TS, Matsumura LK, Maciel RM. Parity is not related to autoimmune thyroid disease in a population-based study of Japanese-Brazilians. Thyroid. 2010;20:1151–1156 [PubMed: 20883173]

223. Brix TH, Hansen PS, Kyvik KO, Hegedüs L. Aggregation of thyroid autoantibodies in twins from opposite-sex pairs suggests that microchimerism may play a role in the early stages of thyroid

autoimmunity. J Clin Endocrinol Metab. 2009;94:4439–4443 [PubMed: 19808849]

224. Greer LG, Casey BM, Halvorson LM, Spong CY, McIntire DD, Cunningham FG. Antithyroid antibodies and parity: further evidence for microchimerism in autoimmune thyroid disease. Am J Obstet Gynecol. 2011;205:471–474 [PubMed: 21944225]

225. Misharin AV, Nagayama Y, Aliesky HA, Rapoport B, McLachlan SM. Studies in mice deficient for the autoimmune regulator (Aire) and transgenic for the thyrotropin receptor reveal a role for Aire in tolerance for thyroid autoantigens. Endocrinology. 2009;150:2948–2956 [PMCID: PMC2689795] [PubMed: 19264867]

226. Pichurin PN, Chen CR, Chazenbalk GD, et al. Targeted expression of the human thyrotropin receptor A-subunit to the mouse thyroid: Insight into overcoming the lack of response to A-subunit adenovirus immunization. J Immunol. 2006;176:668–676 [PubMed: 16365463]

227. Purcell AW, van Driel IR, Gleeson PA. Impact of glycans on T-cell tolerance to glycosylated self-antigens. Immunol Cell Biol. 2008;86:574–579 [PubMed: 18626489]

228. Szabó TG, Palotai R, Antal P, et al. Critical role of glycosylation in determining the length and structure of T cell epitopes. Immunome Res. 2009;5:4. [PMCID: PMC2760507] [PubMed: 19778434]

229. Cloosen S, Arnold J, Thio M, Bos GM, Kyewski B, Germeraad WT. Expression of tumor-associated differentiation antigens, MUC1 glycoforms and CEA, in human thymic epithelial cells: implications for self-tolerance and tumor therapy. Cancer Res. 2007;67:3919–3926 [PubMed: 17440107]

230. Feldt-Rasmussen U, Hyltoft Petersen P, Date J. Sex and age correlated reference values of serum thyroglobulin measured by a modified radioimmunoassay. Acta Endocrinol (Copenh). 1979;90:440–450 [PubMed: 425785]

231. Premawardhana LD, Kiso Y, Phillips DI, Morteo C, Furmaniak J, Rees Smith B. Is TPO detectable in the circulation? Thyroid. 1993;3:225–228 [PubMed: 8257863]

232. Jacobson EM, Tomer Y. The genetic basis of thyroid autoimmunity. Thyroid. 2007;17:949–961 [PubMed: 17824829]

233. Ruan QG, Tung K, Eisenman D, et al. The autoimmune regulator directly controls the expression of genes critical for thymic epithelial function. J Immunol. 2007;178:7173–7180 [PubMed: 17513766]

234. Lima FA, Moreira-Filho CA, Ramos PL, et al. Decreased AIRE expression and global thymic hypofunction in Down syndrome. J Immunol. 2011;187:3422–3430 [PubMed: 21856934]

235. Karlsson B, Gustafsson J, Hedov G, Ivarsson SA, Annerén G. Thyroid dysfunction in Down's syndrome: relation to age and thyroid autoimmunity. Arch Dis Child. 1998;79:242–245 [PMCID: PMC1717691] [PubMed: 9875020]

236. Mizutori Y, Nagayama Y, Flower D, et al. Role of the transgenic human thyrotropin receptor A-subunit in thyroiditis induced by A-subunit immunization and regulatory T cell depletion. Clin Exp Immunol. 2008;154:305–315 [PMCID: PMC2633226] [PubMed: 18811696]

237. Saitoh O, Nagayama Y. Regulation of Graves' hyperthyroidism with naturally occurring CD4+CD25+ regulatory T cells in a mouse model. Endocrinology. 2006;147:2417–2422 [PubMed: 16439459]

238. Saitoh O, Abiru N, Nakahara M, Nagayama Y. CD8+CD122+ T cells, a newly identified regulatory T subset, negatively regulate Graves' hyperthyroidism in a murine model. Endocrinology. 2007;148:6040–6046 [PubMed: 17823258]

239. Johnson KT, Wiesweg B, Schott M, et al. Examination of orbital tissues in murine models of Graves' disease reveals expression of UCP-1 and the TSHR in retrobulbar adipose tissues. Horm Metab Res. 2013;45:401–407 [PubMed: 23386414]

240. Chen CR, Aliesky HA, Rapoport B, McLachlan SM. An attempt to induce “Graves' disease of the gonads” by immunizing mice with the luteinizing hormone receptor provides insight into breaking tolerance to self-antigens. Thyroid. 2011;21:773–781 [PMCID: PMC3123529] [PubMed: 21649471]

241. Kojima A, Tanaka-Kojima Y, Sakakura T, Nishizuka Y. Spontaneous development of autoimmune thyroiditis in neonatally thymectomized mice. Lab Invest. 1976;34:550–557 [PubMed: 947081]

242. Kong YC, Morris GP, Brown NK, Yan Y, Flynn JC, David CS. Autoimmune thyroiditis: a model uniquely suited to probe regulatory T cell function. J Autoimmun. 2009;33:239–246 [PMCID: PMC2783453] [PubMed: 19822405]

243. Morris GP, Brown NK, Kong YC. Naturally-existing CD4(+)CD25(+)Foxp3(+) regulatory T cells are required for tolerance to experimental autoimmune thyroiditis induced by either exogenous or endogenous autoantigen. J Autoimmun. 2009;33:68–76 [PMCID: PMC2706097] [PubMed: 19375891]

244. Nagayama Y, Horie I, Saitoh O, Nakahara M, Abiru N. CD4+CD25+ naturally occurring regulatory T cells and not lymphopenia play a role in the pathogenesis of iodide-induced autoimmune thyroiditis in NOD-H2h4 mice. J Autoimmun. 2007;29:195–202 [PubMed: 17826032]

245. Flynn JC, Meroueh C, Snower DP, David CS, Kong YM. Depletion of CD4+CD25+ regulatory T cells exacerbates sodium iodide-induced experimental autoimmune thyroiditis in human leucocyte antigen DR3 (DRB1*0301) transgenic class II-knock-out non-obese diabetic mice. Clin Exp Immunol. 2007;147:547–554 [PMCID: PMC1810500] [PubMed: 17302906]

246. Morris GP, Yan Y, David CS, Kong YC. H2A- and H2E-derived CD4+CD25+ regulatory T cells: a potential role in reciprocal inhibition by class II genes in autoimmune thyroiditis. J Immunol. 2005;174:3111–3116 [PubMed: 15728527]

247. Horie I, Abiru N, Sakamoto H, Iwakura Y, Nagayama Y. Induction of autoimmune thyroiditis by depletion of CD4+CD25+ regulatory T cells in thyroiditis-resistant IL-17, but not interferon-γ receptor, knockout nonobese diabetic-H2h4 mice. Endocrinology. 2011;152:4448–4454 [PubMed: 21862617]

248. Marazuela M, García-López MA, Figueroa-Vega N, et al. Regulatory T cells in human autoimmune thyroid disease. J Clin Endocrinol Metab. 2006;91:3639–3646 [PubMed: 16804051]

249. Nakano A, Watanabe M, Iida T, et al. Apoptosis-induced decrease of intrathyroidal CD4(+)CD25(+) regulatory T cells in autoimmune thyroid diseases. Thyroid. 2007;17:25–31 [PubMed: 17274744]

250. Strieder TG, Drexhage HA, Lam-Tse WK, Prummel MF, Tijssen JG, Wiersinga WM. A reduced IL2R (CD25) expression level in first and second degree female relatives of autoimmune thyroid disease patients. A sign of a poor capability to preserve tolerance? Autoimmunity. 2006;39:93–98 [PubMed: 16698664]

251. Volpé R, Iitaka M. Evidence for an antigen-specific defect in suppressor T-lymphocytes in autoimmune thyroid disease. Exp Clin Endocrinol. 1991;97:133–138 [PubMed: 1833214]

252. Kohno Y, Naito N, Hiyama Y, et al. Thyroglobulin and thyroid peroxidase share common epitopes recognized by autoantibodies in patients with chronic autoimmune thyroiditis. J Clin Endocrinol Metab. 1988;67:899–907 [PubMed: 2460485]

253. Ruf J, Ferrand M, Durand-Gorde JM, Carayon P. Immunopurification and characterization of thyroid autoantibodies with dual specificity for thyroglobulin and thyroperoxidase. Autoimmunity.

1992;11:179–188 [PubMed: 1571480]

254. Naito N, Saito K, Hosoya T, et al. Anti-thyroglobulin autoantibodies in sera from patients with chronic thyroiditis and from healthy subjects: differences in cross-reactivity with thyroid peroxidase. Clin Exp Immunol. 1990;80:4–10 [PMCID: PMC1535236] [PubMed: 2323101]

255. Ruf J, Ferrand M, Durand-Gorde JM, De Micco C, Carayon P. Significance of thyroglobulin antibodies cross-reactive with thyroperoxidase (TGPO antibodies) in individual patients and immunized mice. Clin Exp Immunol. 1993;92:65–72 [PMCID: PMC1554880] [PubMed: 8467565]

256. Ruf J, Feldt-Rasmussen U, Hegedüs L, Ferrand M, Carayon P. Bispecific thyroglobulin and thyroperoxidase autoantibodies in patients with various thyroid and autoimmune diseases. J Clin Endocrinol Metab. 1994;79:1404–1409 [PubMed: 7962336]

257. Estienne V, Duthoit C, Costanzo VD, et al. Multicenter study on TGPO autoantibody prevalence in various thyroid and non-thyroid diseases; relationships with thyroglobulin and thyroperoxidase autoantibody parameters. Eur J Endocrinol. 1999;141:563–569 [PubMed: 10601957]

258. Latrofa F, Pichurin P, Guo J, Rapoport B, McLachlan SM. Thyroglobulin-thyroperoxidase autoantibodies are polyreactive, not bispecific: analysis using human monoclonal autoantibodies. J Clin Endocrinol Metab. 2003;88:371–378 [PubMed: 12519879]

259. McLachlan SM, Rapoport B. Evidence for a potential common T-cell epitope between human thyroid peroxidase and human thyroglobulin with implications for the pathogenesis of autoimmune thyroid disease. Autoimmunity. 1989;5:101–106 [PubMed: 2485094]

260. Rothbard JB, Taylor WR. A sequence pattern common to T cell epitopes. EMBO J. 1988;7:93–100 [PMCID: PMC454220] [PubMed: 2452085]

261. Hoshioka A, Kohno Y, Katsuki T, et al. A common T-cell epitope between human thyroglobulin and human thyroid peroxidase is related to murine experimental autoimmune thyroiditis. Immunol Lett. 1993;37:235–239 [PubMed: 7505005]

262. Ehlers M, Thiel A, Bernecker C, et al. Evidence of a combined cytotoxic thyroglobulin and thyroperoxidase epitope-specific cellular immunity in Hashimoto's thyroiditis. J Clin Endocrinol Metab. 2012;97:1347–1354 [PubMed: 22259066]

263. McLachlan SM, Rapoport B. Recombinant thyroid autoantigens: the keys to the pathogenesis of autoimmune thyroid disease. J Intern Med. 1993;234:347–359 [PubMed: 8409831]

264. Effraimidis G, Strieder TG, Tijssen JG, Wiersinga WM. Natural history of the transition from euthyroidism to overt autoimmune hypo- or hyperthyroidism: a prospective study. Eur J Endocrinol. 2011;164:107–113 [PubMed: 20956436]

265. de Forteza R, Smith CU, Amin J, McKenzie JM, Zakarija M. Visualization of the thyrotropin receptor on the cell surface by potent autoantibodies. J Clin Endocrinol Metab. 1994;78:1271–1273 [PubMed: 7909819]

266. Jaume JC, Kakinuma A, Chazenbalk GD, Rapoport B, McLachlan SM. Thyrotropin receptor autoantibodies in serum are present at much lower levels than thyroid peroxidase autoantibodies: analysis by flow cytometry. J Clin Endocrinol Metab. 1997;82:500–507 [PubMed: 9024244]

267. Chazenbalk GD, McLachlan SM, Pichurin P, Yan XM, Rapoport B. A prion-like shift between two conformational forms of a recombinant thyrotropin receptor A-subunit module: purification and stabilization using chemical chaperones of the form reactive with Graves' autoantibodies. J Clin Endocrinol

Metab. 2001;86:1287–1293 [PubMed: 11238522]

