Animal Models of Graves' Hyperthyroidism

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Abstract. Graves' disease is a common organ-specific autoimmune disease characterized by overstimulation of the thyroid gland with agonistic anti-thyrotropin (TSH) receptor autoantibodies, which leads to hyperthyroidism and diffuse hyperplasia of the thyroid gland. Several groups including us have recently established several animal models of Graves' hyperthyroidism using novel immunization approaches, such as *in vivo* expression of the TSH receptor by injecting syngeneic living cells coexpressing the TSH receptor, the major histocompatibility complex (MHC) class II antigen and a costimulatory molecule, or genetic immunization using plasmid or adenovirus vectors coding the TSH receptor. This breakthrough has made it possible for us to study the pathogenesis of Graves' disease in more detail and has provided important insights into our understanding of disease pathogenesis. The important new findings that have emerged include: (i) the shed A subunit being the major autoantigen for TSAb, (ii) the significant role played by dendritic cells (DCs) as professional antigen-presenting cells in initiating disease development, (iii) contribution of MHC and particularly non-MHC genetic backgrounds in disease susceptibility, and (iv) influence of some particular infectious pathogens on disease development. However, the data regarding Th1/Th2 balance of TSH receptor-specific immune response or the association of Graves' hyperthyroidism with intrathyroidal lymphocytic infiltration are rather inconsistent. Future studies with these models will hopefully lead to better understanding of disease pathogenesis and help develop novel strategies for treatment and ultimately prevention of Graves' disease in humans.

Key words: Graves' disease, Thyrotropin receptor, Autoimmunity, Adenovirus

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AUTOIMMUNE thyroid diseases include two clinically opposite diseases: Graves' disease and chronic thyroiditis (also known as Hashimoto thyroiditis). Graves' disease is characterized by hyperthyroidism and hyperplasia of the thyroid gland, which is caused by agonistic autoantibodies against the thyrotropin (TSH) receptor, called thyroid stimulating antibodies (TSAb) [1]. Occasionally, in contrast, hypothyroidism and thyroid atrophy are induced by antagonistic anti-TSH receptor autoantibodies that inhibit TSH action, thereby called thyroid blocking antibodies (TBAb). Thus humoral autoimmune response, *i.e.*, T helper cell type 2 (Th2) autoimmune response, is likely to be involved in disease pathogenesis. On the other hand, Hashimoto thyroiditis involves cellular autoimmune response (Th1 autoimmune response) against thyroid specific autoantigens such as thyroglobulin and thyroid peroxidase [2], and/or activation of cytokine-regulated apoptotic pathways [3, 4], leading to thyroid destruction and hypothyroidism.

Although both spontaneous and inducible animal models of experimental autoimmune thyroiditis using thyroglobulin or thyroid peroxidase as autoantigens have long been available as models of Hashimoto thyroiditis in humans [5, 6], no animal model of Graves' hyperthyroidism existed until Shimojo *et al*. established the first inducible mouse model in 1996 [7]. Subsequently several other groups including us have generated different models using distinct approaches [8–12].

This breakthrough has made it possible for us to study the pathogenesis of Graves' disease in more detail. Here I review and discuss these animal models of Graves' hyperthyroidism and the recent advances in our understanding of disease pathogenesis.

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Models of Graves' hyperthyroidism

