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Thyroperoxidase, thyroglobulin, Na^+/I^- symporter, pendrin in thyroid autoimmunity

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1. ABSTRACT

The autoimmune thyroid diseases (AITD), Graves' disease (GD) and Hashimoto's thyroiditis (HT) are most common endocrine disorders in humans. Both disorders are characterized by lymphocytic infiltration of the thyroid gland and the production of autoantibodies (aAb) against proteins that are thyroid-specific or expressed predominantly in the thyroid. The three main autoantigens are thyroperoxidase (TPO), thyroglobulin (Tg), and thyrotropin hormone receptor. Recently, the thyroidal iodide transporters Na^+/I^- symporter (NIS) and pendrin have also been identified as novel antigens in AITD. TPO-aAb and Tg-aAb are hallmarks of AITD, whereas the pathological and clinical relevance of NIS and pendrin aAb are still uncertain. To gain a greater understanding of the pathogenic mechanism(s) of autoimmune thyroid diseases at the molecular level, further characterisation of the autoantigens is required in order to shed light on why and how these molecules are seen by the immune system. This review summarizes current knowledge regarding the patho-physiological function and immunogenic response to the proteins TPO, Tg, NIS, and pendrin.

2. INTRODUCTION

The thyroid gland is the target for a spectrum of autoimmune thyroid diseases (AITD) ranging from the hyperthyroidism of Graves' disease (GD) to destructive Hashimoto's thyroiditis (HT) and hypothyroidism (1). AITD are the most common organ-specific multifactorial autoimmune disorders, affecting up to 3% of the world's population (2-4). Both GD and HT are characterized by lymphocytic infiltration of the thyroid gland by T and B cells reactive to thyroid antigens, the generation of thyroid autoantibodies and clinically abnormal thyroid function. The loss of tolerance results in the generation of an IgG response directed against thyroid-specific proteins: thyroperoxidase (TPO), thyroglobulin (Tg), and thyrotropin receptor (the review on TSH-R is a separate paper in the special issue of FBS) (1, 5-7). Recent studies have shown that the basolateral iodide (I^-) transporter, the Na^+/I^- symporter (NIS) and the apical I^- transporter, pendrin, are also antigens in AITD (8, 9). TPO is a key enzyme involved in the biosynthesis of thyroid hormones and Tg is a pro-hormone and store of T_3 and T_4, and both are recognized as thyroid differentiation markers. NIS and