268. Prummel MF, Laurberg P. Interferon-α and autoimmune thyroid disease. Thyroid. 2003;13:547–551 [PubMed: 12930598]

269. Tomer Y. Hepatitis C and interferon induced thyroiditis. J Autoimmun. 2010;34:J322–J326 [PMCID: PMC2835812] [PubMed: 20022216]

270. Lisker-Melman M, Di Bisceglie AM, Usala SJ, Weintraub B, Murray LM, Hoofnagle JH. Development of thyroid disease during therapy of chronic viral hepatitis with interferon alfa. Gastroenterology. 1992;102:2155–2160 [PubMed: 1587439]

271. Custro N, Montalto G, Scafidi V, et al. Prospective study on thyroid autoimmunity and dysfunction related to chronic hepatitis C and interferon therapy. J Endocrinol Invest. 1997;20:374–380 [PubMed: 9309534]

272. Wong V, Fu AX, George J, Cheung NW. Thyrotoxicosis induced by α-interferon therapy in chronic viral hepatitis. Clin Endocrinol (Oxf). 2002;56:793–798 [PubMed: 12072050]

273. Nagayama Y, Ohta K, Tsuruta M, et al. Exacerbation of thyroid autoimmunity by interferon alpha treatment in patients with chronic viral hepatitis: our studies and review of the literature. Endocr J. 1994;41:565–572 [PubMed: 7889118]

274. Marazuela M, García-Buey L, González-Fernández B, et al. Thyroid autoimmune disorders in patients with chronic hepatitis C before and during interferon-α therapy. Clin Endocrinol (Oxf). 1996;44:635–642 [PubMed: 8759175]

275. Martocchia A, Labbadia G, Paoletti V, et al. Hashimoto's disease during interferon-α therapy in a patient with pre-treatment negative anti-thyroid autoantibodies and with the specific genetic susceptibility to the thyroid disease. Neuro Endocrinol Lett. 2001;22:49–52 [PubMed: 11335880]

276. Baudin E, Marcellin P, Pouteau M, et al. Reversibility of thyroid dysfunction induced by recombinant α interferon in chronic hepatitis C. Clin Endocrinol (Oxf). 1993;39:657–661 [PubMed: 8287583]

277. Carella C, Mazziotti G, Morisco F, et al. Long-term outcome of interferon-α-induced thyroid autoimmunity and prognostic influence of thyroid autoantibody pattern at the end of treatment. J Clin Endocrinol Metab. 2001;86:1925–1929 [PubMed: 11344186]

278. Indolfi G, Stagi S, Bartolini E, et al. Thyroid function and anti-thyroid autoantibodies in untreated children with vertically acquired chronic hepatitis C virus infection. Clin Endocrinol (Oxf). 2008;68:117–121 [PubMed: 17692107]

279. Weetman A. Immune reconstitution syndrome and the thyroid. Best Pract Res Clin Endocrinol Metab. 2009;23:693–702 [PubMed: 19942146]

280. Holland FJ, McConnon JK, Volpé R, Saunders EF. Concordant Graves' disease after bone marrow transplantation: implications for pathogenesis. J Clin Endocrinol Metab. 1991;72:837–840 [PubMed: 2005210]

281. Karthaus M, Gabrysiak T, Brabant G, et al. Immune thyroiditis after transplantation of allogeneic CD34+ selected peripheral blood cells. Bone Marrow Transplant. 1997;20:697–699 [PubMed: 9383236]

282. Au WY, Lie AK, Kung AW, Liang R, Hawkins BR, Kwong YL. Autoimmune thyroid dysfunction after hematopoietic stem cell transplantation. Bone Marrow Transplant. 2005;35:383–388 [PubMed: 15640829]

283. Sinha A, Abinun M, Gennery AR, Barge D, Slatter M, Cheetham T. Graves' immune reconstitution inflammatory syndrome in childhood. Thyroid. 2013;23:1010–1014 [PubMed: 23556479]

284. Zomas A, Marsh JC, Harrison NK, et al. Rapid progression of fibrosing alveolitis and thyrotoxicosis after antithymocyte globulin therapy for aplastic anemia. Ann Hematol. 1995;71:49–51 [PubMed: 7632818]

285. Todd A, Todd J. Graves' disease following successful treatment of severe aplastic anaemia with antilymphocyte globulin. Clin Lab Haematol. 1999;21:69–70 [PubMed: 10197268]

286. Coles AJ, Wing M, Smith S, et al. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. Lancet. 1999;354:1691–1695 [PubMed: 10568572]

287. Gillard P, Huurman V, Van der Auwera B, et al. Graves hyperthyroidism after stopping immunosuppressive therapy in type 1 diabetic Islet cell recipients with pretransplant TPO autoantibodies. Diabetes Care. 2009;32:1817–1819 [PMCID: PMC2752930] [PubMed: 19549735]

288. Gilquin J, Viard JP, Jubault V, Sert C, Kazatchkine MD. Delayed occurrence of Graves' disease after immune restoration with HAART. Highly active antiretroviral therapy. Lancet. 1998;352:1907–1908 [PubMed: 9863795]

289. Jubault V, Penfornis A, Schillo F, et al. Sequential occurrence of thyroid autoantibodies and Graves' disease after immune restoration in severely immunocompromised human immunodeficiency virus-1-infected patients. J Clin Endocrinol Metab. 2000;85:4254–4257 [PubMed: 11095463]

290. Chen F, Day SL, Metcalfe RA, et al. Characteristics of autoimmune thyroid disease occurring as a late complication of immune reconstitution in patients with advanced human immunodeficiency virus (HIV) disease. Medicine (Baltimore). 2005;84:98–106 [PubMed: 15758839]

291. Rasul S, Delapenha R, Farhat F, Gajjala J, Zahra SM. Graves' disease as a manifestation of immune reconstitution in HIV-infected individuals after initiation of highly active antiretroviral therapy. AIDS Res Treat. 2011;2011:743597. [PMCID: PMC3144671] [PubMed: 21804938]

292. Visser R, de Mast Q, Netea-Maier RT, van der Ven AJ. Hashimoto's thyroiditis presenting as painful acute thyroiditis and as manifestation of an immune reconstitution inflammatory syndrome in a human immunodeficiency virus-seropositive patient. Thyroid. 2012;22:853–855 [PubMed: 22784301]

293. Duarte JH, Zelenay S, Bergman ML, Martins AC, Demengeot J. Natural Treg cells spontaneously differentiate into pathogenic helper cells in lymphopenic conditions. Eur J Immunol. 2009;39:948–955 [PubMed: 19291701]

294. King C, Ilic A, Koelsch K, Sarvetnick N. Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. Cell. 2004;117:265–277 [PubMed: 15084263]

295. Gallegos AM, Bevan MJ. Driven to autoimmunity: the nod mouse. Cell. 2004;117:149–151 [PubMed: 15084253]

296. Coles AJ, Compston DA, Selmaj KW, et al. Alemtuzumab vs. interferon β-1a in early multiple sclerosis. N Engl J Med. 2008;359:1786–1801 [PubMed: 18946064]

297. Yang L, Anderson DE, Baecher-Allan C, et al. IL-21 and TGF-β are required for differentiation of human T(H)17 cells. Nature. 2008;454:350–352 [PMCID: PMC2760130] [PubMed: 18469800]

298. Jones JL, Phuah CL, Cox AL, et al. IL-21 drives secondary autoimmunity in patients with multiple sclerosis, following therapeutic lymphocyte depletion with alemtuzumab (Campath-1H). J Clin Invest. 2009;119:2052–2061 [PMCID: PMC2701868] [PubMed: 19546505]

299. Kuchen S, Robbins R, Sims GP, et al. Essential role of IL-21 in B cell activation, expansion, and plasma cell generation during CD4+ T cell-B cell collaboration. J Immunol. 2007;179:5886–5896 [PubMed: 17947662]

300. Peluso I, Fantini MC, Fina D, et al. IL-21 counteracts the regulatory T cell-mediated suppression of human CD4+ T lymphocytes. J Immunol. 2007;178:732–739 [PubMed: 17202333]

301. Thompson SA, Jones JL, Cox AL, Compston DA, Coles AJ. B-cell reconstitution and BAFF after alemtuzumab (Campath-1H) treatment of multiple sclerosis. J Clin Immunol. 2010;30:99–105 [PubMed: 19763798]

302. Chen K, Wei Y, Sharp GC, Braley-Mullen H. Induction of experimental autoimmune thyroiditis in IL-12−/− mice. J Immunol. 2001;167:1720–1727 [PubMed: 11466396]

303. Nagayama Y, Saitoh O, McLachlan SM, Rapoport B, Kano H, Kumazawa Y. TSH receptor-adenovirus-induced Graves' hyperthyroidism is attenuated in both interferon-γ and interleukin-4 knockout mice; implications for the Th1/Th2 paradigm. Clin Exp Immunol. 2004;138:417–422 [PMCID: PMC1809247] [PubMed: 15544617]

304. Furtado GC, Marinkovic T, Martin AP, et al. Lymphotoxin β receptor signaling is required for inflammatory lymphangiogenesis in the thyroid. Proc Natl Acad Sci USA. 2007;104:5026–5031 [PMCID: PMC1829258] [PubMed: 17360402]

305. Verginis P, Li HS, Carayanniotis G. Tolerogenic semimature dendritic cells suppress experimental autoimmune thyroiditis by activation of thyroglobulin-specific CD4+CD25+ T cells. J Immunol. 2005;174:7433–7439 [PubMed: 15905592]

306. Braley-Mullen H, Sharp GC, Tang H, Chen K, Kyriakos M, Bickel JT. Interleukin-12 promotes activation of effector cells that induce a severe destructive granulomatous form of murine experimental autoimmune thyroiditis. Am J Pathol. 1998;152:1347–1358 [PMCID: PMC1858565] [PubMed: 9588903]

307. Nagayama Y, Mizuguchi H, Hayakawa T, Niwa M, McLachlan SM, Rapoport B. Prevention of autoantibody-mediated Graves'-like hyperthyroidism in mice with IL-4, a Th2 cytokine. J Immunol. 2003;170:3522–3527 [PubMed: 12646613]

308. Saitoh O, Mizutori Y, Takamura N, et al. Adenovirus-mediated gene delivery of interleukin-10, but not transforming growth factor β, ameliorates the induction of Graves' hyperthyroidism in BALB/c mice. Clin Exp Immunol. 2005;141:405–411 [PMCID: PMC1809459] [PubMed: 16045729]

309. Nagayama Y, Watanabe K, Niwa M, McLachlan SM, Rapoport B. *Schistosoma mansoni* and α-galactosylceramide: prophylactic effect of Th1 Immune suppression in a mouse model of Graves' hyperthyroidism. J Immunol. 2004;173:2167–2173 [PubMed: 15265954]

310. El Fassi D, Nielsen CH, Hasselbalch HC, Hegedüs L. Treatment-resistant severe, active Graves' ophthalmopathy successfully treated with B lymphocyte depletion. Thyroid. 2006;16:709–710 [PubMed: 16889501]

311. El Fassi D, Nielsen CH, Bonnema SJ, Hasselbalch HC, Hegedüs L. B lymphocyte depletion with the monoclonal antibody rituximab in Graves' disease: a controlled pilot study. J Clin Endocrinol Metab. 2007;92:1769–1772 [PubMed: 17284622]

312. Gilbert JA, Kalled SL, Moorhead J, et al. Treatment of autoimmune hyperthyroidism in a murine model of Graves' disease with tumor necrosis factor-family ligand inhibitors suggests a key role for B cell activating factor in disease pathology. Endocrinology. 2006;147:4561–4568 [PubMed: 16794009]

313. McMaster PR, Kyriakos M. The prevention of autoimmunity to the thyroid and allergic thyroiditis by antigenic competition. J Immunol. 1970;105:1201–1205 [PubMed: 4099051]

314. Sharp GC, Wortis HH, LaRoque RL, Mullen H, Kyriakos M. Protection against autoimmune thyroiditis in guinea pigs by thyroglobulin antiserum. J Immunol. 1974;112:468–477 [PubMed: 4813899]

315. Silverman DA, Rose NR. Inhibition of genetically determined autoimmune disease by organ-specific antigen. Lancet. 1974;1:1257–1258 [PubMed: 4134144]

316. Whitmore DB, Irvine WJ. Prevention of autoimmune thyroiditis in T cell-depleted rats by injections of crude thyroid extract. Clin Exp Immunol. 1977;29:474–479 [PMCID: PMC1541055] [PubMed: 303967]

317. Lewis M, Giraldo AA, Kong YC. Resistance to experimental autoimmune thyroiditis induced by physiologic manipulation of thyroglobulin level. Clin Immunol Immunopathol. 1987;45:92–104 [PubMed: 3113790]

318. Lewis M, Fuller BE, Giraldo AA, Kong YC. Resistance to experimental autoimmune thyroiditis is correlated with the duration of raised thyroglobulin levels. Clin Immunol Immunopathol. 1992;64:197–204 [PubMed: 1643752]

319. Hutchings PR, Parish NM, Cooke A. Abnormalities in the SJL mouse provide evidence for different mechanisms for the induction and transfer of tolerance to mouse thyroglobulin. Immunology. 1989;66:106–110 [PMCID: PMC1385128] [PubMed: 15493271]

