



Determinants of thyroid autoantibody production in Hashimoto's thyroiditis

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Hashimoto's thyroiditis (HT) is the most prevalent thyroid autoimmune disorder, characterized by the presence of specific thyroid autoantibodies (TAb). The development of autoimmunity, including TAb production and clinical presentation of HT, is determined by a complex interplay of genetic susceptibility and several endogenous and environmental factors, which are discussed in this article. During the progression of the disease, TAb production precedes clinical manifestations, although the correlation between TAb concentrations and thyroid function is weak. We do not treat euthyroid HT patients despite elevated TAb; while in hypothyroidism, replacement therapy with L-thyroxine is required. Until now, an effective approach to prevent TAb production and the development of clinical disease has not yet been established. However, further identification of risk factors and their interaction may help in the prevention of thyroid autoimmunity.

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Hashimoto's thyroiditis (HT) was first described in 1912 by Hakaru Hashimoto. Today, it is known as the most prevalent autoimmune thyroid disease (AITD). It is characterized by lymphocytic infiltration of the thyroid gland and by parenchymal destruction and fibrosis, which gradually lead to variable clinical outcomes. Most patients with HT are euthyroid or hypothyroid, although short periods of mild hyperthyroidism are sometimes detected in the course of the disease. The hallmark of HT is the presence of thyroid-directed antibodies in patients' sera. In spite of being a useful marker to identify patients with HT, there is limited evidence that these antibodies have an important role in thyroid destruction. More likely, the key mechanism of tissue damage involves diverse pathways of T-cell-mediated cytotoxicity, such as direct action of cytotoxic CD8⁺ T cells, apoptosis caused by Fas and Fas ligand interaction on target thyroid cells and the effects of a wide array of cytokines produced by the infiltrating T cells [1]. Until now, the reasons for initiation and progression of HT have not been fully understood. It is plausible that the failure of T-cell tolerance owing to interplay of different genetic and nongenetic

factors leads to T-cell activation in the event of thyroid antigen presentation. Subsequently, activated T cells induce B cells and the production of autoantibodies against thyroid antigens [2].

Thyroid antibodies in Hashimoto's thyroiditis Anti thyroglobulin antibodies

Approximately 50 years have passed since Doniach and Roitt discovered autoantibodies against thyroglobulin (Tg) in patients with HT. Tg is a very large, 660-kDa, homodimeric glycoprotein produced by thyroid cells, which is involved in the synthesis and storage of thyroid hormones. It is situated in colloid within thyroid follicles, although small amounts can also be detected in serum. In this respect, Tg may be the target of autoimmune reactivity in predisposed individuals. As demonstrated by experimental models of autoimmune thyroiditis, the large Tg molecule contains several cryptic pathogenic T-cell epitopes, promoted by processing of complexes between Tg and anti-Tg antibodies (TgAb) in antigen-presenting cells or by enhanced iodination of Tg. They may be involved in the pathogenesis of thyroiditis [3]. Furthermore, TgAb enhance

the formation of Tg–TgAb complexes, their recognition by B cells and the subsequent proliferation of B and T cells, thus representing a possible mechanism for the maintenance of thyroid autoimmunity [4]. However, TgAb are not able to activate complement and presumably do not possess the ability to cause an important biological effect in the pathogenesis of HT. Among patients with HT, up to 80% have high concentrations of TgAb, mostly of the IgG class. Besides HT, TgAb are present in other autoimmune thyroid disorders, such as Graves' disease (GD) or postpartum thyroiditis [5]. According to large surveys performed in the past, approximately 13% of the total population presents with positive TgAb, 22% of whom are women over the age of 60 years [6,7].

