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A Unique Mouse Strain That Develops Spontaneous, Iodine-Accelerated, Pathogenic Antibodies to the Human Thyrotropin Receptor

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Abs that stimulate the thyrotropin receptor (TSHR), the cause of Graves’ hyperthyroidism, only develop in humans. TSHR Abs can be induced in mice by immunization, but studying pathogenesis and therapeutic intervention requires a model without immunization. Spontaneous, iodine-accelerated, thyroid autoimmunity develops in NOD.H2h4 mice associated with thyroglobulin and thyroid-peroxidase, but not TSHR, Abs. We hypothesized that transferring the human TSHR A-subunit to NOD.H2h4 mice would result in loss of tolerance to this protein. BALB/c human TSHR A-subunit mice were bred to NOD. H2h4 offspring were repeatedly backcrossed to NOD. H2h4. However, only TSHR-transgenic NOD.H2h4 mice (TSHR/NOD.H2h4) developed pathogenic TSHR Abs as detected using clinical Graves’ disease assays. As in humans, TSHR/NOD.H2h4 female mice were more prone than male mice to developing pathogenic TSHR Abs. Fortunately, in view of the confounding effect of excess thyroid hormone on immune responses, spontaneously arising pathogenic human TSHR Abs cross-react poorly with the mouse TSHR and do not cause thyrotoxicosis. In summary, the TSHR/NOD.H2h4 mouse strain develops spontaneous, iodine-accelerated, pathogenic TSHR Abs in female mice, providing a unique model to investigate disease pathogenesis and test novel TSHR Ag-specific immunotherapies aimed at curing Graves’ disease in humans.

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G raves’ disease is the prototypic autoimmune disease in which the humoral arm of the immune system directly causes organ overactivity (reviewed in Ref. 1). The phenotypic expression of hyperthyroidism results from the stimulatory effect of a single type of autoantibody on a single autoantigen, the thyrotropin (TSH) receptor (TSHR). Graves’ disease is one of the most common autoimmune diseases, affecting ~1% of the population in their lifetimes, with a very strong predilection toward females (3–7:1 female/male ratio in different countries) (2). There is no cure for the disease. Hyperthyroidism can be treated, either by inhibiting thyroid hormone synthesis with thiouamide drugs or by radio-iodine or surgical thyroid ablation, all with the attendant risks of negative side effects or, even more commonly, permanent hypothyroidism requiring lifelong thyroid hormone ingestion.

Immune intervention to cure Graves’ disease by inducing immune tolerance to the TSHR has been a long-standing goal, but it is very difficult to approach experimentally. An important barrier to studying the pathogenesis of Graves’ disease, as well as investigating novel therapies, is that this disease only occurs in humans. Not even the closely related great apes (chimpanzees, gorillas, and orangutans) experience Graves’ disease (3). For 40 y, immunization of different animal species with thyroid extracts, and later with recombinant TSHR protein together with adjuvant, did generate Abs, but none had the conformational specificity capable of activating the TSHR. In 1996, a breakthrough occurred with the demonstration that in vivo expression of the TSHR was necessary to induce thyroid-stimulating Abs (TSAb) in mice, with resultant hyperthyroidism (4). Subsequently, different vectors and immunization approaches have been used to express TSHR in vivo leading to TSAb induction and hyperthyroidism, for example, in some mouse strains (5–9), hamsters (10) and rhesus monkeys (11).

All the foregoing approaches involving in vivo TSHR expression in animals are of limited use in studying approaches to induce tolerance to the TSHR, a necessary and essential requirement for eliminating TSAb and consequent hyperthyroidism without suppressing or ablating normal thyroid function. A suitable animal model to study potential immunotherapeutic strategies requires TSAb to arise spontaneously and stably to self (syngeneic)-Ag. In contrast, the majority of previous induced animal models have used xenogeneic (human) TSHR with a transient TSAb response. An important barrier to the goal of using immunotherapy to induce tolerance to the...
Mice Spontaneously Secreting Thyrotropin Receptor Abs

TSHR, and thereby reverse the development of TSAb to cure, not treat, Graves’ disease in humans.