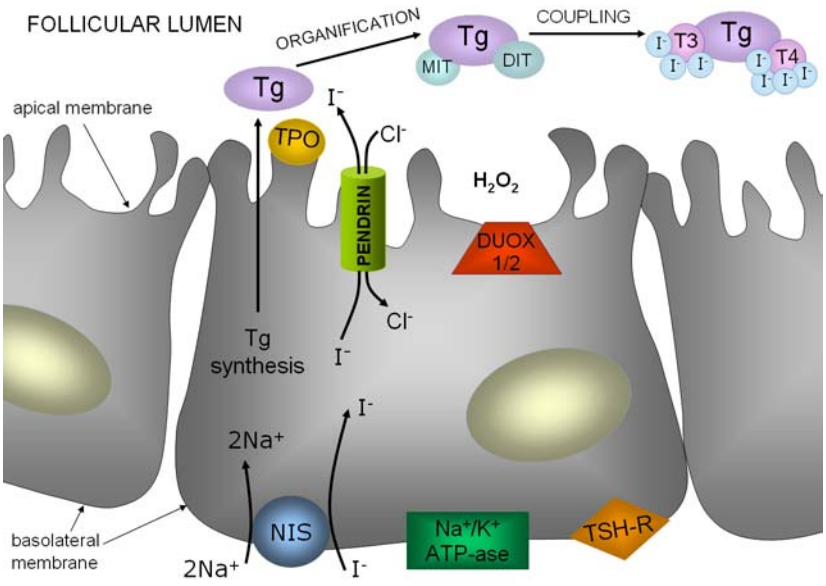

Figure 1. Schematic illustration of a follicular cell showing the cellular localization of proteins involved in the thyroid hormone biosynthesis. TSH-R, thyrotropin receptor; NIS, sodium iodide symporter, ATPase, sodium-potassium adenosine triphosphatase; TPO, thyroperoxidase; DUOX 1/2, calcium and NADPH dependent oxidases; Tg, thyroglobulin. Iodide is transported into the cell by the NIS in a process dependent of the sodium gradient generated by the ATPase. Then at the apical membrane iodide translocation is mediated by pendrin. At the membrane/colloid interface iodide is oxidized by the TPO in the presence of H_2O_2 produced by DOUX 1/2. Tg secreted into colloidal lumen is subsequently iodinated in the reactions catalyzed by the TPO. Thyroperoxidase catalyzes organification of iodide - iodination of selected tyrosyl residues to form MIT and DIT followed by coupling two iodotyrosines to form T_3 and T_4.

pendrin, functioning as iodide (I^-) transporters located, respectively, on the basolateral and apical membranes of the thyroid follicular cells, are proteins found predominantly, but not exclusively, in the thyroid tissue. Antibodies against TPO are present in the vast majority of patients with GD and HT, and represent an invaluable marker of thyroid autoimmunity. Tg antibodies, although less frequent, are also recognized as a marker of AITD. NIS antibodies are less commonly found in AITD patients and their pathogenic and diagnostic role is still unclear. A novel type of thyroid antibodies, detected in majority of patients with AITD, react with the protein pendrin. Pendrin antibodies may represent a diagnostic tool that is as useful as TPO- and Tg-Ab, but their occurrence and clinical and pathogenic importance require further study. To gain some understanding of the autoimmune response against the aforementioned autoantigens at the molecular level, current knowledge concerning the nature of these proteins and their antigenic activity will be summarized.

3. THYROID AUTOANTIGENS IN AUTOIMMUNE THYROID DISORDERS

3.1. Thyroperoxidase (TPO)

3.1.1. TPO protein structure and function

The thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) play an important role in the control of the body metabolism. Thyroperoxidase is a key enzyme involved in the thyroid hormone biosynthesis and a major autoantigen in AITD. TPO is a type I glycosylated transmembrane protein located in the apical membrane of the thyrocytes facing the follicular lumen, where other proteins involved in thyroid hormone biosynthesis are also localized and where the main steps of hormonogenesis normally occur (Figure 1) (10). The single-copy human TPO gene (2pter – p12) encodes a protein of 933 amino acids with a single membrane spanning region, a large extracellular domain orientated towards the follicular lumen, and a cytoplasmic tail of 61 amino acids in length (11, 12). TPO expression is under the control of thyroid-specific transcription factors such as TTF-1, TTF-2 and PAX-8 (13). This enzyme catalyzes two reactions within the thyroid: oxidation of inorganic I^- and coupling of iodinated tyrosines to generate T_3 and T_4. Thus, TPO plays a key role in thyroid hormone biosynthesis and is essential for normal thyroid function (14). TPO synthesized on polysomes is inserted into the endoplasmic reticulum where glycosylation of the protein core take place, and this subsequently becomes mature in the Golgi apparatus. The majority of this enzyme is found in the perinuclear membrane, endoplasmic reticulum and intracellular vesicles. Most of this intracellular TPO pool is incorrectly folded and rapidly degraded (15). Mature, properly folded, enzymatically active TPO is transported to the apical pole of the thyrocytes. Only about 2% of the enzymatically active TPO is found at the apical cell surface (16, 17). After being targeted to the thyrocyte apical membrane, TPO exposes its prosthetic group to the colloidal lumen (18).

The human TPO molecule of 105-110 kDa in size contains five asparagine-linked potential glycosylation sites