Antithyroid peroxidase antibodies

Some 3 years after the discovery of TgAb, antibodies against a microsomal antigen were described and the antigen was later identified as thyroid peroxidase (TPO). The 100-kDa TPO antigen is situated at the apical membrane and is responsible for thyroid hormone synthesis, including iodine oxidation and iodination of thyrosyl residues on Tg. Similar to TgAb, anti-TPO antibodies (TPOAb) are predominantly of the IgG class. They are able to activate complement and are directly cytotoxic, although the correlation between TPOAb concentration and complement fixation is weak and, therefore, less likely to be essential for tissue damage [5,8]. Nevertheless, a recent study by Karanikas and colleagues demonstrated the correlation between TPOAb level and the production of cytokines interferon (IFN)- γ and tumor necrosis factor (TNF)- α , both of which enhance cellular cytotoxic immunity [9]. TPOAb are present in the majority (>90%) of HT patients; likewise, they are frequently elevated in other autoimmune thyroid disorders, including GD and postpartum thyroiditis. TPOAb concentration is independent of the type of thyroid disorder, yet a positive correlation with thyrotropin (TSH) level has been found [10,11]. In euthyroid subjects, Strieder and colleagues observed the correlation between TPOAb value and serum TSH, possibly predicting the future thyroid failure [12]. In the general population, the prevalence of TPOAb is 13%, while the prevalence in females over the age of 60 years increases to 27% [6,7].

Other antibodies

Among other antibodies related to HT, most relevant are blocking antibodies (BAb) against the TSH receptor (TSH-R), or TSHR-BAb, and Tg–TPO antibodies that bind to both Tg and TPO. TSHR-BAb inhibit the interaction between TSH and TSH-R, which is crucial for intracellular signaling. Therefore, the action of TSHR-BAb results in reduced thyroid hormone synthesis and cell growth with consequent hypothyroidism [13]. These antibodies have been described in up to 20% of HT patients and correlate with severity of hypothyroidism and reduction of thyroid tissue [10,14,15]. In GD, blocking antibodies may coexist with stimulating antibodies against TSH-R counteracting their agonistic activity.

Tg–TPO antibodies differ from monospecific TgAb and TPOAb and their diagnostic applicability and clinical importance have not been clearly defined. According to a large multicentric study, high Tg–TPO antibodies are present in 41% of HT patients, 35% of GD and 16% of patients with postpartum thyroiditis. Likewise, they can be detected in various other disorders, such as thyroid cancer, multinodular goiter, pernicious anemia, viral hepatitis treated with IFN- α , systemic lupus erythematosus and Type 1 diabetes mellitus [16].

Approximately 10 years ago, the antibodies against the sodium iodide symporter (NIS) were described and they are currently still the subject of controversy. While initial reports indicated a significant iodide uptake inhibiting activity of NIS-directed autoantibodies, later studies of patients with AITD established a low prevalence of these antibodies, possessing no ability to modulate NIS activity. Besides, the detection of NIS-directed autoantibodies has not been shown to increase the diagnostic strength for AITD [17].

Risk factors for thyroid autoantibody production

Genetic background

Genetic epidemiology

The importance of genetic susceptibility to thyroid autoantibody (TAB) production was initially indicated several decades ago by Hall and colleagues who reported the presence of TAB in 56% of siblings of patients with AITD [18]. Subsequent epidemiological studies confirmed this association; elevated TAB have been found more frequently in the first-degree relatives of patients with AITD [12,19,20]. Those family studies were supported further by segregation analyses of families with TAB, suggesting an autosomal dominant inheritance pattern for both TgAb and TPOAb, with reduced penetrance in men [21–23]. According to a study of siblings of patients with AITD, based on the prevalence data from National Health And Nutrition Examination Survey (NHANES) III survey [6], the calculated sibling risk ratio for HT is 28 [24]. Additional data on HT, provided by twin studies, indicate a 55% concordance rate in monozygotic (MZ) twins compared with no concordance in dizygotic (DZ) twins [25]. Regarding TAB, the concordance rates were reported to be higher in MZ compared with DZ twins, 74 versus 37% for TgAb and 64 versus 44% for TPOAb, respectively [26]. Similarly, Brix and colleagues have demonstrated that the prevalence of TPOAb and TgAb in healthy twin siblings of affected individuals was 34 and 26%, respectively, which was higher than the corresponding prevalence of 9 and 7% in a control population [27]. Recently, the genetic background has been estimated to contribute as much as 73% to the predisposition of being TAB positive [28].