Materials and Methods

Generating NOD.H-2<sup>b4</sup> mice expressing the human TSHR A-subunit

NOD.H<sup>b4</sup> mice (The Jackson Laboratory, Bar Harbor, ME) and transgenic BALB/c mice expressing low intrathyroidal levels of the human TSHR A-subunit (line 51.9; subsequently referred to as TSHR-Tgic) (12) were bred at Cedars-Sinai Medical Center. Male TSHR-Tgics were crossed to female NOD.H<sup>b4</sup> females to generate N1 progeny. Expression of the transgene was determined by PCR (13). Tranogenic male N1 pups were bred to wild-type NOD.H<sup>b4</sup> females to generate N2 mice, and the same procedure was repeated to produce the N3 and N4 generations. At this stage, to introduce the NOD.H<sup>b4</sup> Y chromosome, we crossed wild-type NOD.H<sup>b4</sup> males to female N4 Tgic-NOD.H<sup>b4</sup> mice. Thereafter, we reverted to crossing Tgic-NOD.H<sup>b4</sup> male offspring with wild-type NOD.H<sup>b4</sup> females. Genome scanning (The Jackson Laboratory) was performed on tail DNA from the N2, N3, N5, and N6 generations to select males with the highest proportion of NOD.H<sup>b4</sup> genes to breed the next generation. N7 mice were bred from two N6 males with 99.3% or 99.5% NOD.H<sup>b4</sup> genes (Supplemental Fig. 1).

Data are reported for parental strains and offspring from N1 to N8 backcrosses. Unless indicated otherwise (and excluding all breeding mice), from wk 8 of age was supplemented with 0.05% sodium iodide (NaI) for 16 wk at which time (age 24 wk) TSHR-Tgic and non-Tgic offspring (N1-N8) as well as parental strains were euthanized to harvest blood for 16 wk at which time (age 24 wk) TSHR-Tgic and non-Tgic offspring (N1-N8) as well as parental strains were euthanized to harvest blood and thyroid tissue. Where indicated, additional NOD.H<sup>b4</sup> mice were maintained on regular or NaI water for up to 32 wk. All mouse studies were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee at Cedars-Sinai Medical Center. TSHR/NOD.H<sup>b4</sup> mice have been墙面 by the Mutant Mouse Resource Center under the designation NOD.Cg-Tg(TSHR)51.9Smcl. MMRRC:037586.

TSHR Ab assays

TSHR Abs were measured using three assays: ELISA, TSH binding inhibition (TBI) assay, and TSAb bioassay.

ELISA. The assay for TSHR Abs (IgG class) has been reported previously (7). Recombinant TSHR A-subunit protein secreted by CHO cells with an amplified transgenome (14) was purified from culture supernatants by affinity chromatography (15). ELISA wells were coated with A-subunit protein (5 μg/ml) and incubated with test sera (1:100 dilution; duplicate aliquots). The positive control used in this assay was serum from BALB/c mice immunized with TSHR A-subunit adenovirus. Ab binding was detected with HRP-conjugated mouse anti-IgG (A3673; Sigma Chemical, St. Louis, MO), and the signal developed with o-phenylenediamine and H<sub>2</sub>O<sub>2</sub>. Data are reported as the percentage inhibition of [<sup>125</sup>I]-TSH binding to the TSH holoreceptor.

TBI assay. TBI levels were measured in 25 μl mouse serum using a clinical assay kit (Kronus, Star, ID). The data are reported as the percentage inhibition of [<sup>125</sup>I]-TSH binding to the TSH holoreceptor.

TSAb bioassay. An in-house bioassy was used to measure cAMP generation by CHO cells expressing the human TSHR (7, 16). To permit testing double the volume of serum normally used in immunized mice (10 versus 5 μl), we modified the assay as follows: 25 μl test mouse serum + 75 μl normal human serum was precipitated with 300 μl 20% polyethylene glycol 4000 (Sigma-Aldrich, St. Louis, MO) in water and resuspended in 240 μl Ham’s F12 medium supplemented with 10 nM Hepes, pH 7.4, 1 mM isobutylmethylxanthine and 0.3% BSA. Duplicate aliquots (110 μl) were incubated with the LANCE cAMP kit (Perkin Elmer, Boston MA). TSAb activity was expressed as a percentage of cAMP values attained with polyethylene glycol 4000 precipitated IgG from wild-type BALB/c mice. As a positive control, IgG from normal BALB/c mice was supplemented with monoclonal TSHR M22 (17).