1. Comparison of different animal models

A comparison of the characterizations of different mouse models so far reported is summarized in Table 1. The first animal model of Graves' hyperthyroidism was reported by Shimojo *et al.* [7] and involved multiple injections with a fibroblast cell line (L cells) genetically engineered to stably co-express the TSH receptor and the major histocompatibility complex (MHC) class II antigen of syngeneic AKR/N mice (H-2k) and other H-2k-bearing mouse strains. Because these cells also express a costimulatory molecule B7-1 [13], they likely function as non-professional antigen-presenting cells. After repetitive immunizations, most of the mice developed anti-TSH receptor antibodies detected by TSH binding inhibiting antibody (TBIAb) assay, and approximately 20% of the mice developed TSAb and elevated serum thyroxine. Reproducibility of this model was confirmed by other groups [14–16]. Neither sex bias nor intrathyroidal lymphocytic infiltration was observed [7, 14]. This model is, however, not suitable for detailed *in vitro* studies with splenocytes or sera because of the non-specific adjuvant activity of this cell line; for example, injection of untransfected L cells itself induced a robust, antigen-nonspecific interferon-γ (IFN-γ) production from splenocytes [13]. Importantly, L cells expressing the TSH receptor alone (without MHC class II expression) failed to induce disease [7].

A similar approach was used for two other models. In the model reported by Davies *et al.* [8], outbred hamsters were repeatedly immunized with Chinese hamster ovary cells stably expressing the TSH receptor and conventional Th2 adjuvants (alum and pertussis

toxin). These cells also express endogenous MHC class II (expression of co-stimulatory molecules is unknown). Approximately 30% mice developed hyperthyroidism with intrathyroidal lymphocytic infiltration. The Prabhakar's group [9] used BALB/c mice and a syngeneic B lymphoma cell line (M12) stably expressing the TSH receptor. These cells likely function as professional antigen-presenting cells, because they express endogenous MHC class II antigen and a costimulatory molecule B7-1. Surprisingly, disease reportedly developed vertically in all the mice immunized with intrathyroidal lymphocytic infiltration [9]. One possible reason for the greater efficacy of this model *versus* the Shimojo and hamster models is that it uses a susceptible BALB/c mouse strain [11, 17]. Of interest, they also reported that either injection of xenogeneic human embryonal kidney 293 cells stably expressing the extracellular domain of the TSH receptor alone or in combination with soluble TSH receptor extracellular domain protein and a Th2 adjuvant (cholera toxin B, CTB) or immunization with TSH receptor extracellular domain protein and CTB was equally effective at inducing disease [9]. However, importantly, thyroid histology in these murine models did not correspond to that in Graves' disease in humans ("*thinning* of the thyroid epithelium" in the former *versus* "*cuboidal* thyroid epithelium" in the latter). Another negative aspect of this model is the much longer time period (more than 6 months) required to induce Grave' disease, compared to 2–3 months in other models. Furthermore, most critically, we could not replicate their data (Nagayama, unpublished data).

The next model was DNA vaccination, *i.e.*, intramuscular injections of the eukaryotic expression vector coding for the TSH receptor, reported by the Vassart's group. In their original reports [10, 18], this approach

Table 1. Comparison of different mouse models of Graves' hyperthyroidism

Authors [refs.]	Immunogen	Susceptible mouse strains	Disease incidence (%)	Reproduction	Sex bias	Intrathyroidal lymphocyte infiltrations
Shimojo [7]	Fibroblasts expressing TSHR and MHC class II	AKR/N	21	Yes	No	No
Prabhakar [9]	B lymphoma cells expressing TSHR	BALB/c	100	No	?	Yes
Vassart [10]	Plasmid coding TSHR	NMRI	16	No	Yes	Yes
Nagayama [11]	Adenovirus coding TSHR	BLAB/c BALB.K	55 (~70*)	Yes	No	No
Kita [12]	DCs expressing TSHR	BALB/c	37 (70*)	?	?	No

*, disease incidence in the optimized models (ref. 22 and our unpublished data).

was only effective for outbred NMRI mice with low disease induction rate (~15%), but not for inbred BALB/c mice. Of interest, thyroid histology showed some intrathyroidal lymphocytic infiltration in both NMRI and BALB/c mice. However, this model could not be replicated by other groups including us [11, 16, 19]. Later, it was shown that TSH receptor-DNA vaccination was more effective for HLA-DR3 transgenic mice with non-obese diabetes (NOD) background that had a disease incidence of ~30% [20].