Candidate gene regions & genes in HT

The genetic susceptibility to AITD is very likely to be determined by the interaction of several different genes having various alleles. Extensive studies of different populations were carried out to identify those genes employing two main approaches, association studies and whole genome linkage analyses [29]. Until

now, numerous putative genetic regions linked with AITD and putative AITD-susceptibility genes have been reported, some of which are distinctive for GD or HT and some of them being associated with both. In HT, the loci that demonstrated evidence for linkage are located on chromosomes 5q, 6p, 8q, 10q and 12q [30,31]. Among candidate genes associated with HT, the most notable are the major histocompatibility (MHC) gene complex on chromosome 6p21, cytotoxic T lymphocyte-associated 4 (*CTLA-4*) gene on chromosome 2q33 and *Tg* gene located on chromosome 8q24 [32].

MHC encodes human leukocyte antigens (HLAs), which act via the binding of antigenic peptide and presenting it to T cells. Certain HLA alleles presumably have higher affinity for autoantigen peptides and are, therefore, stronger candidates for being involved in autoimmune response. However, the data on the influence of MHC on the development of HT are not conclusive, although a weak association with HLA DR3, DR4 and DR5 has been suggested [19].

CTLA-4 is a molecule expressed on activated T cells, which functions as a key inhibitor of T-cell activation and proliferation. Several *CTLA-4* gene polymorphisms were described that could lead to impaired expression and function of *CTLA-4* molecule and thus contribute to exaggerated immune response [33–35]. Among them, polymorphisms -318 C/T in the promoter [36], 49 A/G in exon 1 [37,38] and CT60 in 3' untranslated region [35] were found to be most associated with HT.

No significant association has been observed between HT and several other putative susceptibility genes, including *TSH-R* gene, T-cell receptor gene, cytokine-related genes, *CD40* gene, protein tyrosine phosphatase-22 (*PTPN-22*) gene, *Fas* gene or *Fas* ligand gene [32].

Candidate gene regions & genes in TAb production

Until now, fewer studies have been published on genes involved in TAb production. A whole genome linkage analysis, performed by Tomer and colleagues, demonstrated that the major TAb susceptibility region was located on chromosome 2q33 and the key TAb gene was most likely the *CTLA-4* gene [39]. This observation was later confirmed in an expanded data set [34]. When we studied different *CTLA-4* gene polymorphisms in AITD, we established significantly higher TAb concentrations in HT patients with 49A/G and -318 C/T polymorphisms [40]. These results confirmed our previous observation in GD patients, where the association between TAb production and 49A/G *CTLA-4* gene polymorphism was found [41]. Interestingly, in both groups, the genetic influence was stronger in TPOAb production than in TgAb production. However, a similar study by Park and colleagues did not confirm this association in patients with GD [42].

Endogenous factors of TAb production

Female sex

Undoubtedly, women have a much stronger predisposition to AITD; it is diagnosed in women five to ten times more

often than in men [43]. Similar observations hold for the predisposition to TAb production [6,7,43,44]. According to a large population survey, NHANES III, the prevalence of TPOAb is 17% in women and 8.7% in men, while the prevalence of TgAb is 15.2 and 11.5%, respectively [6]. The prevalence rates of TAb increase with age, since approximately 30% of women and 11% of men over the age of 60 years present with positive TAb [7]. In women, the presence of TPOAb was found to be associated with the number of prior pregnancies [12]. This observation might be explained by the presence of fetal microchimerisms, where semi-allogenic fetal cells, transmitted into the mother's circulation during pregnancy, engraft in thyroid. There, they might be involved in triggering thyroid autoimmunity. That presumption is also supported by the finding of fetal cells in thyroid specimens of patients with HT more frequently than in thyroids of control subjects [45]. However, in a subsequent study on a larger group of women, the association of TAb with parity was not confirmed [46]. Recently, Hansen and colleagues provided evidence that the genetic susceptibility for serum TAb production was greater in women than in men. For TPOAb, the estimated genetic influence was 72% in women compared with 61% in men and, for TgAb, it was 75 and 39%, respectively [28]. The possible explanations relate to the role of the X chromosome and hormonal influences, yet they remain to be clarified. The putative involvement of the X chromosome is also indicated by higher TAb prevalence in X-chromosome aneuploidies, as in Turner's or Klinefelter's syndrome. An interesting recent report, possibly explaining the female predisposition to AITD, demonstrated higher frequency of skewed X-chromosome inactivation in relation to thyroid autoimmunity. The background might be the insufficient expression and tolerance of self antigens on one X chromosome leading to autoimmune response [47].