Autoantibodies to thyroglobulin and thyroid peroxidase

Thyroglobulin (Tg) was isolated from murine thyroid glands as previously described (18). ELISA wells (Immulon 4HBX; Thermo Scientific, Rochester NY) were coated with mouse Tg (1.5 μg/ml) and incubated with test sera (duplicate aliquots, 1:100 dilution). Ab binding was detected with horseradish peroxidase-conjugated goat anti-mouse IgG (A3673; Sigma Chemical), the signal developed with o-phenylenediamine, and the reaction stopped using 20% H<sub>2</sub>SO<sub>4</sub>. The negative control serum was from 8-wk-old NOD.H<sup>b4</sup> mice on regular water; the positive control serum was from BALB/c mice immunized with mouse Tg and CFA (19). Thyroglobulin Ab (TgAb) data are presented as the OD at 490 nm.

Thyroid peroxidase (TPO) Abs (TPOAbs) were measured using CHO cells stably expressing mouse-TPO (18). Sera (1:50 dilution) were incubated with mouse TPO-CHO cells, and binding was detected with FITC-conjugated affinity-purified goat anti-mouse IgG (M30101; Invitrogen, Carlsbad, CA). Cells staining with propidium iodide (1 μg/ml) were excluded from analysis. The negative control for IgG class Ab binding to mouse TPO-CHO cells was serum from 8-wk-old NOD.H<sup>b4</sup> mice. Positive controls were mouse IgGs 15 and 64 to human TPO (20), provided to us by Dr. J. Rau (Marseille, France), that recognize mouse TPO (18, 20). Flow cytometry was performed (10,000 events) using a FACScan with CELLQUEST Software (Becton Dickinson, San Jose, CA). Data are reported as the geometric mean.

Serum thyroxine, TSH, and thyroid histology

Serum total thyroxine (T4) was measured in 25 μl mouse serum by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). T<sub>4</sub> values were computed from kit standards and expressed as micrograms per deciliter (μg/dl). TSH was measured by radioimmunoassay (21) (Dr. S. Refetoff, University of Chicago; fee for service). Thyroid glands were preserved in zinc fixative (BD Pharmingen, San Diego CA), paraffin-embedded, and serial sections stained with H&E (IDEXX BioResearch Lab Animal and Biological Materials Diagnostic Testing, Columbia, MO).

FIGURE 1. Concepts underlying the hypothesis that expressing the human TSHR A-subunit transgene in NOD.H<sup>b4</sup> mice would generate a strain that produces spontaneous, iodine-accelerated, TSHR Abs. Recipient NOD.H<sup>b4</sup> mice experience thyroiditis associated with TgAb and TPOAb (18, 22–24), but not to the TSHR. The donor BALB/c mice do not develop thyroid autoimmunity, and the human TSHR A-subunit targeted to the thyroid gland is a self-Ag (13). However, regulatory T cell depletion before human TSHR A-subunit adenovirus immunization breaks tolerance to endogenous mouse thyroid Ags Tg and TPO (12).
Statistics

Significant differences between responses in different groups were determined by Mann–Whitney rank sum test or, when normally distributed, by Student t test. Multiple comparisons were made using ANOVA. Tests were performed using SigmaStat (Jandel Scientific Software, San Rafael, CA).

Results

The concept of developing a mouse that fulfills the criteria described earlier arose from a number of factors (Fig. 1). First, unlike the conventional NOD mouse that develops diabetes, the NOD.H2bd strain develops spontaneous, iodine-accelerated, autoimmune thyroiditis in association with autoantibodies to Tg (22–24) and, at a later stage, to TPO (18), but not to the TSHR. Second, we previously generated transgenic BALB/c mice with the human TSHR A-subunit selectively expressed in the thyroid gland, a self-Ag as evident by tolerance to human TSHR A-subunit immunization (13). This component of the TSH holoreceptor is the antigenic target of TgAbs that causes Graves’ disease (7, 14, 25). Regulatory T cell depletion with anti-CD25 before TSHR A-subunit adenovirus immunization led to massive thyroiditis associated with Ab spreading from the TSHR to the other two thyroid autoantigens, Tg and TPO (12). We therefore hypothesized that transferring the TSHR A-subunit transgene locus from the BALB/c transgenics to the NOD.H2bd strain that spontaneously develops thyroid autoimmunity would lead to the spontaneous generation of pathogenic TSHR autoantibodies.