320. Kong YM, Okayasu I, Giraldo AA, et al. Tolerance to thyroglobulin by activating suppressor mechanisms. Ann NY Acad Sci. 1982;392:191–209 [PubMed: 6215879]

321. Hawiger D, Inaba K, Dorsett Y, et al. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J Exp Med. 2001;194:769–779 [PMCID: PMC2195961] [PubMed: 11560993]

322. Misharin AV, Nagayama Y, Aliesky HA, Mizutori Y, Rapoport B, McLachlan SM. Attenuation of induced hyperthyroidism in mice by pretreatment with thyrotropin receptor protein: deviation of thyroid-stimulating to nonfunctional antibodies. Endocrinology. 2009;150:3944–3952 [PMCID: PMC2717879] [PubMed: 19389831]

323. Weiner HL, da Cunha AP, Quintana F, Wu H. Oral tolerance. Immunol Rev. 2011;241:241–259 [PMCID: PMC3296283] [PubMed: 21488901]

324. Peterson KE, Braley-Mullen H. Suppression of murine experimental autoimmune thyroiditis by oral administration of porcine thyroglobulin. Cell Immunol. 1995;166:123–130 [PubMed: 7585972]

325. Guimaraes VC, Quintans J, Fisfalen ME, et al. Suppression of development of experimental autoimmune thyroiditis by oral administration of thyroglobulin. Endocrinology. 1995;136:3353–3359 [PubMed: 7543043]

326. Guimaraes VC, Quintans J, Fisfalen ME, et al. Immunosuppression of thyroiditis. Endocrinology. 1996;137:2199–2207 [PubMed: 8641166]

327. Rapoport B, McLachlan SM. Food for thought–is induction of oral tolerance feasible and practical in human thyroid autoimmunity? Endocrinology. 1996;137:2197–2198 [PubMed: 8641165]

328. Weetman AP, Yateman ME, Ealey PA, et al. Thyroid-stimulating antibody activity between different immunoglobulin G subclasses. J Clin Invest. 1990;86:723–727 [PMCID: PMC296786] [PubMed: 2168443]

329. Latrofa F, Chazenbalk GD, Pichurin P, Chen CR, McLachlan SM, Rapoport B. Affinity-enrichment of thyrotropin receptor autoantibodies from Graves' patients and normal individuals provides insight into their properties and possible origin from natural antibodies. J Clin Endocrinol Metab. 2004;89:4734–4745 [PubMed: 15356088]

330. Lee S, Scherberg N, DeGroot LJ. Induction of oral tolerance in human autoimmune thyroid disease. Thyroid. 1998;8:229–234 [PubMed: 9545109]

331. Saegusa K, Ishimaru N, Haneji N, et al. Mechanisms of neonatal tolerance induced in an animal model for primary Sjögren's syndrome by intravenous administration of autoantigen. Scand J Immunol. 2000;52:264–270 [PubMed: 10972902]

332. Wu L, Xun L, Yang J, et al. Induction of murine neonatal tolerance against Graves' disease using recombinant adenovirus expressing the TSH receptor A-subunit. Endocrinology. 2011;152:1165–1171 [PubMed: 21285313]

333. Prummel MF, Strieder T, Wiersinga WM. The environment and autoimmune thyroid diseases. Eur J Endocrinol. 2004;150:605–618 [PubMed: 15132715]

334. Brent GA. Environmental exposures and autoimmune thyroid disease. Thyroid. 2010;20:755–761 [PMCID: PMC2935336] [PubMed: 20578899]

335. Burek CL, Talor MV. Environmental triggers of autoimmune thyroiditis. J Autoimmun. 2009;33:183–189 [PMCID: PMC2790188] [PubMed: 19818584]

336. Mori K, Nakagawa Y, Ozaki H. Does the gut microbiota trigger Hashimoto's thyroiditis? Discov Med. 2012;14:321–326 [PubMed: 23200063]

337. Dang MN, Buzzetti R, Pozzilli P. Epigenetics in autoimmune diseases with focus on type 1 diabetes. Diabetes Metab Res Rev. 2013;29:8–18 [PubMed: 23180441]

338. Ingbar SH. Autoregulation of the thyroid. Response to iodide excess and depletion. Mayo Clin Proc. 1972;47:814–823 [PubMed: 4678499]

339. Braverman LE, Ingbar SH, Vagenakis AG, Adams L, Maloof F. Enhanced susceptibility to iodide myxedema in patients with Hashimoto's disease. J Clin Endocrinol Metab. 1971;32:515–521 [PubMed: 4926257]

340. Stanbury JB, Ermans AE, Bourdoux P, et al. Iodine-induced hyperthyroidism: occurrence and epidemiology. Thyroid. 1998;8:83–100 [PubMed: 9492158]

341. Wolff J. Iodide goiter and the pharmacologic effects of excess iodide. Am J Med. 1969;47:101–124 [PubMed: 4307521]

342. Pedersen IB, Knudsen N, Jørgensen T, Perrild H, Ovesen L, Laurberg P. Thyroid peroxidase and thyroglobulin autoantibodies in a large survey of populations with mild and moderate iodine deficiency. Clin Endocrinol (Oxf). 2003;58:36–42 [PubMed: 12519410]

343. Bagchi N, Brown TR, Urdanivia E, Sundick RS. Induction of autoimmune thyroiditis in chickens by dietary iodine. Science. 1985;230:325–327 [PubMed: 4048936]

344. Li HS, Carayanniotis G. Induction of goitrous hypothyroidism by dietary iodide in SJL mice. Endocrinology. 2007;148:2747–2752 [PubMed: 17347303]

345. Carayanniotis G. Molecular parameters linking thyroglobulin iodination with autoimmune thyroiditis. Hormones (Athens). 2011;10:27–35 [PubMed: 21349803]

346. Li HS, Verginis P, Carayanniotis G. Maturation of dendritic cells by necrotic thyrocytes facilitates induction of experimental autoimmune thyroiditis. Clin Exp Immunol. 2006;144:467–474 [PMCID: PMC1941967] [PubMed: 16734616]

347. Kawashima A, Yamazaki K, Hara T, et al. Demonstration of innate immune responses in the thyroid gland: potential to sense danger and a possible trigger for autoimmune reactions. Thyroid. 2013;23:477–487 [PMCID: PMC3610444] [PubMed: 23234343]

348. McLachlan SM, Rapoport B. Why measure thyroglobulin autoantibodies rather than thyroid peroxidase autoantibodies? Thyroid. 2004;14:510–520 [PubMed: 15307940]

349. Li HS, Carayanniotis G. Iodination of tyrosyls in thyroglobulin generates neoantigenic determinants that cause thyroiditis. J Immunol. 2006;176:4479–4483 [PubMed: 16547286]

350. Kolypetri P, Noel NA, Carayanniotis KA, Carayanniotis G. Iodine content of thyroglobulin in Nod.H2h4 mice developing iodine-accelerated autoimmune thyroiditis. Hormones (Athens). 2010;9:151–160 [PubMed: 20687399]

351. Duntas LH. The role of selenium in thyroid autoimmunity and cancer. Thyroid. 2006;16:455–460 [PubMed: 16756467]

352. Bülow Pedersen I, Knudsen N, Carlé A, et al. Serum selenium is low in newly diagnosed Graves' disease: a population-based study. Clin Endocrinol Oxf. 2013;79(4):584–590 [PubMed: 23448365]

353. Duntas LH. Selenium and the thyroid: a close-knit connection. J Clin Endocrinol Metab. 2010;95:5180–5188 [PubMed: 20810577]

354. Chesney AM, Clawson TA, Webster B. Endemic goiter in rabbits. Bull Johns Hopkins Hosp. 1928;43:261

355. Chu M, Seltzer TF. Myxedema coma induced by ingestion of raw bok choy. N Engl J Med. 2010;362:1945–1946 [PubMed: 20484407]

356. Eheman CR, Garbe P, Tuttle RM. Autoimmune thyroid disease associated with environmental thyroidal irradiation. Thyroid. 2003;13:453–464 [PubMed: 12855012]

357. Pacini F, Vorontsova T, Molinaro E, et al. Prevalence of thyroid autoantibodies in children and adolescents from Belarus exposed to the Chernobyl radioactive fallout. Lancet. 1998;352:763–766 [PubMed: 9737280]

358. Imaizumi M, Usa T, Tominaga T, et al. Radiation dose-response relationships for thyroid nodules and autoimmune thyroid diseases in Hiroshima and Nagasaki atomic bomb survivors 55–58 years after radiation exposure. JAMA. 2006;295:1011–1022 [PubMed: 16507802]

359. Agate L, Mariotti S, Elisei R, et al. Thyroid autoantibodies and thyroid function in subjects exposed to Chernobyl fallout during childhood: evidence for a transient radiation-induced elevation of serum thyroid antibodies without an increase in thyroid autoimmune disease. J Clin Endocrinol Metab. 2008;93:2729–2736 [PubMed: 18430771]

360. McGregor AM, Petersen MM, Capiferri R, Evered DC, Smith BR, Hall R. Effects of radioiodine on thyrotrophin binding inhibiting immunoglobulins in Graves' disease. Clin Endocrinol (Oxf). 1979;11:437–444 [PubMed: 583262]

361. Sklar C, Whitton J, Mertens A, et al. Abnormalities of the thyroid in survivors of Hodgkin's disease: data from the Childhood Cancer Survivor Study. J Clin Endocrinol Metab. 2000;85:3227–3232 [PubMed: 10999813]

362. Dunkelmann S, Wolf R, Koch A, Kittner C, Groth P, Schuemichen C. Incidence of radiation-induced Graves' disease in patients treated with radioiodine for thyroid autonomy before and after introduction of a high-sensitivity TSH receptor antibody assay. Eur J Nucl Med Mol Imaging. 2004;31:1428–1434 [PubMed: 15221291]

363. McGregor AM, McLachlan SM, Smith BR, Hall R. Effect of irradiation on thyroid-autoantibody production. Lancet. 1979;2:442–444 [PubMed: 89502]

364. Laurberg P, Wallin G, Tallstedt L, Abraham-Nordling M, Lundell G, Tørring O. TSH-receptor autoimmunity in Graves' disease after therapy with anti-thyroid drugs, surgery, or radioiodine: a 5-year prospective randomized study. Eur J Endocrinol. 2008;158:69–75 [PubMed: 18166819]

365. Nagayama Y, Kaminoda K, Mizutori Y, Saitoh O, Abiru N. Exacerbation of autoimmune thyroiditis by a single low dose of whole-body irradiation in non-obese diabetic-H2h4 mice. Int J Radiat Biol. 2008;84:761–769 [PubMed: 18821390]

366. Nagayama Y, Ichikawa T, Saitoh O, Abiru N. Induction of late-onset spontaneous autoimmune thyroiditis by a single low-dose irradiation in thyroiditis-prone non-obese diabetic-H2h4 mice. J Radiat Res. 2009;50:573–577 [PubMed: 19755803]

367. Wiersinga WM. Smoking and thyroid. Clin Endocrinol (Oxf). 2013;79:145–151 [PubMed: 23581474]

368. Effraimidis G, Tijssen JG, Wiersinga WM. Discontinuation of smoking increases the risk for developing thyroid peroxidase antibodies and/or thyroglobulin antibodies: a prospective study. J Clin Endocrinol Metab. 2009;94:1324–1328 [PubMed: 19141579]

369. Nizri E, Irony-Tur-Sinai M, Lory O, Orr-Urtreger A, Lavi E, Brenner T. Activation of the cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via suppression of Th1 and Th17 responses. J Immunol. 2009;183:6681–6688 [PubMed: 19846875]

370. Lazarus JH. Lithium and thyroid. Best Pract Res Clin Endocrinol Metab. 2009;23:723–733 [PubMed: 19942149]

371. Silverman DA, Rose NR. Spontaneous and methylcholanthrene-enhanced thyroiditis in BUF rats. I. The incidence and severity of the disease, and the genetics of susceptibility. J Immunol. 1975;114:145–147 [PubMed: 1078675]

372. Tomer Y, Davies TF. Infection, thyroid disease, and autoimmunity. Endocr Rev. 1993;14:107–120 [PubMed: 8491150]

373. Pozzilli P, Signore A, Williams AJ, Beales PE. NOD mouse colonies around the world–recent facts and figures. Immunol Today. 1993;14:193–196 [PubMed: 8517916]

374. Pichurin P, Yan XM, Farilla L, et al. Naked TSH receptor DNA vaccination: a TH1 T cell response in which interferon-γ production, rather than antibody, dominates the immune response in mice. Endocrinology. 2001;142:3530–3536 [PubMed: 11459799]

375. Nagayama Y, McLachlan SM, Rapoport B, Niwa M. A major role for non-major histocompatibility complex genes but not for microorganisms in a novel murine model of Graves' hyperthyroidism. Thyroid. 2003;13:233–238 [PubMed: 12729471]