Our group established the following two models [11, 12]. The first model involved repeated intramuscular injections of recombinant, replication-defective adenovirus vector coding the full-length TSH receptor (AdTSHR) [11]. This model is reproducible in different groups using mice or hamsters [21, 22] and highly efficient with virtually 100% anti-TSH receptor antibody induction and 50–60% disease incidence in BALB/c mice. Non-stimulating antibodies, but not TSAb, were induced at variable levels in other mouse strains examined (see below). Of interest, as determined with the chimeric TSH-lutropin receptors, TSAbs in sera from hyperthyroid mice recognized epitope(s) similar to those of TSAbs in human Graves' sera. Our second model used bone marrow-derived DCs transduced with AdTSHR. Disease incidence was ~35% [12]. DCs are well known to be the most potent professional antigen-presenting cells and are prerequisite for the initiation of primary immune responses [23], thereby indicating that this model may be highly relevant physiologically. These two models were later modified and optimized further by using adenovirus expressing the TSH receptor A subunit (AdTSHR289), coding approximately two thirds of the N-terminal receptor extracellular domain (289 amino acids) instead of the full-length receptor (22; Mizutori and Nagayama, unpublished data). Thus disease incidence is now 60–80% in BALB/c mice in both of the modified models. However, sex bias and intrathyroidal lymphocytic infiltration were not observed in either of these models.

Finally, a spontaneous mouse model of Graves' hyperthyroidism was also generated by transgenic expression of the variable region of a monoclonal anti-TSH receptor autoantibody (B6B7) isolated from Graves' peripheral blood lymphocytes [24], although TSAb activity of this monoclonal antibody was extremely weak. Approximately 70% mice developed hyperthyroidism, in which elevated thyroidal uptake of technetium pertechnetate was observed.

Taken together, each model has its own advantages and limitations. However, in my opinion, in terms of high reproducibility and disease incidence, and the applicability to any mouse strain, our models, particularly intramuscular injection of AdTSHR289, appear to be the most useful Graves' models at present, although these models lack sex bias and intrathyroidal lymphocytic infiltrates [11, 12], the common features in Graves' disease in humans.

2. Implications of animal models in disease pathogenesis

Some important implications in the pathogenesis of Graves' disease in humans were provided by generations of these animal models. Firstly, the Shimojo model supports the hypothesis proposed by Bottazzo *et al*. more than two decades ago [25] that has since been evaluated extensively by others [26]. In their hypothesis, thyroid cells in autoimmune thyroid diseases aberrantly express MHC class II antigen and, as non-professional antigen-presenting cells, present thyroid-specific autoantigens to naïve T cells, leading to thyroid autoimmune reaction. However, the lack of expression of costimulatory molecules B7 on thyrocytes [27] suggests induction of tolerance rather than immunity [28]. More recently, thyroid-specific MHC class II transgenic mice did not spontaneously develop autoimmune thyroid disease [29, 30]. Thus the cells likely involved in the initiation of thyroid autoimmunity are professional antigen-presenting cells, *i.e.*, DCs [31], as we have shown in our second model [12]. A recent study shows that the mannose receptor expressed on DCs may play a role in their capturing the minute amounts of TSH receptor protein shed from the thyroid [32].

Secondly, all but one [9] successful mouse model of Graves' hyperthyroidism exploited the *in vivo* expression of the TSH receptor. In contrast, an attempt to induce Graves' disease using conventional immunization method with conformationally intact full-length TSH receptor protein and complete Freund adjuvant (CFA) failed [33]. These discrepant data can possibly be best explained by the recent novel finding that TSAbs in sera from Graves' patients preferentially recognized the free A subunit rather than the two-subunit receptor or the full-length single polypeptide receptor, indicating that the free A subunit may be the better autoantigen than the other two forms of the receptor [34]. It is worthy noting here that the TSH receptor is cleaved

into two subunits, a TSH binding "A" subunit and a transmembrane and cytoplasmic "B" subunit, on the cell surface, from which the free A subunit is spontaneously released by shedding [1] (Fig. 1). In the successful mouse models mentioned above [7–12], the free A subunit very likely sheds from the full-length TSH receptor expressed *in vivo* and can be recognized as autoantigen to TSAb. In contrast, it is unlikely that the full-length receptor used for the conventional immunization [33] undergoes cleavage. This hypothesis was reinforced by the fact that our two models were optimized with AdTSHR289 as mentioned above.