TSHR Abs develop in TSHR transgenic (not wild-type) NOD.H2bd offspring

TSHR Abs detected by ELISA were present in some transgenic offspring derived by crossing human TSHR A-subunit–expressing BALB/c mice to the NOD.H2bd strain and repeated backcrossing to NOD.H2bd mice (N1-N8 generations; Fig. 2A). Neither wild-type offspring lacking the TSHR transgene nor the parental NOD.H2bd mice developed TSHR Abs. In contrast, TgAbs were detectable in some parental NOD.H2bd mice and N1-N8 backcross offspring regardless of whether they contained the TSHR A-subunit transgene (Fig. 2B). TPOAbs also develop in some N1-N8 backcross offspring independent of the A-subunit transgene (Supplemental Fig. 2).

Consistent with the standard protocol for NOD.H2bd mice, from 8 wk of age mice were provided with NaI-supplemented water for 16 wk. However, we maintained a smaller number of animals on regular water. As for the Nal group, only transgenic backcross offspring, not the parent NOD.H2bd strain or the nontransgenic offspring, developed TSHR Abs detectable by ELISA (Supplemental Fig. 3). TSHR Abs were undetectable in the N1+N2 generation on regular water but were clearly present in the same generation of mice on NaI water (Supplemental Fig. 3 versus Fig. 2A). These data confirm previous observations for Tg autoantibodies that dietary iodide enhances (or accelerates) but is not responsible for the development of thyroid autoimmunity in NOD.H2bd mice (18, 23). In particular, TgAbs are detectable much earlier than TPOAbs in NOD.H2bd mice maintained on NaI (18). Because NaI accelerates thyroid autoimmunity, backcrossed mice after the N6 generation were maintained on NaI-supplemented water.

High TBI in male NOD.H2bd mice associated with elevated TSH

Although measuring TSHR Abs by ELISA is a convenient initial screening method to determine whether tolerance to the receptor is maintained or broken, this assay only detects nonfunctional TSHR Abs. Pathogenic, functional TSHR Abs do not recognize adherent, purified TSHR Ag on the ELISA plate (26). The latter autoantibodies can only be detected by competition for TSH binding to the native holoreceptor or by activating the receptor expressed on the surface of intact cells, thereby generating cAMP.

In preliminary studies, sera from male and female parental NOD.H2bd mice without the A-subunit transgene were tested for TBI Abs. Unexpectedly, high TBI values were detected in male, but not in female, mice (Fig. 3A). Greater TSH levels in males than in females is a recognized phenomenon in a number of mouse strains with normal thyroid function and without thyroid autoimmunity (21, 22). Because the high TBI levels observed in
Among the female mice, none of the parental NOD.H2h4 TSHR autoantibodies detected by the TBI assay限定了Graves’ disease in humans。Although reducing the number of mice available for study, this limitation was not a handicap in view of the far greater female background values in male mice severely limited interpretation of the data, after the N6 generation we focused primarily on females. A similar TSH-dependent high background was also observed in the bioassay for TSAbs (data not shown).

The foregoing observations indicated the need to study male and female mice separately when testing sera for pathogenic TSHR autoantibodies. In addition, because the high TBI and TSAb background values in male mice severely limited interpretation of the data, after the N6 generation we focused primarily on females. Although reducing the number of mice available for study, this limitation was not a handicap in view of the far greater female incidence of Graves’ disease in humans.

TSHR autoantibodies detected by the TBI assay

Among the female mice, none of the parental NOD.H2h4 or nontransgenic backcrossed offspring in the N1+2 to N7+8 generations had elevated TBI autoantibodies (Fig. 4A). In contrast, TBI autoantibodies were detected in some female mice expressing the TSHR A-subunit transgene from the N1+2 to the N7+8 backcrosses (Fig. 4A). In these female mice, the proportion of TBI positivity increased from 2 of 13 in the N1+N2 generation to 5 of 10 in the N7+N8 generation.

Turning to the male mice, because the high background levels in the TBI assay limited interpretation of the data, we studied fewer male mice in later generations. With this proviso, none of the male TSHR/NOD.H2h4 transgenic mice had TBI values greater than those observed in their nontransgenic littermates (Fig. 4B). Moreover, none of the male TSHR/NOD.H2h4 transgenics attained TBI values increasing above the high background to approach values observed in some of the female transgenics (Fig. 4A). These data suggest that the human TSHR A-subunit locus is more conducive to the spontaneous development of pathogenic TSHR autoantibodies in females than in male TSHR/NOD.H2h4 transgenic mice.