376. Weiss M, Ingbar SH, Winblad S, Kasper DL. Demonstration of a saturable binding site for thyrotropin in *Yersinia enterocolitica*. Science. 1983;219:1331–1333 [PubMed: 6298936]

377. Bech K, Larsen JH, Hansen JM, Nerup J. Letter: *Yersinia enterocolitica* infection and thyroid disorders. Lancet. 1974;2:951–952 [PubMed: 4138430]

378. Wenzel BE, Heesemann J, Wenzel KW, Scriba PC. Antibodies to plasmid-encoded proteins of enteropathogenic *Yersinia* in patients with autoimmune thyroid disease. Lancet. 1988;1:56. [PubMed: 2891918]

379. Yamamoto M, Rapoport B. Studies on the binding of radiolabeled thyrotropin to cultured human thyroid cells. Endocrinology. 1978;103:2011–2019 [PubMed: 218797]

380. Luo G, Fan JL, Seetharamaiah GS, et al. Immunization of mice with *Yersinia enterocolitica* leads to the induction of antithyrotropin receptor antibodies. J Immunol. 1993;151:922–928 [PubMed: 8335920]

381. Luo G, Seetharamaiah GS, Niesel DW, et al. Purification and characterization of *Yersinia enterocolitica* envelope proteins which induce antibodies that react with human thyrotropin receptor. J Immunol. 1994;152:2555–2561 [PubMed: 7510749]

382. Zhang H, Kaur I, Niesel DW, et al. Lipoprotein from *Yersinia enterocolitica* contains epitopes that cross-react with the human thyrotropin receptor. J Immunol. 1997;158:1976–1983 [PubMed: 9029141]

383. Zhang H, Kaur I, Niesel DW, et al. *Yersinia enterocolitica* envelope proteins that are crossreactive with the thyrotropin receptor (TSHR) also have B-cell mitogenic activity. J Autoimmun. 1996;9:509–516 [PubMed: 8864826]

384. Gangi E, Kapatral V, El-Azami El-Idrissi M, Martinez O, Prabhakar BS. Characterization of a recombinant *Yersinia enterocolitica* lipoprotein; implications for its role in autoimmune response against thyrotropin receptor. Autoimmunity. 2004;37:515–520 [PubMed: 15621579]

385. Wang Z, Zhang Q, Lu J, et al. Identification of outer membrane porin f protein of *Yersinia enterocolitica* recognized by antithyrotopin receptor antibodies in Graves' disease and determination of its epitope using mass spectrometry and bioinformatics tools. J Clin Endocrinol Metab. 2010;95:4012–4020 [PubMed: 20484489]

386. Guarneri F, Carlotta D, Saraceno G, Trimarchi F, Benvenga S. Bioinformatics support the possible triggering of autoimmune thyroid diseases by *Yersinia enterocolitica* outer membrane proteins homologous to the human thyrotropin receptor. Thyroid. 2011;21:1283–1284 [PubMed: 21877932]

387. Corapçioğlu D, Tonyukuk V, Kiyan M, et al. Relationship between thyroid autoimmunity and *Yersinia enterocolitica* antibodies. Thyroid. 2002;12:613–617 [PubMed: 12193307]

388. Effraimidis G, Tijssen JG, Strieder TG, Wiersinga WM. No causal relationship between *Yersinia enterocolitica* infection and autoimmune thyroid disease: evidence from a prospective study. Clin Exp Immunol. 2011;165:38–43 [PMCID: PMC3110319] [PubMed: 21488870]

389. Brix TH, Hansen PS, Hegedüs L, Wenzel BE. Too early to dismiss *Yersinia enterocolitica* infection in the aetiology of Graves' disease: evidence from a twin case-control study. Clin Endocrinol (Oxf). 2008;69:491–496 [PubMed: 18284638]

390. Hargreaves CE, Grasso M, Hampe CS, et al. *Yersinia enterocolitica* provides the link between thyroid-stimulating antibodies and their germline counterparts in Graves' disease. J Immunol. 2013;190:5373–5381 [PubMed: 23630351]

391. Kraemer MH, Donadi EA, Tambascia MA, Magna LA, Prigenzi LS. Relationship between HLA antigens and infectious agents in contributing towards the development of Graves' disease. Immunol Invest. 1998;27:17–29 [PubMed: 9561915]

392. Hammerstad SS, Jahnsen FL, Tauriainen S, et al. Inflammation and Increased Myxovirus Resistance Protein A Expression in Thyroid Tissue in the Early Stages of Hashimoto's Thyroiditis. Thyroid.

2013;23:334–341 [PubMed: 22998463]

393. Blackard JT, Kong L, Huber AK, Tomer Y. Hepatitis C virus infection of a thyroid cell line: implications for pathogenesis of hepatitis C virus and thyroiditis. Thyroid. 2013;23:863–870 [PMCID: PMC3704108] [PubMed: 23259732]

394. Vaccaro M, Guarneri F, Borgia F, Cannavò SP, Benvenga S. Association of lichen sclerosus and autoimmune thyroiditis: possible role of Borrelia burgdorferi? Thyroid. 2002;12:1147–1148 [PubMed: 12593730]

395. Benvenga S, Guarneri F, Vaccaro M, Santarpia L, Trimarchi F. Homologies between proteins of Borrelia burgdorferi and thyroid autoantigens. Thyroid. 2004;14:964–966 [PubMed: 15671776]

396. Benvenga S, Santarpia L, Trimarchi F, Guarneri F. Human thyroid autoantigens and proteins of Yersinia and Borrelia share amino acid sequence homology that includes binding motifs to HLA-DR molecules and T-cell receptor. Thyroid. 2006;16:225–236 [PubMed: 16571084]

397. Kamath C, Young S, Kabelis K, et al. Thyrotrophin receptor antibody characteristics in a woman with long-standing Hashimoto's who developed Graves' disease and pretibial myxoedema. Clin Endocrinol (Oxf). 2012;77:465–470 [PubMed: 22891608]

398. Gregoric E, Gregoric JA, Guarneri F, Benvenga S. Injections of Clostridium botulinum neurotoxin A may cause thyroid complications in predisposed persons based on molecular mimicry with thyroid autoantigens. Endocrine. 2011;39:41–47 [PubMed: 21061092]

399. Benoist C, Mathis D. Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry? Nat Immunol. 2001;2:797–801 [PubMed: 11526389]

400. Mangalam AK, Taneja V, David CS. HLA class II molecules influence susceptibility versus protection in inflammatory diseases by determining the cytokine profile. J Immunol. 2013;190:513–518 [PMCID: PMC3545203] [PubMed: 23293357]

401. von Herrath MG, Fujinami RS, Whitton JL. Microorganisms and autoimmunity: making the barren field fertile? Nat Rev Microbiol. 2003;1:151–157 [PubMed: 15035044]

402. Di Genova G, Savelyeva N, Suchacki A, Thirdborough SM, Stevenson FK. Bystander stimulation of activated CD4+ T cells of unrelated specificity following a booster vaccination with tetanus toxoid. Eur J Immunol. 2010;40:976–985 [PubMed: 20104490]

403. Kawashima A, Tanigawa K, Akama T, Yoshihara A, Ishii N, Suzuki K. Innate immune activation and thyroid autoimmunity. J Clin Endocrinol Metab. 2011;96:3661–3671 [PubMed: 21956420]

404. Enouz S, Carrié L, Merkler D, Bevan MJ, Zehn D. Autoreactive T cells bypass negative selection and respond to self-antigen stimulation during infection. J Exp Med. 2012;209:1769–1779 [PMCID: PMC3457731] [PubMed: 22987800]

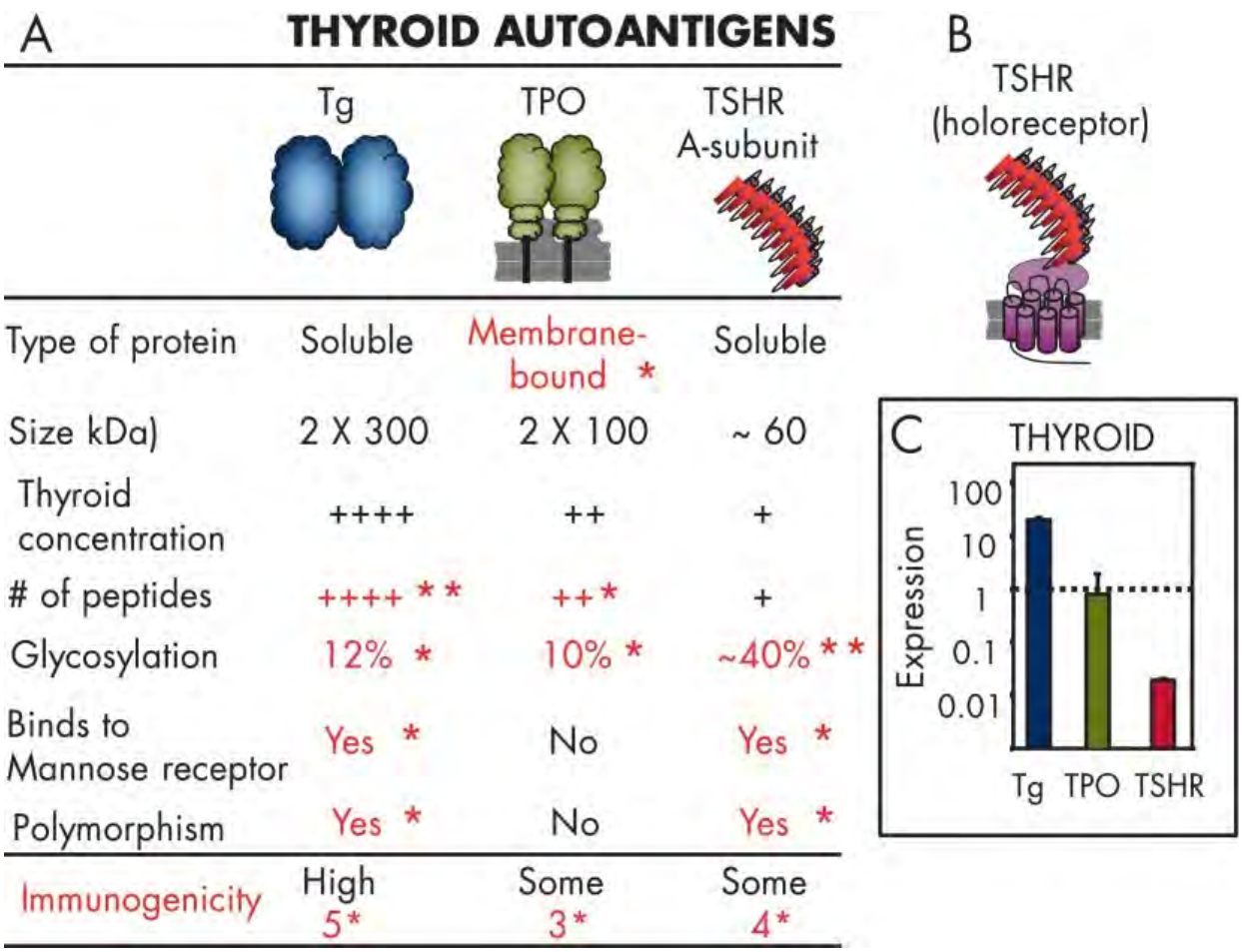
405. Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. Nat Rev Immunol. 2001;1:69–75 [PubMed: 11905816]

406. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. Science. 2002;296:490–494 [PubMed: 11964470]

407. Cox SP, Phillips DI, Osmond C. Does infection initiate Graves disease? A population based 10 year study. Autoimmunity. 1989;4:43–49 [PubMed: 2491641]

Figures and Tables

Figure 1.



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Characteristics and intrathyroidal concentrations of Tg, TPO, and the TSHR. A, Characteristics of thyroid autoantigens that may contribute to their immunogenicity. Individual factors are indicated by an asterisk, and the sum of the asterisks is shown as an "immunogenicity score". # indicates number. B, Schematic representation of the TSH holoreceptor, including its transmembrane domain. Red, A-subunit; purple, hinge, transmembrane, and intracellular domains. C, Expression of Tg, TPO, and TSHR mRNA in mouse thyroid tissue measured by real-time PCR and normalized to the housekeeping gene β-actin (value of 1.0, indicated by dotted line). These data have been adapted from Ref. 25.

Table 1.