3. Th1 versus Th2 immune balance in development of Graves' hyperthyroidism

It is well known that adaptive immunity can be differentiated into two distinct directions: IFN-γ-dominated Th1 and IL-4-dominated Th2 responses [35, 36] (Fig. 2). IL-12 promotes differentiation of naïve T cells to IFN-γ and IL-2-producing Th1 cells that mediate cell-mediated immunity. The main source of IL-12 is the DCs. In contrast, IL-4 skews the development of naïve T cells toward IL-4 producing Th2 cells that mediate antibody-mediated immunity. Antibodies can be produced in either conditions, but the former produces complement-fixing isotypes (IgG2a and IgG2b sub-

classes in mice) and the latter non-complement-fixing ones (IgE and IgG1 in mice).

Therefore, it has long been thought that Graves' disease is an autoantibody-mediated, Th2-dominant autoimmune disease. Features of atopy in Graves' disease [37] and induction of Graves' disease by treatment with anti-CD52 monoclonal antibody (a Th2-inducer) [38] are among the supporting evidence. However, studies on cytokine profiles in thyroid tissues and sera provided controversial data regarding Th1 to Th2 balance [39]. Furthermore, of importance, TSAbs in human sera are of IgG1 subclass (Th1 type in humans) [40].

Even in mouse models, the data regarding the Th1/Th2 balance of TSH receptor-specific immune response are also uncertain. In the Shimojo model, the induction of hyperthyroidism was enhanced by Th2 adjuvants (alum and pertussis toxin) and delayed by a Th1 adjuvant (CFA) [15]. However, splenocytes from mice immunized with untransfected L cells themselves spontaneously produced substantial amounts of a Th1 cytokine IFN-γ [13].

The Prabhakar model was characterized by mixed Th1 and Th2 immune responses, as demonstrated by IgG1 and IgG2a subclasses (Th2 and Th1, respectively, in mice) of anti-TSH receptor antibodies and TSH receptor-specific splenocyte production of both Th1
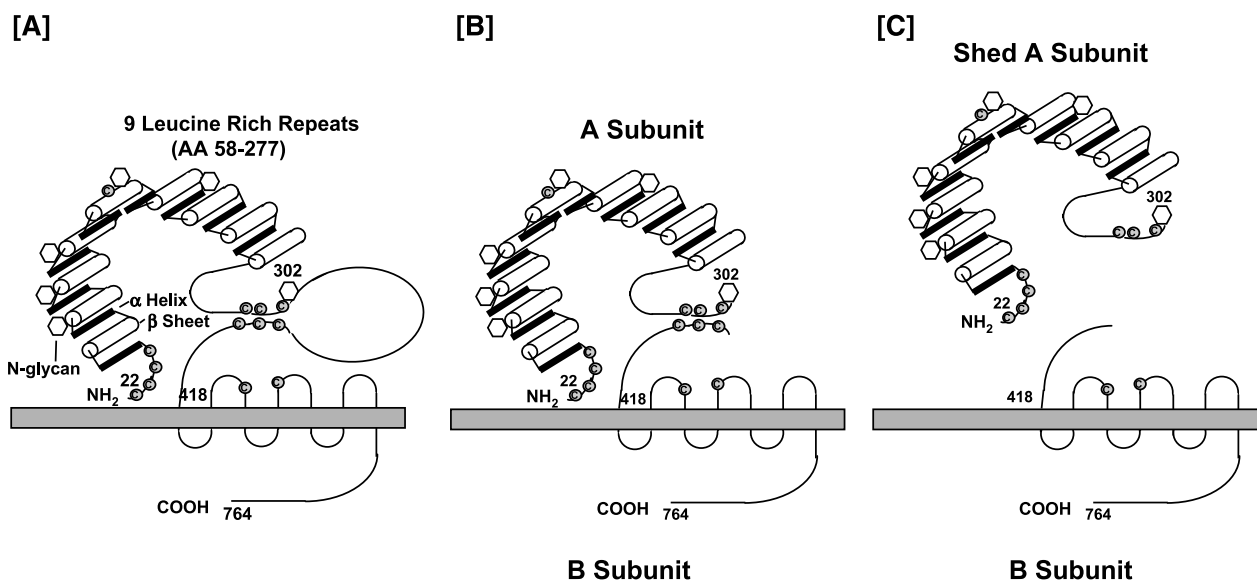