TSHR autoantibodies detected in the TSAb bioassay

Additional evidence for pathogenic TSHR Ab levels arising spontaneously in NOD.H2h4 mice transgenic for the human TSHR A-subunit came from functional bioassays for TSAbs. As mentioned earlier, because of the confounding influence of high TSH levels even in nontransgenic male NOD.H2h4 mice, we tested sera from female TSHR/NOD.H2h4 transgenic mice for their ability to stimulate cAMP generation in monolayers of CHO cells expressing the TSH holoreceptor. TSAb was clearly increased in some (4/9) female transgenic offspring in the N7+N8 generation versus 0/13 in the nontransgenic offspring (Fig. 5). TSAb values correlated with TBI levels in the transgenic NOD.H2h4 N7+N8 offspring ($r = 0.865$, $p = 0.003$).

Serum T4 levels in NOD.H2h4 mice with the human TSHR A-subunit transgene

Spontaneously arising autoantibodies to the human TSHR transgene had little, if any, effect on the murine TSHR. Three female NOD.H2h4 mice transgenic for the TSHR A-subunit in the N5-N8 generations had serum T4 levels greater than the normal range established in nontransgenic NOD.H2h4 littersmates (Fig. 6A). However, for a number of reasons, a diagnosis of hyperthyroidism cannot be clearly established. First, an elevated serum T4 of similar magnitude was also observed in one female non-
transgenic littermate. Second, although the two females with the highest T4 levels were positive for TSAb, the former levels are disproportionately low relative to those (12–20 μg/dl) attained in BALB/c females with similar TSAb levels after TSHR A-subunit adenovirus immunization (e.g., 6, 7, 27, 28). Finally, on histological examination, the mouse thyroid follicular cells were not hypertrophic (cuboidal or columnar) as typically observed in hyperthyroid mice in the induced model of Graves’ disease. The extent of thyroid lymphocytic infiltration did not differ between the NOD.H2b4 males with and without the A-subunit transgene (up to 35% of total thyroid volume; representative histology shown in Fig. 7) and was insufficient to severely decrease thyroid reserve.

Consistent with serum T4 sex differences observed in other mouse strains (21, 29), the normal range for this parameter in nontransgenic NOD.H2b4 male mice was higher than in female mice. On this basis, none of the NOD.H2b4 males with the TSHR A-subunit transgene had increased serum T4 levels (Fig. 6B). The goal of immunotherapy using this novel strain (that does not develop hyperthyroidism) is shown schematically in Fig. 8.

Discussion

We report the generation of a strain of mice that develop spontaneous, iodine-accelerated, pathogenic TSHR autoantibodies. This TSHR/NOD.H2b4 strain was generated by transferring the human TSHR A-subunit transgene locus from BALB/c mice (12, 13) to NOD.H2b4 mice. In the former BALB/c strain, the human TSHR A-subunit is self, whereas NOD.H2b4 mice are genetically predisposed to spontaneous development of autoimmune thyroiditis in association with autoantibodies to the thyroid-specific Ags Tg and TPO (18, 22–24). However, autoantibodies to the mouse TSHR do not arise spontaneously in NOD.H2b4 mice, which remain euthyroid despite moderate lymphocytic infiltration. The reason for immunological tolerance (at least at the humoral level) to the TSHR in NOD.H2b4 mice is unknown but may relate to the very low level of TSHR expression on thyrocytes. In contrast, Tg, the primary autoantigen in the development of thyroiditis in these animals, is by far the dominant protein generated and secreted by the thyroid. Our hypothesis, validated in this report, was that a higher level of TSHR expression in the thyroid, particularly in a secreted form available for presentation to the immune system, would break tolerance to this self-Ag in a strain, namely, NOD.H2b4 mice, that spontaneously develop thyroid autoimmunity.

The TSHR differs from the closely related luteinizing hormone and follicle-stimulating hormone receptors in undergoing intramolecular proteolytic cleavage leading to disulphide bonded A- and B-subunits. Some of the TSHR autoantibody binding A-subunits are subsequently shed from the cell surface (30, 31), and there is strong evidence that shed A-subunits contribute to the induction and affinity maturation of functional TSHR Abs (7, 32, 33). In studies on the recombinant TSHR, the isolated A-subunit lacking the transmembrane component of the receptor is not retained at the cell surface but is secreted into the extracellular milieu (14). Because the secreted, recombinant TSHR A-subunit is essentially the same as the A-subunit shed from the holoreceptor, we previously generated two lines of transgenic BALB/c mice with the human TSHR A-subunit targeted to the thyroid, one a being a high expressor and the other a low expressor (12, 13). In these mice, the human TSHR A-subunit is a self-Ag. Consequently, tolerance to the human TSHR is difficult to break in the high-expressor transgenics but can be broken by adenovirus immunization in the low expressors (12, 13). Therefore, to optimize the possibility of a spontaneous loss of tolerance to the A-subunit transgene, we...
used the low-expressor transgenic as the parent strain to backcross the A-subunit locus onto an NOD.H2<sup>h4</sup> background.