Thyroid Autoimmunity Arising Spontaneously in Humans and Animals

	Autoimmune Responses			Outcome	Enhancing Factors	Ref.
	TSHR	Tg	TPO			
Graves' disease						
Humans	TSAb	TgAb[a]	TPOAb	Hyper	Iodine	34, rev; 11
Humans	TBAb			Hypo atrophy		35, 36
Humans	TSAb	TgAb[a]	TPOAb	Ophthalmopathy	Smoking	38, rev
	T cells					
	Cytokines					
Thyroiditis						
Humans		TgAb	TPOAb	Hypo	Iodine	40, 41
		T cells	T cells	(Some)		46–51
Marmosets (some						65
not all colonies)						66
Dogs (beagles)		TgAb	TPOAb	Hypo		63, 64
OS chickens		TgAb	Mic Ab	Hypo		61, 62
Rats, Biobreeding		TgAb			Iodine	56
NOD mice		TgAb	Mic Ab			57
NOD.H2h4 mice		TgAb	TPOAb	Eu/hypo	Iodine	58–60

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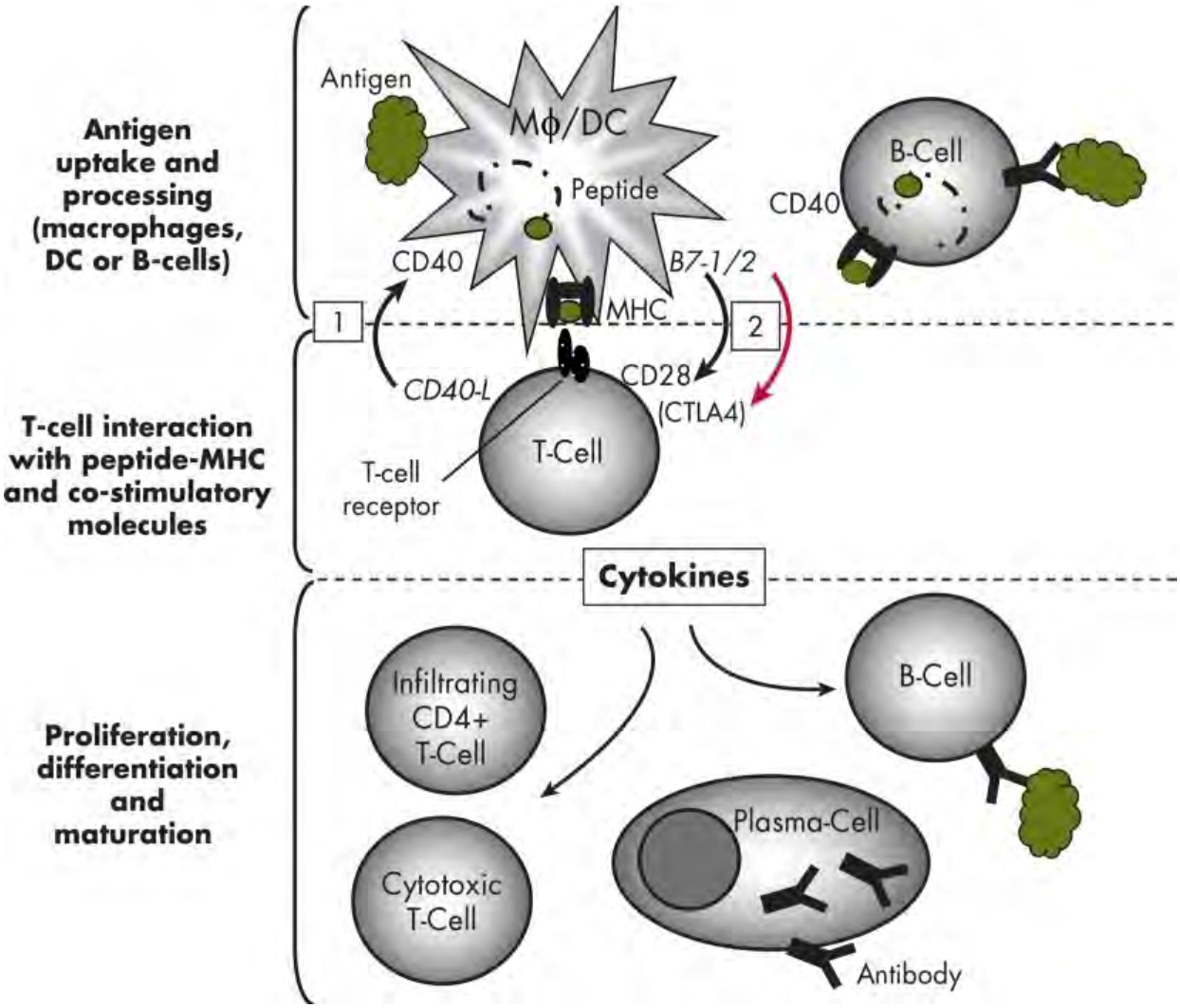
Abbreviations: ref, review; outcome of thyroid autoimmunity is given as hyperthyroid (Hyper), hypothyroid (Hypo), euthyroid (Eu), or thyroid atrophy. Antibodies (Ab) against an unidentified thyroid membrane component (Mic for microsomes) were observed in NOD mice (57).

[a]In some Graves' patients.

Figure 2.



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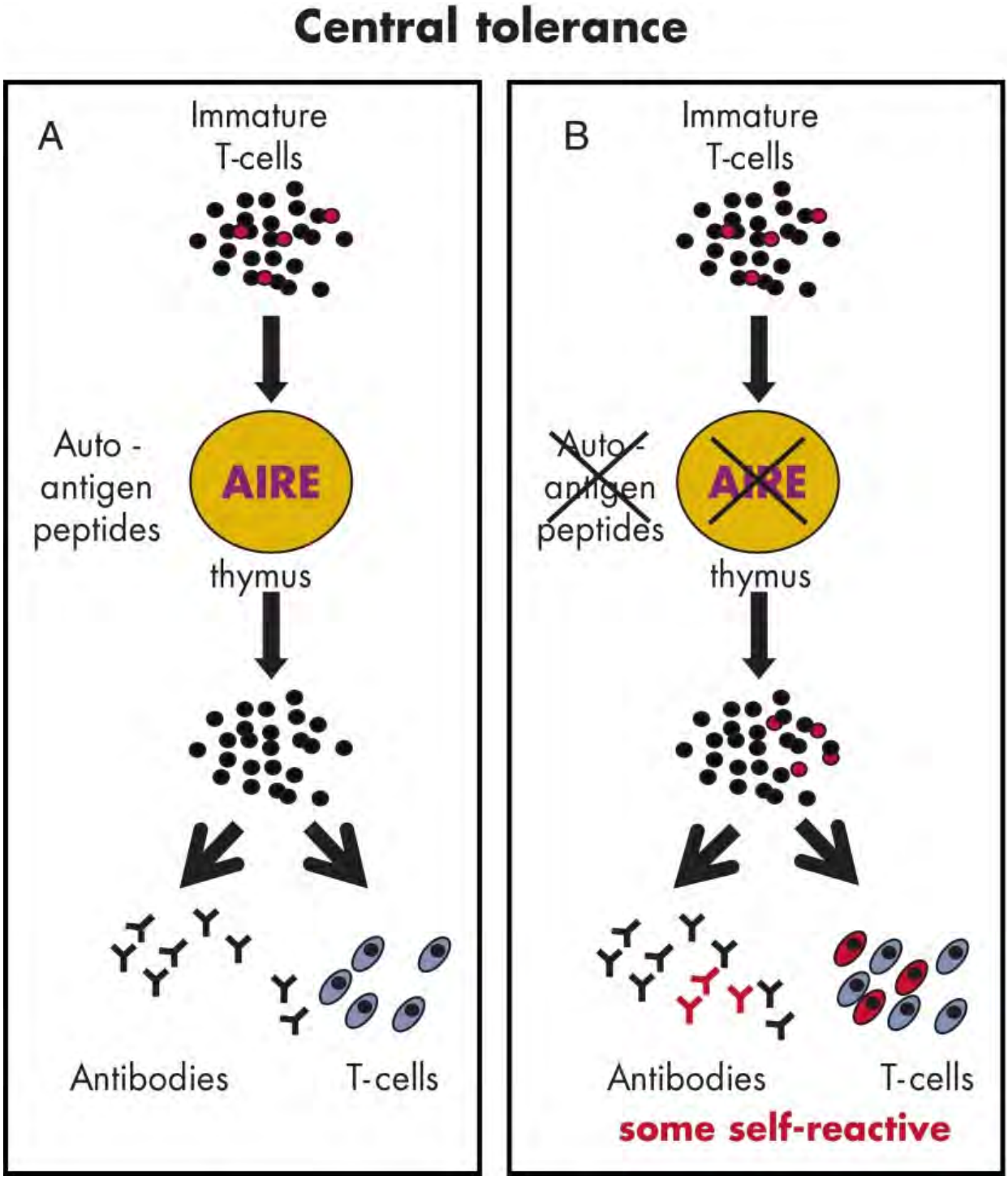
Overview of cellular interactions leading to effector T cells and antibody-producing B cells. Upper panel, Antigen uptake and processing by professional APCs, macrophages (Mφ)/DCs, or B cells. These cells proteolyze antigens and present derived peptides in MHC molecules. Middle panel, T-cell interaction with peptide-MHC complexes on APC. Engagement of costimulatory molecules on APC and T cells (CD40 and CD40-L; signal 1) followed by B7–1/2 and CD28/CTLA4 (signal 2) (as described in *Section III.C.*) leads to cytokine generation. The red arrow represents inhibition. Lower panel, Proliferation, differentiation, and maturation of T cells leads to infiltrating CD4+ T cells and cytotoxic T cells. B cells undergoing this process develop into plasma cells secreting antibodies. Some B cells remain as memory B cells and/or function as APC by virtue of cell surface Ig that function as specific antigen receptors (see upper panel).

Figure 3.



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Central tolerance and T-cell “education” in the thymus in the presence of the AIRE (A) and in the absence of Aire (B).

Table 2.

Induction of Thyroiditis and Thyroid Antibody (Ab) by Conventional Immunization

	Source	Adjuvant	Strain	MHC	Thyroiditis	Ab to	Ref.
i. Thyroid extract							
Dogs	Dog	CFA			Yes	Tg	108, 109
Guinea pigs	Guinea pig	CFA			Yes	Tg	108, 109
Monkeys	Monkey	CFA	Rhesus		Yes	Mic	110
Monkeys	Human	CFA	Vervet		Yes	Tg, Mic	111
Rabbits	Rabbit	CFA			Yes	Tg	108, 109
Rabbits	Human	CFA			No	Tg	108
ii. Tg							
Rabbits	Rabbit	CFA			Yes	Tg	108, 109
Mice	Mouse	CFA, LPS	CBA ##	IA-k	Yes	Tg	112, 113
	Mouse	LPS	BALB #	IA-d	No	Tg	112
	Mouse	LPS	DR3-tg	DR3	Yes	Tg	114
	Human	LPS	DR3-tg	DR3	Yes	Tg	114
iii. TPO (or peptide, pep)							
Mice	Mouse	CFA	BL/6; F1	IA-b, k	Yes	TPO	117
	m-pep	CFA	BL/6	IA-b	Yes; hypo	TPO	119
	Porcine	CFA	BL/6	IA-b	Yes	TPO	115
	p-pep	CFA	BL/6	IA-b	Yes		118
	Porcine	CFA	CBA	IA-k	No	TPO	116
	Porcine	CFA	BALB/c	IA-d	No	TPO	116
	Human	CFA	AKR/N	IA-k	No	TPO	130
	Human	DNA	BALB/c	IA-b	No	TPO	132
	Human	Aden	DR3-tg	DR3	No	TPO	134

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Abbreviations: m-pep, mouse peptide; p-pep, porcine peptide; Aden, adenovirus; F1, offspring of B6 × CBA mice; ##, good responder (or # or poor) responder mouse strains in terms of thyroiditis; DR3-tg, mouse transgenic for human HLA-DR3. Adjuant, CFA, LPS, Al, Mg, Per, Al_2OH_3 + $Mg(OH)_2$ + Bordetella pertussis toxin. The antigen source,

adjuvant, mammal strain, and MHC class II are included. Mic, microsomes.

Table 3.