Fig. 1. Schematic representation of different structures of the TSH receptor. [A] The uncleaved, full-length holoreceptor consisting of the large extracellular domain with leucine-rich repeats flanked with two cysteine-rich regions and the cleavage domain (C-peptide), and the transmembrane/cytoplasmic region. [B] The receptor with two subunit-structure upon cleavage of C-peptide. [C] The shedding of the A subunit after disruption of the disulfide bonds or the further cleavage process [66].

(IFN-γ and Th2 (interleukin-4; IL-4) cytokines [41]. However, studies with knockout mice revealed that protection of IFN-γ or Stat-4 (a Th1 signaling molecule) deficient mice from Graves' hyperthyroidism, indicating the importance of Th2 [41, 42]. However, transient immune deviation toward either Th1 or Th2 by Flm-like tyrosine kinase 3 ligand (Flt3L) or granulocyte macrophage colony stimulating factor (GMCSF), respectively, had no effect on disease induction [41].

In the DNA vaccination model, intrathyroidal lymphocytic infiltrates consist of B cells and IL-4-producing T cells, the characteristics of Th2 immune response [10], whereas splenocytes produced the Th1 cytokines IFN-γ, TNF-α and IL-2, but not Th2 cytokines, when challenged with TSH receptor antigen [43], and the monoclonal antibody isolated is of IgG2a subclass (Th1) [18]. Furthermore, recent data show attenuation of disease induction by intradermal injection of TSH receptor-DNA and IL-4-DNA [44].

In the dendritic cell model, TSH receptor-specific immune response induced was mixed Th1 and Th2 responses, because of induction of IgG1 and IgG2a subclass antibodies [12]. Th2 adjuvants (alum and pertussis toxin) completely suppressed antibody pro-

duction and hyperthyroidism, but a Th1 adjuvant (polyinosinic-polycytidylic acid) had little effect.