Postpartum period

Pregnancy is a period of immune suppression, in which several autoimmune disorders, including AITD, improve and serum TAb concentrations partly decline. After delivery, re-establishment of immune responsiveness is followed by occurrence or exacerbation of AITD and an increase of TAb concentration [48–51]. It is established that 50% of pregnant women with positive TPOAb develop postpartum thyroid dysfunction (PPTD). Higher TPOAb levels in early pregnancy appear to be associated with a stronger likelihood of PPTD after delivery [52]. Besides, in patients with PPTD the TPOAb levels are significantly higher compared with euthyroid TPOAb-positive postpartum women [53,54]. The concentration of TPOAb is increased in approximately 70% of patients with PPTD and represents a risk factor for permanent thyroid dysfunction [51]. According to a recent review, the prevalence of PPTD in the general population is approximately 8%, occurring 5.7-times more in women with elevated TPOAb, while women with Type 1 diabetes have a three-fold incidence of PPTD [55].

Low birth weight

The influence of reduced fetal growth on the presence of TAB was first indicated by Phillips and colleagues in a study of adult women, where the prevalence of TPOAb was 2.4-fold higher and the prevalence of TgAb was 5.4-fold higher in association with low birth weight [56]. In a subsequent twin study, only TPOAb was observed as being more prevalent in the smaller twin [26]. Also, in a recent twin study, Brix and colleagues found no association between the birth weight and serum concentrations of TAB [57]. Also, in their previous study of a connection between the birth weight and overt AITD, no correlation was found [58].

Environmental factors of TAB production

Iodine supply

It is known from epidemiological studies and from experiments on animal models that excessive iodine supply increases the risk for AITD and for thyroid dysfunction [59–61]. Presumably, iodination of Tg increases its antigenicity by altering its stereochemical structure and enhancing the appearance of cryptic pathogenic T-cell epitopes [3]. Iodine also stimulates the cells of the immune system, including macrophages, dendritic cells, and B and T lymphocytes. Besides, large amounts of iodine are toxic to thyroid cells, probably owing to free oxygen radicals produced in the TPO-mediated process of iodine oxidation [1]. However, the data regarding the effects of iodine supplementation on the prevalence of positive serum TPOAb concentrations in humans are contradictory, since some researchers reported even higher TAB prevalence in iodine-deficient areas [44], while others observed only a transient increase of TAB prevalence after introduction of iodine supplementation [62].

Smoking

Despite the undeniably harmful influence of smoking on the development of GD and especially Graves' ophthalmopathy, there are only a few contradictory studies relating to the role of smoking in the pathogenesis of HT and TAB production. Apart from one study [63], the association of smoking with HT or hypothyroidism was not confirmed by a recently performed meta analysis [64]. Even a lower prevalence of hypothyroidism in smokers was described in a report based on a NHANES III survey [65]. Furthermore, the smokers presented significantly less often with positive serum TAB value, indicating the protective influence of smoking on the development of TAB and HT [12,65].

Drugs

Some drugs are known to influence thyroid autoimmunity in genetically predisposed individuals. Lithium therapy, used in patients with affective disorders, is thought to induce TAB production. According to some reports, patients receiving lithium present with higher prevalence of TAB [66], while in patients with previous thyroid autoimmunity, an increase of TAB titers was demonstrated after initiation of lithium treatment [67]. However, these observations have not been confirmed by other

studies, although an increased risk of development of hypothyroidism was reported [68,69]. Furthermore, amiodarone with high iodine content and cytotoxic effect of both amiodarone and desethylamiodarone on thyrocytes may induce thyroid autoimmunity. In patients receiving amiodarone, a transient increase of TPOAb was reported, but this observation was not confirmed by subsequent studies [70]. In addition, IFN- α , which stimulates the T-helper (Th)1 immune response, was shown to induce TAB production when used for the treatment of patients with hepatitis C virus infection [71].