Alternative or Novel Approaches to Induce Thyroiditis and Thyroid Antibody (Ab)

	Source	Adjuvant	Strain	MHC	Thyroiditis	Ab	Ref.
i. Neonatal thymectomy and whole-body irradiation							
Rats			Wistar		Yes		123
Rats		3-MCA	Buffalo		Yes		122, 124
ii. Injecting lymphocytes sensitized to Tg or sensitized to thyrocytes							
Guinea pigs	Guinea pig	Tg	Strain 13		Yes		125
Mice	Mouse	LPS	CBA	IA-k	Yes		126, 127
Mice	Mouse	Thyrocytes	CBA	IA-k	Yes	TgAb	128
iii. Injecting DCs pulsed with Tg or fibroblasts that express TPO							
Mice	Mouse	DC-Tg	CBA	IA-k	Yes		129
Mice	Human	RT-TPO	AKR/N	IA-k	No	TPOAb	130
iv. Implanting thyroid gland + LPS							
Mice	Mouse	LPS	B10(BR)	IA-k	Yes		131
			B10B2	IA-d	No		
v. Tg or TPO expressed in vivo (plasmid or adenovirus vectors)							
Mice	Human	TPO DNA	DR3-tg	DR3	Yes	TPOAb	132
Mice	Mouse	TPO-Ad	BL/6	IA-b	Yes	TPOAb	135
Mice	Human	Tg-DNA	C3H/He	IA-k	No	TgAb	133
vi. Intrathyroidal chemokine/ cytokine expression or chemokine receptor knockout							
Mice-tg	CCL21		BL/6	IA-b	Yes		136
Mice-ko	CCR7		NOD	IA-gp7	Yes	TgAb	138
Mice-tg	IFN-α		BL/6	IA-b	Yes		137
vii. Transgenic expression of human T-cell receptor specific for TPO (TAZ10 mice)							
Mic-tg	T cell		CBA	IA-k	Yes		143, 144
viii. Enhanced tumor regression							
Mice, TUBO	Mouse	Treg depl	BALB/c	IA-d	Yes	TgAb	145
Tumor	Rat	DNA-neu					
	Mouse	Tg, LPS					
ix. Inadvertent thyroiditis (human-TSHR A-subunit transgenics)							
Mice-tg		Treg depl	BALB/c	IA-d	Yes	TgAb	147

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Abbreviations: Ad, adenovirus; B6, C57/BL6; B10, C57BL/10 mice; DNA-neu, DNA encoding rat neu, homolog of human herceptin-2; DR3-tg, transgenic for human HLA-DR3; human A-sub tg, transgenic for human TSHR A-subunit; lymphs, lymphocytes; n/a, not applicable; RT-TPO, fibroblasts coexpressing MHC class II and TPO;

TUBO, mouse mammary cell tumor that expresses neu. The antigen source, animal strain, and mouse/human MHC class II (IA or DR) are included.

Table 4.

TSHR Antibodies and Graves' Disease Induced by Novel Approaches

	TSHR Species	Approach	Strain	MHC Class II	Status	Ab	Thyr	Ref.
i. TSAb H & L chain V region genes		Transgenic	C57BL/6	I-Ab	Hyper	TSAb	No	151
ii. TSHR-expressing cells								
Mice	Human	Fibros	AKR/N	I-Ak	Hyper	TSAb	No	158, 183
Mice	Human	B cells	BALB/c	I-Ad	Hyper	TSAb	Yes	159
Mice	Human	DCs	BALB/c	I-Ad	Hyper	TSAb	Yes	160
Hamsters	Human	CHO cells	Armen.	na	Hyper	TSAb	Yes	161
iii. TSHR expressed in vivo by DNA or adenovirus vectors								
Mice	Human	cDNA	BALB/c	I-Ad	Eu	TSAb	Yes	162
Mice	Human	cDNA	Outbred		Hyper	TSAb	Yes	163
Mice	Human	Adeno	BALB/c	I-Ad	Hyper	TSAb	No	164
Mice			C57BL/6	I-Ab	Eu	TSAb	No	164
			Others	Many	Eu	TSAb	No	164
Mice	Human	DCs	BALB/c	IA-d	Hyper	TSAb	No	160
iv. TSHR A-subunit expressed in vivo by adenovirus or by cDNA + electroporation								
Mice	Human	Adeno	BALB/c	IA-d	Hyper	TSAb	No	165
Mice	Human	cDNA + E	BALB/c	IA-d	Hyper	TSAb	No	166, 167
Mice		Treg depl,	A-sub-tg	IA-d	Hypo	TgAb	Yes	147
	Human	Adeno				TPOAb		147
v. Transfer of mouse TSHR A-subunit primed splenocytes								
Priming	Mouse	Adeno	TSHR-ko	IA-d		TSAb		172
Recipient	Mouse	Spleen	Athymic	IA-d	Hyper,	TSAb	Yes	174
		Transfer	BALB/c		hypo	TBAB		174
vi. TSHR variant expressed in vivo using plasmid DNA								
Mice	Mouse	cDNA + E	BALB/c	IA-d	Hyper	TSAb	No	176

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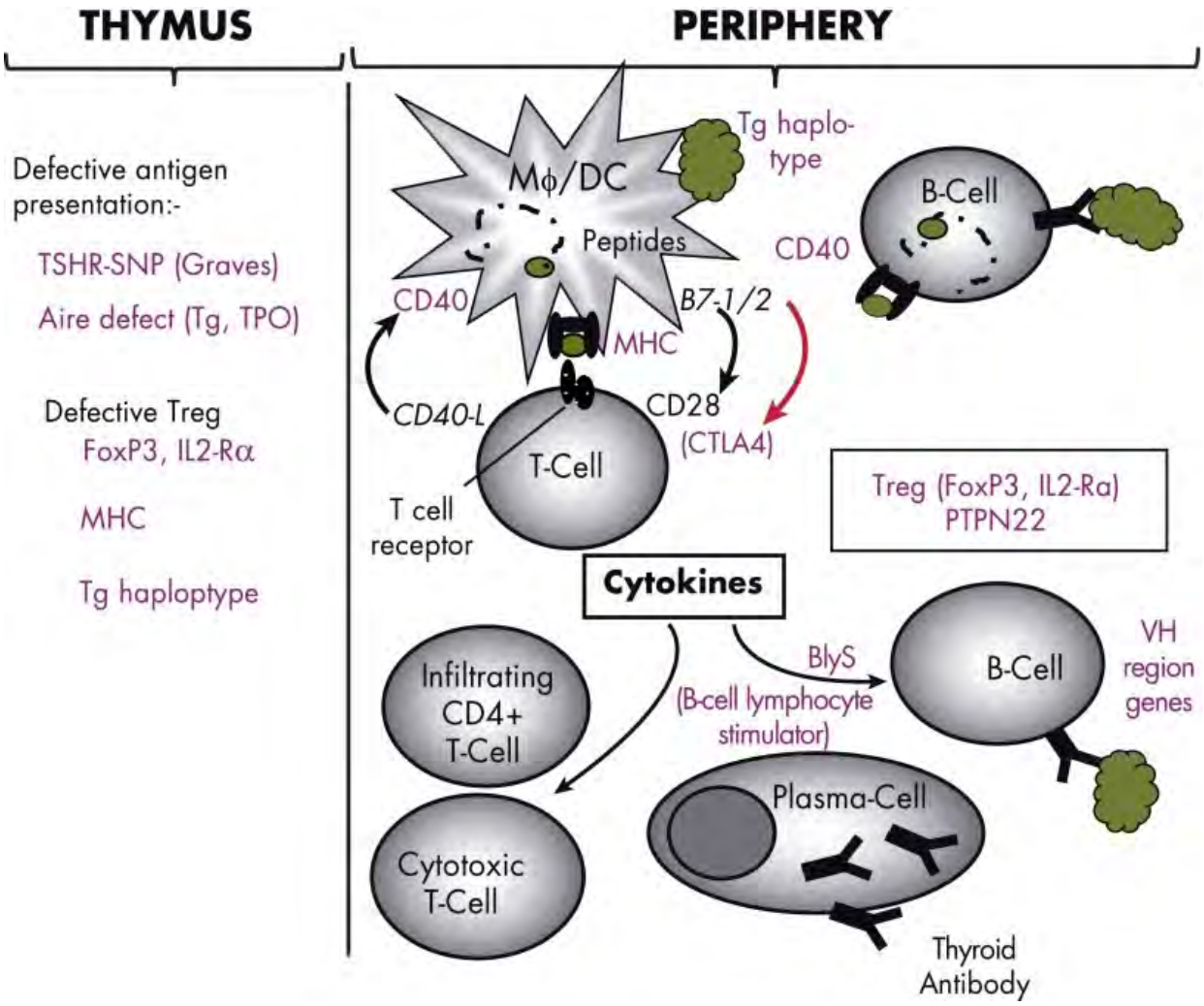
Abbreviations: Armen, Armenian hamsters; depl, depleted; Fibros, fibroblasts; E, electroporation; Eu, euthyroid; Hyper, elevated T_4 levels; Hypo, low or absent T_4; A-sub-tg Lo exp, low human A-subunit transgene expressor; TSHR ko, TSHR coexpressing MHC class II and TSHR knockout mice (173). The outcome is shown for thyroid status

(hyper, hypo, euthyroid), thyroid antibodies (Ab), and thyroiditis (Thyr).

Figure 4.



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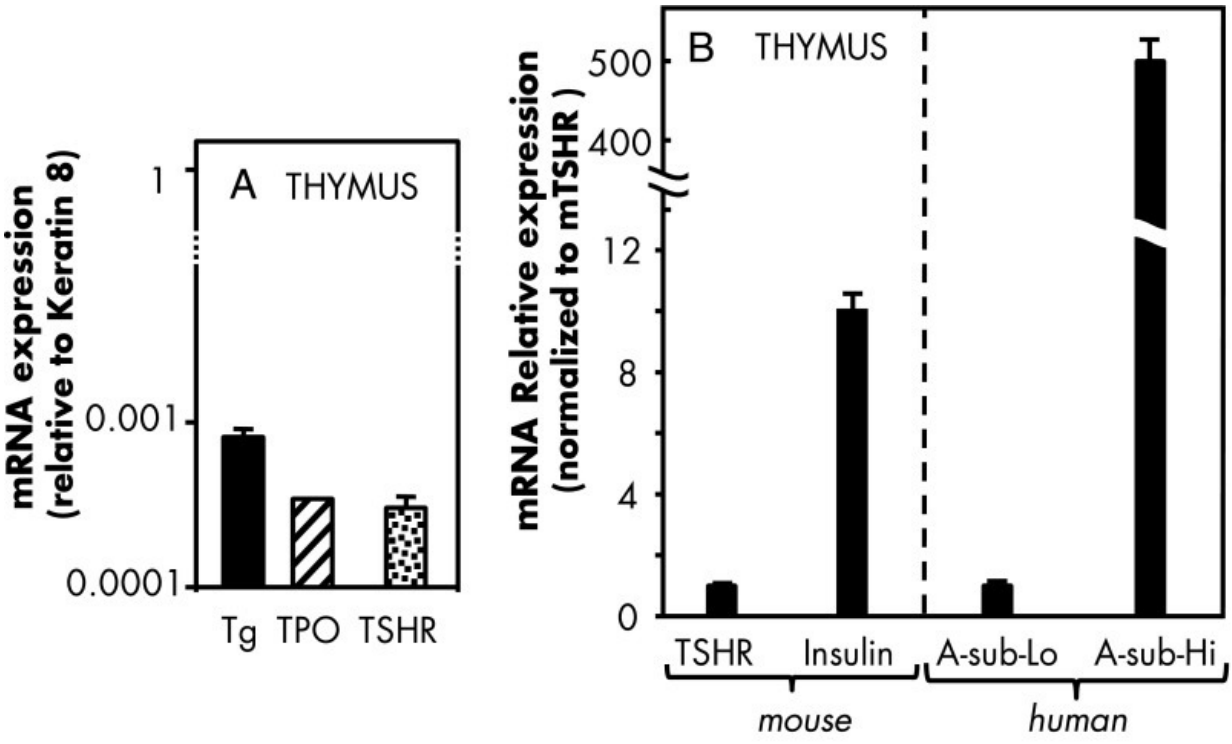
Genes associated with thyroid autoimmunity and their role in central tolerance or peripheral control of autoimmune responses. Evidence for the genes illustrated and their identification are cited in *Section VI*. BlyS, B-cell lymphocyte stimulator. Mφ, macrophage.

Figure 5.