In NOD.H2<sup>h4</sup> mice expressing the human TSHR A-subunit transgene, autoantibodies to this Ag measured by ELISA arose spontaneously in both males and females. Such Abs lack bioactivity (26). Only TSHR Abs measured in the TBI and TSAb assays are of pathogenic significance and clinically relevant. However, unlike the ELISA, the TBI and TSAb assays also detect TSH. As reported in some other mouse strains (21, 29), TSH levels in male NOD.H2<sup>h4</sup> mice were much higher than in females, thereby introducing spuriously high background levels that limit interpretation of the TBI and TSAb data in male mice. For this reason, in later backcrosses, we focused on female TSHR/NOD.H2<sup>h4</sup> mice. It is, therefore, fortunate for the future study of these mice that Graves’ disease, like many autoimmune conditions, primarily affects women (2). Indeed, although the confounding influence of endogenous TSH precludes making firm conclusions, our data suggest a bias toward pathogenic TSHR autoantibody generation in female TSHR/NOD.H2<sup>h4</sup> mice (Fig. 4). By the N7/N8 backcross, 40–50% of female TSHR/NOD.H2<sup>h4</sup> mice developed TBI and TSAb, a sufficient proportion for future investigations described later.

The variability in developing pathogenic TSHR Abs by N7/N8 backcross mice is not likely to be due to extensive heterogeneity in their genetic background for two reasons: 1) genome-wide screens of the N6 males selected to generate N7 mice revealed 99.3 and 99.5% NOD.H2<sup>h4</sup> genes, implying 1% BALB/c genes; and 2) virtually all N7+N8 mice produced TgAb in comparable amounts

FIGURE 7. Thyroiditis in transgenic and nontransgenic NOD.H2<sup>h4</sup> mice. Typical examples of lymphocytic infiltration, varying from minimal to moderate, are shown on thyroid histology (H&E, original magnification ×10) from N5 and N6 mice (three transgenic and one nontransgenic). Arrows (white) indicate lymphocytic infiltrates.

FIGURE 8. The goal of immunotherapy using TSHR/NOD.H2<sup>h4</sup> mice is the restoration of tolerance to the TSHR. Methods used to induce hyperthyroidism in mice, unsuitable for inducing self-tolerance to the TSHR, include TSH injection (e.g., 48), TSAb mAb injection (45); hamster TSAb mAb hybridoma injection (44); TSHR immunization approaches to express TSHR in mice (5–9), hamsters (10), and rhesus monkeys (11); and expressing TSAb (B6B7) in a transgenic mouse (42, 43). Confounding effects of hyperthyroidism on the immune system include altering the phenotype and function of Ag-presenting dendritic cells (46) and polarizing dendritic cells leading to impaired function of regulatory T cells, a major change that may influence the emergence of pathogenic autoantibodies (47).
with the parent NOD.H2h4 strain. More likely, the variability is an inherited (albeit unexplained) characteristic of the NOD strain used to generate NOD.H2h4 mice; NOD mice are well-known to exhibit variability in diabetes and breeding (e.g., 34), and variable degrees of spontaneous thyroiditis develop in another NOD-derived strain, NOD.H2H (35).