Expert commentary

Despite numerous investigations, the determinants of TAB production in HT are still not well known, with plenty of unresolved questions remaining to be clarified. Considering that there is an extensive genetic contribution to the development of TAB, it is understandable that our chance of taking control over TAB production is limited. However, as indicated in this article, a variety of other influences are essential for triggering the disease in genetically predisposed individuals. Given this view, our understanding of all contributing elements and their interactions is of crucial importance, since a genetically predisposed individual might escape the disease development simply by avoiding certain other risk factors. Fortunately, several patients with HT are euthyroid, with positive TAB being only a marker of the disease and a predictor of eventual future thyroid dysfunction. In these patients, nothing but a regular follow-up is required. However, in the course of HT, subclinical or overt hypothyroidism may occur at any time and then the replacement therapy with L-thyroxine becomes indispensable. In hypothyroid patients, L-thyroxine therapy is associated with the decrease of TAB, especially TSHR-BAb and TPOAb concentrations [10,72], although this effect has not always been observed [49,73]. The decrease of TAB levels appears to be associated with the fall of TSH values, as an elevation of TAB levels, together with the rise of TSH, follows the discontinuation of L-thyroxine supplementation [10]. Therefore, it is feasible that TSH stimulates the thyroid antigen presentation and, thus, increases the antigen availability to the immune system. In addition to L-thyroxine, the selenium intake has been shown to influence TAB production. Selenium is an essential constituent of selenoproteins. Among them, deiodinases and glutathione peroxidase are involved in thyroid hormone metabolism, while some other selenium-containing enzymes are crucial for adequate immune competence. Nevertheless, selenium supplementation influences the decrease of TPOAb, but it has no impact on TgAb level and thyroid function [74–76], which, therefore, reduces its clinical usefulness.

Recently, researchers have investigated the therapeutic options for the autoimmune diseases that interfere with autoantigen immune reactivity. A promising approach is targeting the CTLA-4 molecule on activated T cells to achieve the reinforcement of an inhibitory signal with subsequent suppression of T-cell activation [77–79]. Until now, several *in vitro* and *in vivo* studies have been performed, but an efficacious

therapeutic approach applicable to clinical practice has not yet been developed. Another interesting tool is the therapy with anti-CD3 monoclonal antibodies, which were shown to induce immune tolerance and antigen-specific unresponsiveness [80]. Although these investigations raise hope for patients with devastating autoimmune disorders, they seem to be an unlikely solution in HT, where the treatment of hypothyroidism, which is the ultimate outcome, is easy, inexpensive and safe.

Five-year view

Most probably, treatment of thyroid dysfunction in HT will remain unchanged within the next 5 years, since L-thyroxine replacement in the hypothyroid phase of the disease is simple and safe for the patient, and is also associated with low expenses. Furthermore, several patients with HT are euthyroid

and do not require treatment despite being TAB positive. However, a lot of progress has been made in the last few years in our knowledge regarding the impacts on disease development. Nevertheless, the precise mechanisms leading to the TAB production and clinical disease presentation remain to be identified. It is feasible that the next 5 years will bring understanding of precise genetic and other influences, their interaction and the way they trigger the impaired immune response. Therefore, in the future, we may hope to identify the individuals prone to developing AITD and to increase our knowledge of how to prevent disease development. Moreover, the high prevalence of the disease and good experimental models and thyroid tissue available for studies might enable researchers to discover specific and safe therapeutic agents that target the autoimmune reaction, not only relevant to AITD, but also to more serious autoimmune disorders.

Key issues

- The hallmark of Hashimoto's thyroiditis (HT) is the presence of thyroid peroxidase antibodies and thyroglobulin antibodies.
- The development of thyroid autoantibody (TAB) production and subsequent clinical disease is the sequel of a complex interaction of genetic background (cytotoxic T lymphocyte-associated 4 gene, major histocompatibility complex region, thyroglobulin gene), endogenous factors (female sex, postpartum period) and environmental factors (iodine, lithium, amiodarone, interferon- α).
- In HT, we treat hypothyroidism by replacing L-thyroxine; while in euthyroid TAB-positive patients, treatment is not necessary, since no clinical advantage is expected.
- Further knowledge on the background of thyroid autoimmunity might contribute to the prevention of disease development.

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