C	Thymic expression	Expectation for Breaking tolerance
A-sub-Hi (*human*)	+++++	Most difficult
Insulin (*mouse*)	+++	
Tg (*mouse*)	++	
A-sub-Lo (*human*)	+	
TSHR (*mouse*)	+	
TPO (*mouse*)	+	Easier

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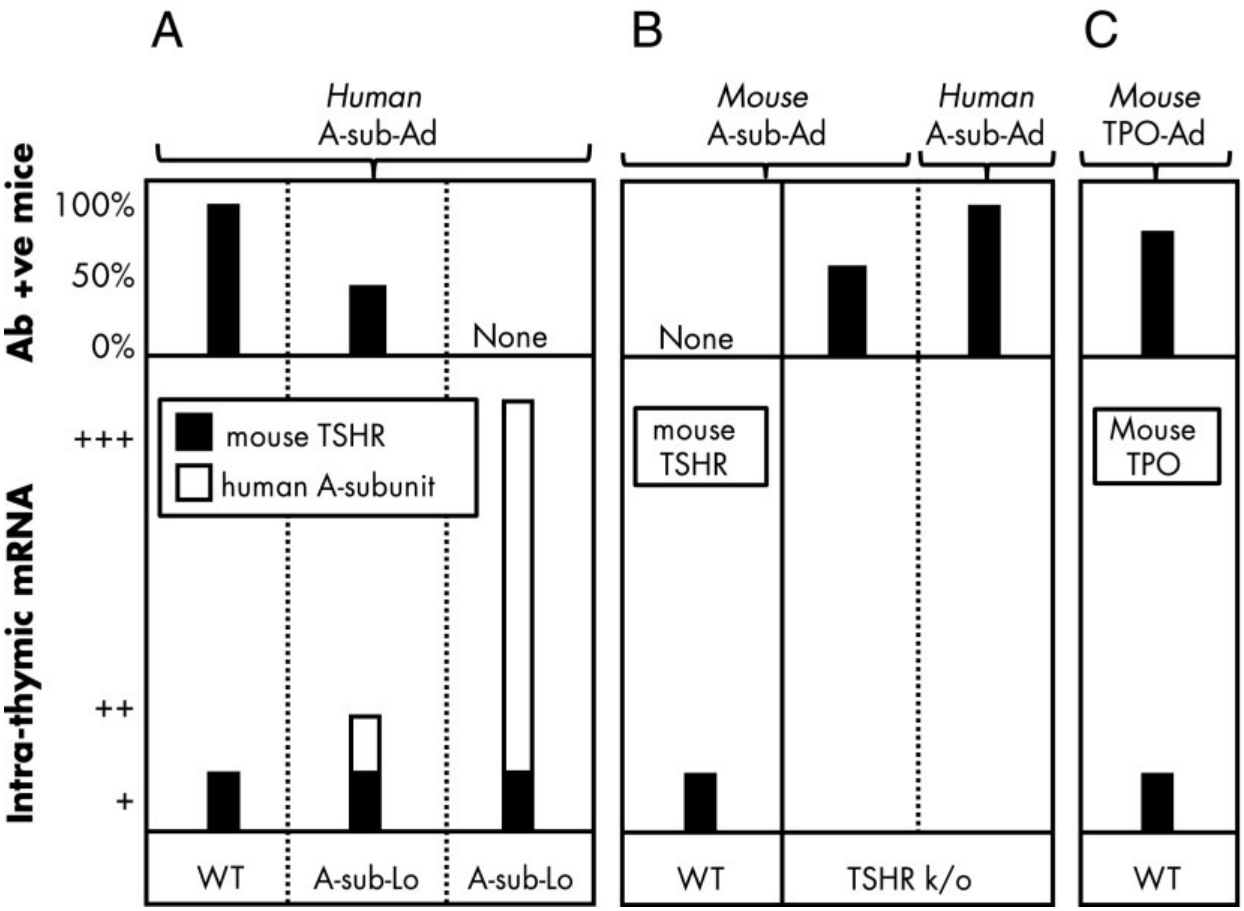
Central tolerance to thyroid autoantigens. A, Intrathymic expression in mice of mouse Tg, TPO, and the TSHR. Expression levels measured by real-time PCR were normalized to keratin-8, a marker for thymic epithelial cells. B, Intrathymic expression in mice of mouse TSHR, Tg, TPO, the transgenic human TSHR A-subunit and mouse insulin measured by real-time PCR. Data normalized to the level in wild-type mice for Tg, TPO, and TSHR. Data for panels A and B were drawn from Ref. 25, 225. A-sub-Lo, Human TSHR A-subunit low expressor; A-sub-Hi, human TSHR A-subunit high expressor. C, Expectations for breaking central tolerance to thyroid autoantigens.

Figure 6.



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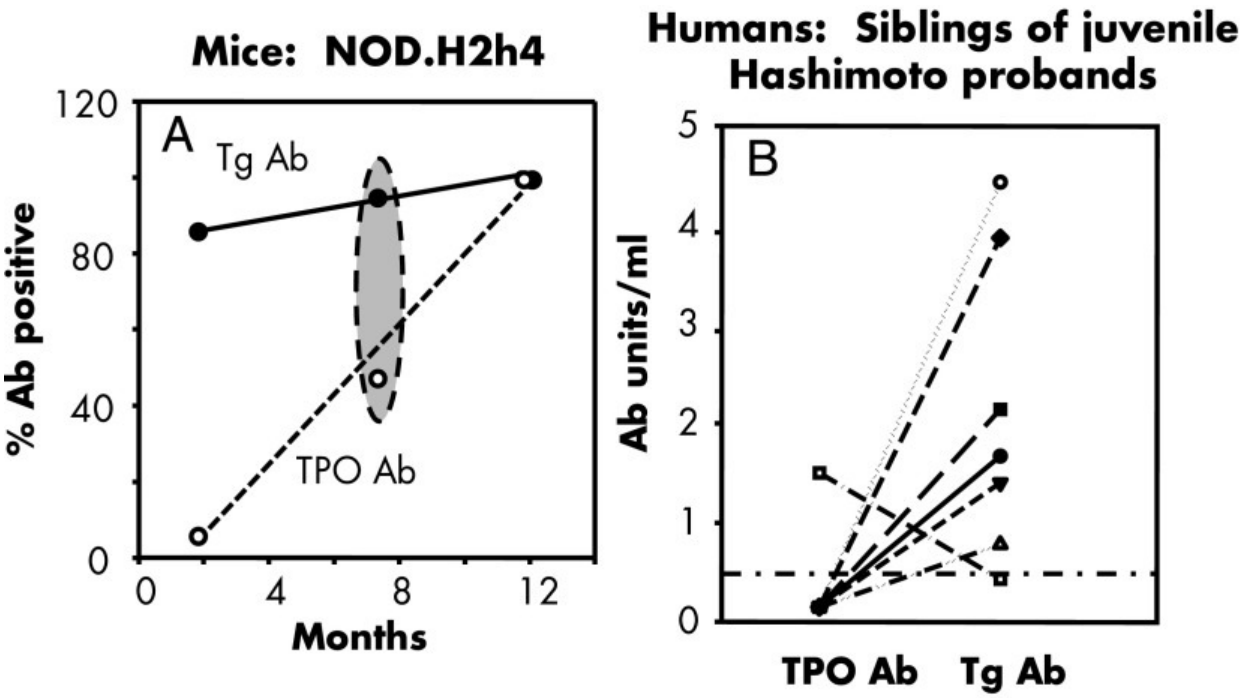
Breaking central tolerance to the TSHR and TPO. Schematic depiction of the relationship between the efficacy of antibody generation (upper panels) and intrathymic mRNA expression (lower panels). This efficacy is expressed as the percentage of mice that become antibody positive after immunization with indicated adenoviruses (Ad) encoding the TSHR or TPO. A, TSHR antibody responses induced in wild-type and Lo- and Hi-expressor human A-subunit transgenic mice by immunization with human TSHR-A-subunit Ad. B, TSHR antibody responses in wild-type and TSHR knockout (k/o) mice after immunization with mouse TSHR A-subunit-Ad and (in knockouts) with human TSHR A-subunit-Ad. C, TPO antibodies induced in wild-type mice with mouse-TPO-Ad. Data for panels A to C are derived from Refs. 135, 172, 225, and 236.

Figure 7.



C

		Tg	TPO
	Thymus	++	+
Prediction	Tolerance	High	Low
Prediction	Immunogenicity	High	Some
Genetics	Polyphorphisms	Yes	No
Observed	AUTO Ab	1st	2nd

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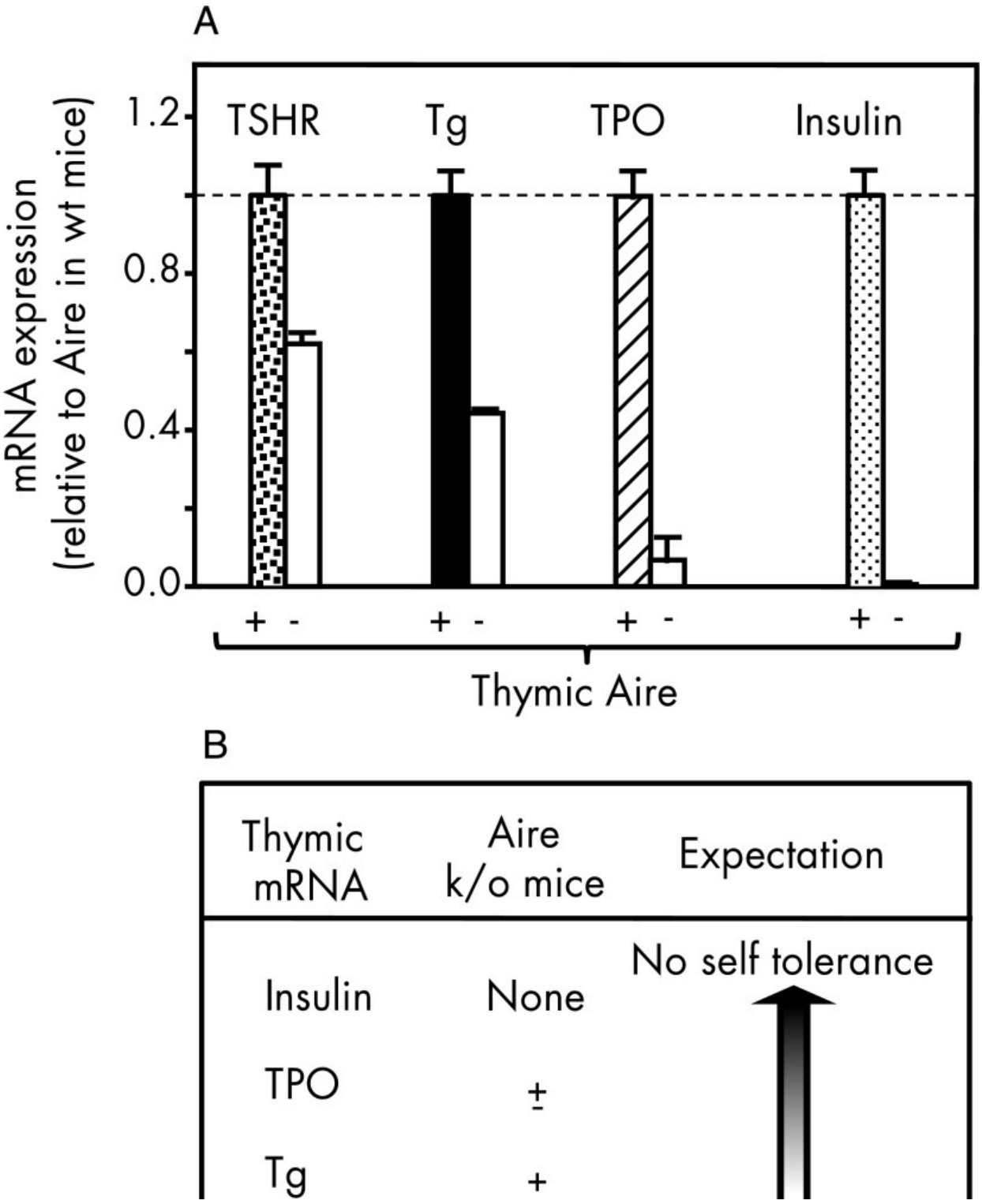
Spontaneous development of antibodies to Tg and TPO. A, NOD.H2h4 mice; B, human siblings with juvenile Hashimoto's thyroiditis. From Chen et al (135), with permission from The Endocrine Society. C, Predictions compared with observations for the sequential appearance of autoantibodies to Tg and TPO.

Figure 8.



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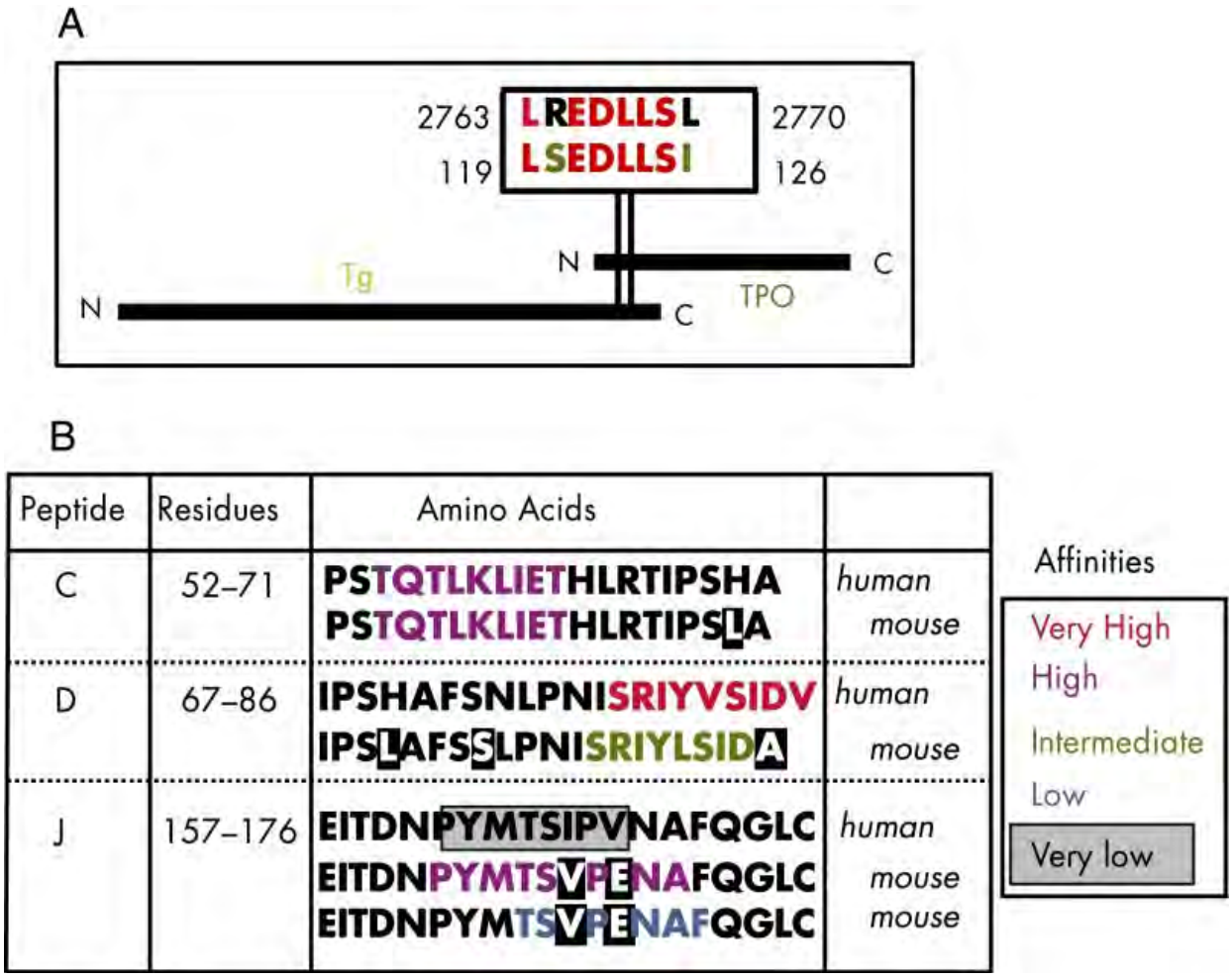
Effects of the lack of Aire on central tolerance. A, Quantitative intrathymic expression of mRNA (real time PCR) for different self-antigens in wild-type and Aire knockout ("–," k/o) mice (225). Data were normalized to expression of the mouse TSHR in Aire "+" (wild type; wt) mice. B, Expectations for the change in self-tolerance in the absence of Aire.