In the adenovirus model, anti-TSH receptor immune response induced was also mixed Th1 and Th2 responses, as indicated by IgG1 and IgG2a subclass antibodies and TSH receptor-specific splenocyte secretions of IFN-γ and a Th2 cytokine IL-10 [45, 46]. However, Th2 immune deviation (increased IgG1 to IgG2a (Th2 to Th1) ratios and/or impaired secretion of IFN-γ from splenocytes) and suppression of development of Graves' hyperthyroidism by co-injection of adenovirus expressing IL-4 (AdIL4), prior infection of *Schistosoma mansoni,* and simultaneous administration of α-galactosylceramide [45, 46] indicate the crucial role played by TSH receptor-specific Th1 immune response in disease induction. Of interest, Th2 immune deviation selectively suppressed TSAb titers, not non-stimulating antibody titers determined by TBIAb and ELISA assay, suggesting suppression of clinically overt hyperthyroidism, but not TSH receptor autoimmunity by these Th2-inducers. Disappointingly, however, these suppressive effects of AdIL4, *Schistosoma mansoni* and α-galactosylceramide are preventive, but not therapeutic, *i.e.,* once anti-TSH receptor immune
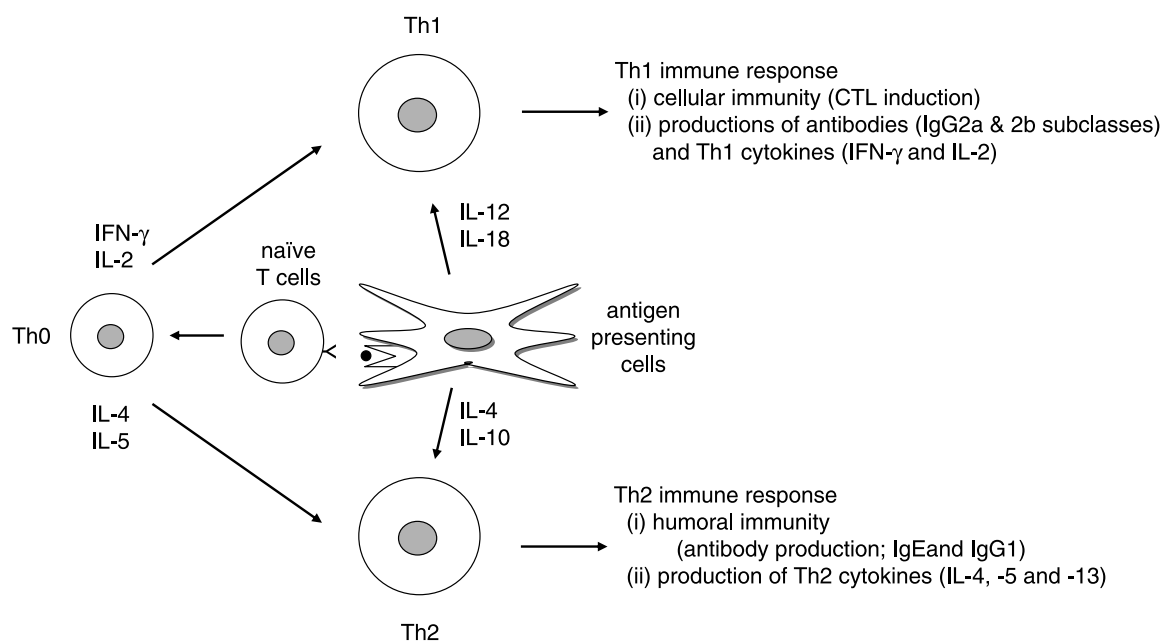

Fig. 2. Th1 and Th2 differentiation pathways. Upon stimulation by the T cell receptor-peptide complex on antigen-presenting cells, naïve T cells differentiate into Th1 or Th2 cells via Th0 cells. Two cytokines, IL-12 and IL-4, exert dominant influences on these differentiations. Th1 immune response promotes cellular immunity and productions of complement fixing antibodies and Th1 cytokines (IFN-γ and IL-2), whereas Th2 immune response enhances antibody-mediated immunity (non-complement fixing antibodies) and produces Th2 cytokines (IL-4 and -5).

response is fully induced, Th2-immune deviation has little or no effect on disease development [45, 46].

Without contradicting the above-mentioned data on the importance of Th1 response in AdTSHR-induced Graves' disease, adenovirus expressing IL-12 enhanced a Th1 immune response (enhancement of IFN-γ secretion from splenocytes) without affecting disease incidence [45]. The optimal Th1 immune response would already have been induced by AdTSHR infection. However, unexpectedly, prior infection of *Mycobacterium bovis* BCG biased anti-TSH receptor immune response to a Th1 phenotype (augmented IFN-γ secretion and impaired IL-10 secretion from splenocytes) and significantly protected mice from AdTSHR-induced hyperthyroidism [47]. Considering the effect of IL-12, this protective effect of *Mycobacterium bovis* BCG infection cannot solely be explained by Th1 immune deviation. The possible explanation of these unexpected data is discussed below.