An important, and initially puzzling, feature of the TSHR/ NOD.H2h4 mice was the minimal degree of hyperthyroidism despite the presence of TSAb activity in their sera. Serum T4 levels greater than the normal range were observed in three female TSHR/NOD.H2h4 mice, but increased values also occurred in some nontransgenic NOD.H2h4 female littermates. Lympohytic thyroiditis was not extensive and was similar in NOD.H2h4 mice with and without the A-subunit transgene. Coexisting thyroiditis could, therefore, not explain this lack of hyperthyroidism. Rather, there is strong evidence for two other factors that limit the thyroid response to pathogenic TSHR autoantibodies. First, the transgenic human A-subunit, lacking the transmembrane component of the receptor, cannot respond functionally to the TSHR autoantibodies that it induces. Only activation of the endogenous mouse TSH holoreceptor can cause hyperthyroidism. Second, different mouse strains do not respond equally well to autoantibodies induced by human TSHR adenovirus immunization, used in most models. For example, despite similar TSAb activities in serum, severe hyperthyroidism occurs readily in BALB/c mice, whereas C57BL/6 mice largely remain euthyroid (6, 28, 36). The parental NOD.H2h4 mouse strain used in this study is similar to C57BL/6 mice. In a previous study, only a small proportion of mice of the former strain became hyperthyroid, with modest serum T4 elevations that were disproportionately low relative to the high TSHR autoantibody values (37). Therefore, TSAb to the human TSHR in NOD.H2h4 mice, whether induced (37) or arising spontaneously with iodine-acceleration (as in this model), appear to cross-react minimally with the mouse TSHR.

Genome-wide array studies in recombinant inbred mice derived from BALB/c and C57BL/6 mice (strongly and weakly responsive to induced TSHR Abs, respectively) have revealed linkage of this phenotype to the IgH V region gene locus (16). These genes are, in turn, linked to H chain C region polymorphisms (or allootypes) (e.g., 38–40). It may be relevant that C57BL/6 and NOD.H2h4 mice (both low responders to TSAb generated to the human TSHR) have the IgG2a IgH-C allotype [b], whereas BALB/c mice (strong responders to human TSHR-specific TSAb) bear the [a] allotype (41).

In addition to TSHR Abs and hyperthyroidism induced by immunization (see Introduction), a transgenic mouse model of Graves’ disease has been reported with the H and L chain variable regions of a human IgM mAb with weak TSAb activity (B6B7) (42, 43). These TSAb are generated by the inserted IgM transgenes and do not arise spontaneously in response to a self-Ag. Moreover, the IgM TSAb transgenic mouse model requires LPS administration to expand the transgenic B cell population. Other animal models have involved the i.p. injection of hybridoma cells secreting a potent hamster monoclonal TSAb (44) or injection of purified, extremely potent mouse monoclonal TSAb IgG (45). Valuable insights were obtained from all three studies into (for example) immune parameters and/or the pathogenic changes accompanying TSAb-induced hyperthyroidism. However, none of these approaches permits study of the spontaneous loss of tolerance to self-Ag leading to TSHR autoantibody production by normal B cells.

It may not be appreciated that the absence of overt hyperthyroidism in TSHR/NOD.H2h4 mice is not detrimental but, instead, is an advantage for studies for several reasons. TSAb are the proximate cause of Graves’ disease. Therefore, the critical goal in studying the pathogenesis of, or potential therapy for, Graves’ disease using TSHR/NOD.H2h4 mice is not to focus on hyperthyroidism that develops secondary to TSAb, but on the modulation of the primary development of TSHR autoantibodies, preferably by restoring TSHR self-tolerance (Fig. 8). There are many animal models of hyperthyroidism and/or thyrotoxicosis, and numerous therapies can be used to reverse hyperthyroidism. However, it should be noted that thyrotoxicosis (excess thyroid hormone) alters the phenotype and function of Ag-presenting dendritic cells by increasing the expression of costimulatory molecules required to initiate Ab production (46). Thyrotoxicosis also polarizes dendritic cells leading to impaired function of regulatory T cells, a major change that may influence the emergence of pathogenic autoantibodies (47). Consequently, the critical goal in studying the pathogenesis or therapy of our new model is to focus on preventing the development of TSHR autoantibodies without the confounding influence of thyroid hormone fluctuations.

In conclusion, we have developed a unique model, the TSHR/ NOD.H2h4 strain, that develops spontaneous, iodine-accelerated, TSAb detectable by ELISA. High TSH levels interfere with assays for pathogenic Abs in males. However, in female TSHR/ NOD.H2h4 mice, pathogenic Abs can be detected in assays used clinically for human Graves’ disease that involve inhibition of TSH binding to its receptor, as well as activation of the TSHR. This mouse model, which has nontransgenic T and B cells, represents a significant advance for studies of Graves’ pathogenesis and will facilitate investigating potential approaches for TSHR Ag-specific immunotherapy to cure, rather than treat, Graves’ disease in humans.

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Disclosures
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