Figure 9.



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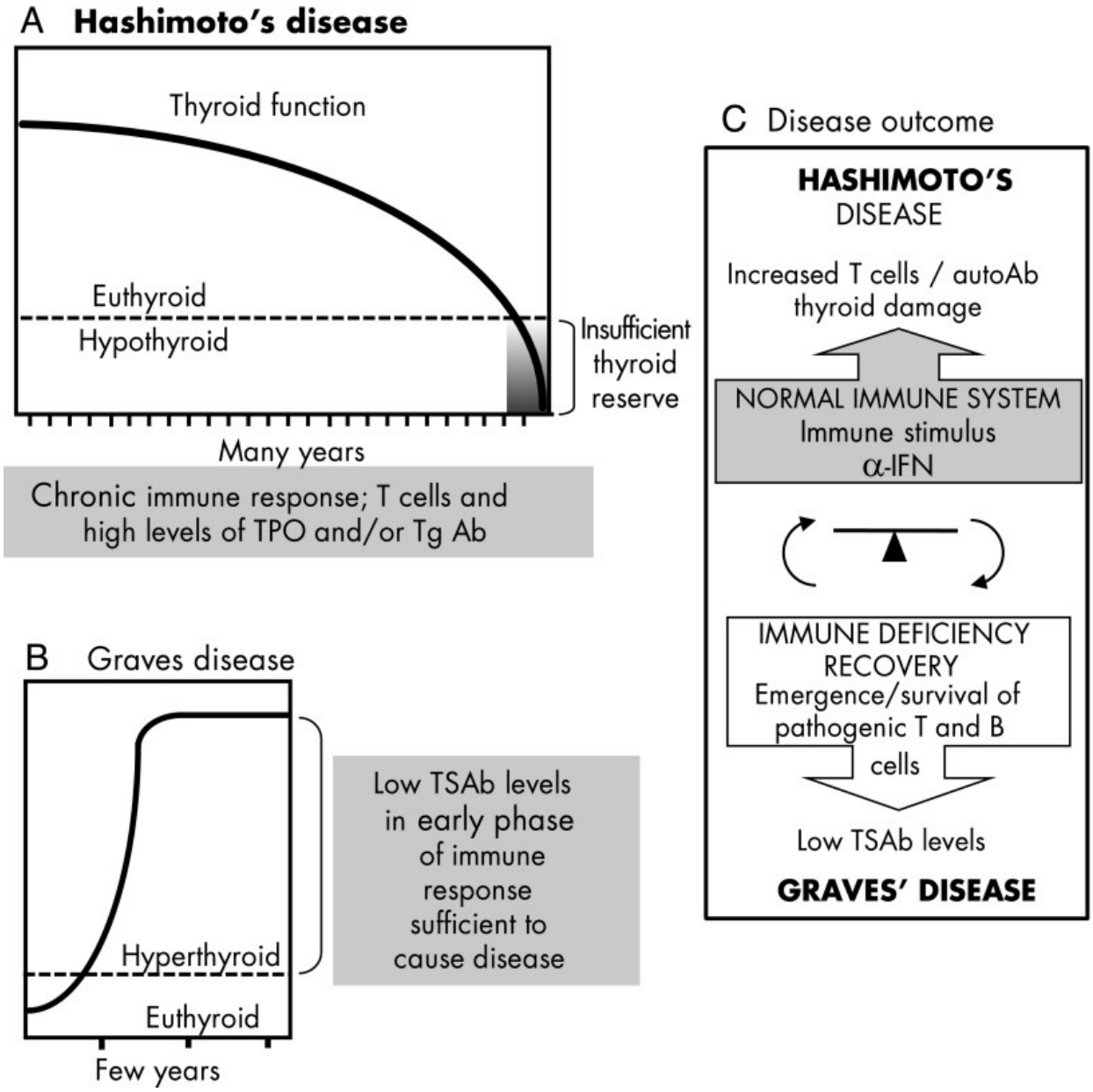
Selected T-cell epitopes on thyroid autoantigens. A, Predicted “TgPO” epitope shared by human Tg and human TPO (259) and demonstrated for recognition by MHC class I lymphocytes in HLA-A2-positive patients (262). B, In silico binding affinities to mouse MHC class II (IA-d) for human and mouse TSHR peptides (236).

Figure 10.



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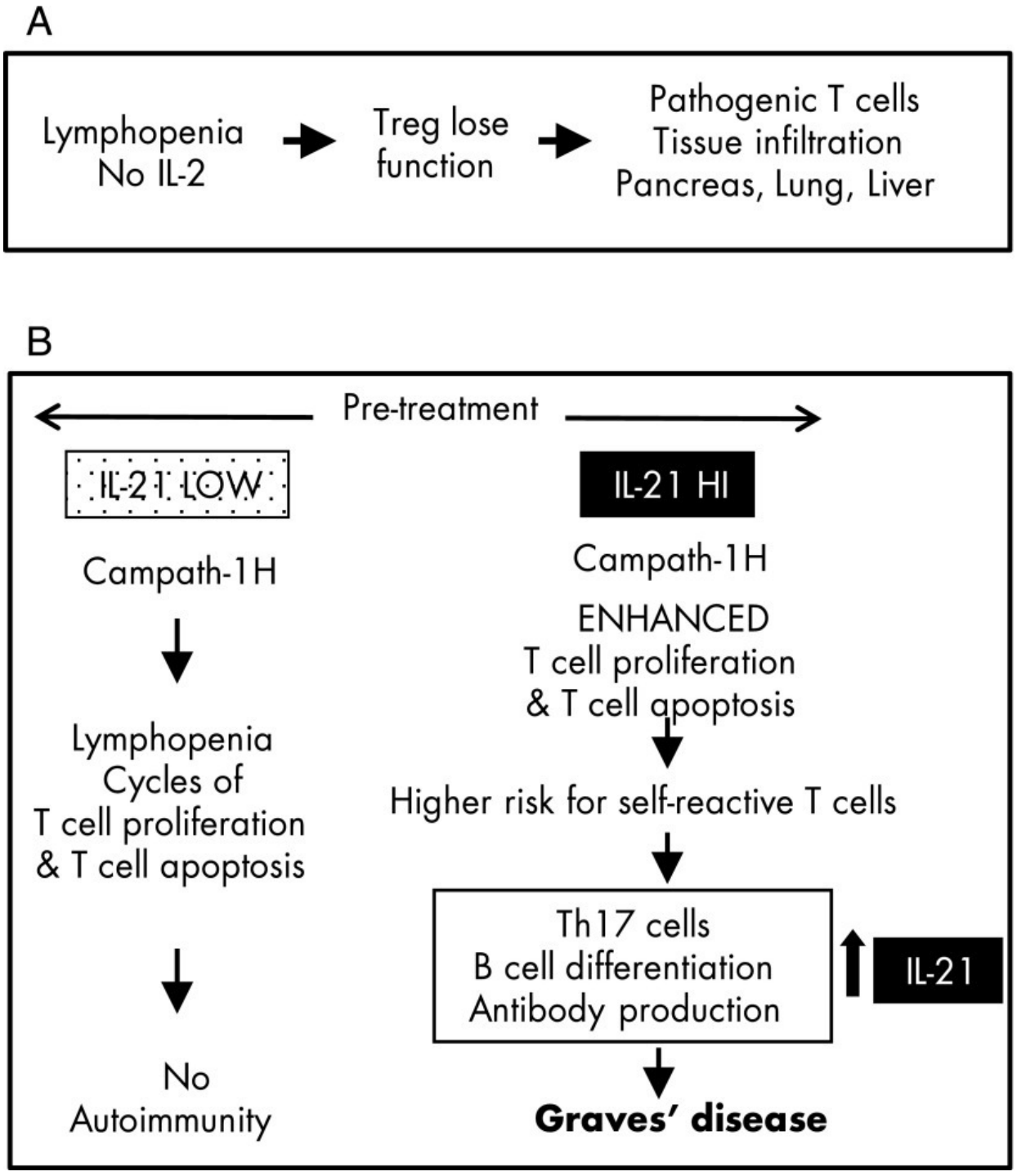
Slow development of Hashimoto's disease compared with the rapid onset of Graves' disease and outcome of immune intervention. A, In Hashimoto patients, immune responses to TPO and/or Tg are present for many years and reach high levels. TSH maintains thyroid reserve until overcome by the extent of thyroid damage. B, In Graves' disease, there is no compensatory mechanism to prevent hyperthyroidism (unlike for hypothyroidism in Hashimoto's disease). TSAbs are potent antibodies and can cause hyperthyroidism at an early stage of the immune response. Consistent with the relatively acute onset of the disease, TSAb concentrations are far lower than those for TPO or Tg antibodies. C, Outcomes of immune intervention for other diseases. IFN-α for hepatitis C infection enhances responses in a “normal” immune system leading to increased reactivity to TPO and/or Tg (T cells or antibodies and subsequent thyroid damage). In contrast, under conditions of immune deficiency, pathogenic T cells emerge or survive, leading to TSHR antibodies that, even at low levels, stimulate the thyroid gland.

Figure 11.



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Mechanisms proposed to explain "reconstitution autoimmunity." A, Spontaneous differentiation of natural Treg into pathogenic helper T cells under conditions of lymphopenia. After the loss of Treg function, pathogenic T cells infiltrate tissues (293). B, IL-21 drives secondary autoimmunity after therapeutic lymphocyte depletion. Recovery from lymphopenia is associated with cycles of T-cell proliferation and T-cell apoptosis. Autoimmunity does not arise if IL-21 concentrations are low. High IL-21 concentrations increase the risk of emerging self-reactive T cells. IL-21 acts on Th17 cells and enhances B-cell differentiation and antibody production (298). In genetically susceptible individuals, TSAbs develop and cause hyperthyroidism.

Table 5.

Protocols to Induce Resistance (Tolerance) to Subsequent Experimental Autoimmune Disease

	Treatment	Source	Strain	Immunization	Ref.
i. Injecting/increasing thyroid antigens					
Rats	Thyroid extract	Rat	BUF	3-MCA	315
Rats	Thyroid extract	Rat	PVG	Thymectomy; irradiation	316
Mice	Tg protein	Mouse	CBA	mTg + LPS	320
	Tg-TSH induced	Mouse	CBA	mTg + LPS	317, 318
Mice	Tg-protein	Mouse	CBA, SJL	mTg + CFA	319
		Mouse	CBA	Splenocyte transfer	319
Mice	TSHR A-subunit	Human	BALB/c	A-subunit adenovirus	322
ii. Oral tolerance					
Mice	Tg	Human	CBA	Human Tg + CFA	325, 326
Mice	Tg	Porcine	CBA	Adoptive transfer of mTg Restimulated splenocytes	324
Humans	Tg	Human		None	330
iii. Neonatal tolerance					
Mice	TSHR	Human	BALB/c	Human A-subunit Ad	332

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Abbreviations: mTg, mouse Tg; gpTg, guinea pig Tg; Ad, adenovirus; BUF, Buffalo. Included are data for one study in humans with ongoing autoimmune thyroid disease (330).