Somewhat unexpectedly, BALB/c mice deficient in IFN-γ or IL-4 by gene disruption are both resistant to AdTSHR-induced hyperthyroidism [48]. These data support the importance of a Th1 cytokine IFN-γ but are apparently inconsistent with the data obtained with AdIL4 [45]. However, these contradictory data may be explained by impairment of both Th1 and Th2 immune responses in IL-4 null mice, as demonstrated by decreased IgG1 to IgG2a ratios and loss of TSH receptor-specific IFN-γ production from splenocytes.

Taken together, although most of the immunization protocols induced mixed Th1 and Th2 immune responses, polarization to either Th1 or Th2 led to variable consequences. In genetic immunization methods, Th2 immune deviation was associated with lower disease incidence [44–46], whereas Th1 immune bias suppressed disease induction in cell-mediated immunization protocols [7, 41, 42]. It is at present unclear which model mimics human Graves' disease more closely. It is indeed suggested that analysis of multiple animal models is necessary to gain insight into the pathogenesis of human autoimmune diseases.

4. Genetic factors in Graves' disease

The etiology of Graves' disease in humans is thought to be multifactorial, *i.e.*, both genetic and environmental factors appear to be involved in disease development [49]. In genetic factors, associations have been observed between Graves' disease and MHC genes (human leukocyte antigen (HLA) in humans) as well as non-MHC genes [50].

Even in mouse models, evidence suggests the importance of both MHC and non-MHC genes. Firstly, the following evidence supports the importance of non-MHC genes. In the Shimojo model, five different mouse strains, all with the same H-2k haplotype but with different non-MHC genetic backgrounds, produced variable levels of anti-TSH receptor antibodies [51]. DNA vaccination was effective in outbred NMRI, but not inbred BALB/c, mice [10, 18]. BALB/c (H-2d) and BALB/k (H-2k) mice are susceptible and DBA/2J (H-2d) and CBA/J (H-2k) mice were resistant to AdTSHR-induced hyperthyroidism [11, 17]. Furthermore, resistant strains can be divided into two subgroups, good and poor responders, in terms of non-stimulating anti-TSH receptor antibody production in the adenovirus model. The former includes C57BL/6, SJL/J and DBA/2J mice, and the latter CBA/J and DBA/1J mice [11, 17]. Thus, there are at least two different types of genetic factors: one group influencing production of thyroid stimulating antibodies, and the other non-stimulating antibody production. A recent study showed that F1 hybrids between BALB/c and C57BL/6 mice are susceptible to AdTSHR289-induced Graves' hyperthyroidism, indicating the dominant role played by susceptibility gene(s) rather than resistant gene(s) in disease development [52].

In contrast, there is also evidence supporting the importance of MHC genes. As mentioned above, HLA-DR3 transgenic NOD mice are more prone to develop AdTSHR-induced Graves' hyperthyroidism than non-transgenic NOD mice [20]. In another study, HLA-D3 transgenic mice on C57BL10 backgrounds developed anti-TSH receptor antibodies and intra-thyroidal lymphocyte infiltration, but HLA-DQ6b transgenic mice did not [53]. Indeed HLA-DR3 is, while -DQ6b is not, reported to be associated with Graves' disease in humans.

Overall, MHC and particularly non-MHC genes appear to be involved in determining the susceptibility to hyperthyroidism. Of interest, contrary to Graves' models, the genetic susceptibility to thyroglobulin- or thyroid peroxidase-induced thyroiditis depends on particular MHC haplotypes [5, 6].

5. Environmental factors in Graves' disease

Environmental factors possibly contributing to dis-

ease development include iodine, smoking, stressful life events, and infectious agents in humans [54]. As described above, the consequences of DNA vaccination were quite varied in different laboratories [10, 11, 16, 18, 19]. A recent study implied that different results may be attributable to the difference in housing conditions, namely conventional *versus* pathogen-free facilities [55]. However, disease incidence was neither different between conventional *versus* pathogen-free housing facilities nor affected by administration of microbial adjuvants, *Escherichia coli* lipopolysaccharide or *Saccharomyces cerevisae* zymosan A (the ligands for Toll-like receptors (TLRs) 4 and 2, respectively) in the adenovirus model [17]. TLRs, together with nucleotide-binding oligomerization domain protein and receptors that induce phagocytosis (*e.g.*, scavenger receptors, mannose receptors and β-glucan receptors), are so-called pattern-recognition receptors (PRRs) that detect evolutionarily conserved signatures from pathogens (pathogen-associated molecular patterns, PAMPs) and play a crucial role in innate immunity [56, 57]. The strong adjuvant activity of adenovirus itself [58] may be one of the reasons for the difference between DNA vaccination and adenovirus model.

However, as mentioned earlier, *Schistosoma mansoni* and *Mycobacterium bovis* BCG infections showed negative impact on disease development, indicating that certain infectious pathogens may play a role in the development of Graves' disease [46, 47]. These data cannot be explained by altered Th1 *versus* Th2 immune response. Instead, these results fit the "hygiene hypothesis" or "counter regulatory model" [59, 60], which propose that reduced exposure to certain microorganisms, irrespective of their ability to induce a Th1- or a Th2-biased immune response, during childhood in developed countries impairs the development of an appropriately educated immune system, causing increased rates in development of not only Th1-type autoimmune diseases but also Th2-type allergic diseases in adults. Indeed the incidence of autoimmune and allergic diseases in humans is increasing in developed countries in general [61] and Graves' disease is relatively uncommon in developing countries in particular [62, 63]. Overall, the development of Graves' hyperthyroidism may be negatively affected by certain infectious pathogens regardless of their ability to modify Th1 *versus* Th2 balance.

Iodide was used to clarify the association between Graves' disease and intrathyroidal lymphocytic infiltration that is frequently observed in Graves' thyroid glands in humans [64]. Immunization of NOD.H-2h4 mice, which spontaneously develop autoimmune thyroiditis on high-iodide diet, with AdTSHR289 revealed that AdTSHR289 immunization did not affect on the degree of intrathyroidal lymphocytic infiltration or anti-thyroglobulin antibody titers, and that anti-TSH receptor antibody titers were not influenced by iodide administration. Although iodide did somewhat suppress hyperthyroidism, this effect was considered to be a non-immune mechanism. Thus the immune response against the TSH receptor does not appear to have any effect on thyroiditis, *i.e.*, intrathyroidal lymphocytic infiltration, at least in this mouse strain. However, we have recently found that depletion of naturally occurring $CD4^+CD25^+$ regulatory T lymphocytes [65] by anti-CD25 antibody renders resistant C57BL/6 mice susceptible to Graves' hyperthyroidism to some extent, and that the thyroid glands from hyperthyroid C57BL/6 mice show extensive intrathyroidal lymphocytic infiltration (Saitoh and Nagayama, unpublished data), indicating that anti-TSH receptor autoimmunity can recruit lymphocytes into the thyroid glands in C57BL/6 genetic background. Further studies will be necessary to study relationship between hyperthyroidism and thyroiditis.

Conclusion

Several mouse models of Graves' hyperthyroidism were established and are being extensively used for evaluating the pathogenesis of disease. Several interesting observations have been reported regarding the role of receptor cleavage, antigen presenting DCs, and genetic and environmental factors. However, the data on Th1/Th2 balance and thyroidal lymphocytic infiltration are rather inconsistent among the different models. We are at present uncertain which model most closely resembles Graves' disease in humans, or what data most adequately represent the pathogenesis of human disease. Therefore we should be cautious when interpreting the data from *animal* models to gain the insight into the pathogenesis of *human* Graves' disease. Nevertheless, further studies with these models will hopefully lead to better understanding of disease pathogenesis, and ultimately lead to the development of new approaches for treatment and also prevention of human Graves' disease in the future.

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