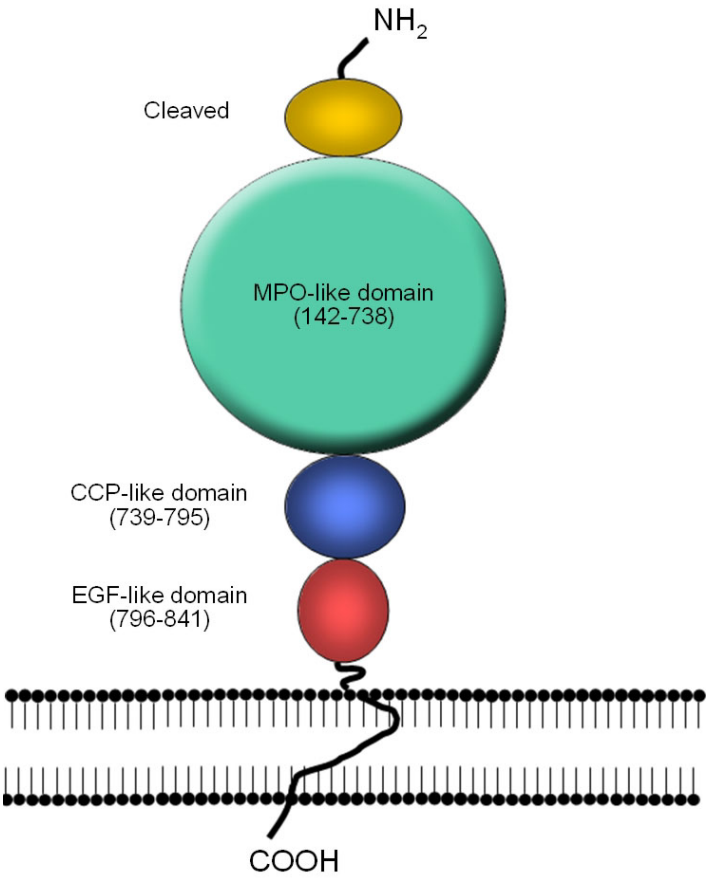

Figure 2. Schematic structure of the TPO molecule. The MPO-like module (amino acid 142-738); CCP-like (amino acid 739-795), and EGF-like (amino acid 796-841) domains.

in the ectodomain (residues 1-848) and carbohydrate constitutes ~10% of its mass (19, 20). The molecule contains a heme prosthetic group derived from ferroprotoporphyrin IX in the catalytic site located in the central part of the protein core (21-23). It has been suggested that the heme-protein bonds are formed through a self-processing mechanism. TPO belongs to the animal peroxidase superfamily (oxidoreductases, EC 1.7.1.11) and shares highest homology with members of mammalian myeloperoxidase family (24-26). The primary structure of hTPO exhibits a high degree of sequence similarity (42% identity, residues 1-738) to granulocyte myeloperoxidase (MPO) and the extracellular C-terminal region shares structural homology with complement control protein (CCP-like, residues 739-795), C4b-beta$_2$ glycoprotein, and an epidermal growth factor (EGF-like domain, residues 796-841) has also been identified (24, 27-29). Secondary structure prediction of hTPO reveals that the protein structure is mainly alpha-helical with relatively little beta-sheet and is organized into distinct domains (30). Although hTPO crystals have been obtained, they were too small for crystallographic analysis or suitable only for low X-ray diffraction, and so the three-dimensional structure has yet to be solved (31,32). However, the crystallization of MPO and the determination of its three-dimensional structure has permitted the modeling of the human TPO protein and partial elucidation of the molecule structure and the arrangement of its domains (33, 34). Three-dimensional modeling indicates that the TPO ectodomain is composed of three distinct modules: from the N-terminus there is an MPO-like region, while towards the C-terminus there is a CCP-like region and then an EGF-like region at the boundary with the transmembrane domain (Figure 2) (34). However, determination of the precise arrangement of these three modules on the membrane surface awaits the resolution of the three-dimensional TPO structure. This also means that accurate localization of the autoantigenic epitopes or immunodominant regions of hTPO is currently difficult to achieve.

3.1.2. TPO as an autoantigen

It is now 50 years since the presence of autoantibodies to a thyroid specific autoantigen distinct from thyroglobulin, named the thyroid microsomal antigen, was described (35). Despite intensive investigation, the nature of microsomal antigen was not established for almost three decades. The first evidence identifying TPO as the microsomal antigen was presented in 1985 (5, 20, 36). Thyroperoxidase expressed on the thyrocyte surface is now recognized as one of the main thyroid autoantigens, and both humoral and cell-mediated immune responses against TPO are thought to be involved in thyroid autoimmunity.

TPO autoantibodies are a hallmark of AITD (37). They have been detected in the sera of the majority of patients with GD (~80%), HT (> 90%) and postpartum thyroiditis (two-thirds), as well as in up to 26% of euthyroid subjects (7, 38-41). The antibodies are mainly produced by B lymphocytes infiltrating the thyroid gland and their titers reflect the severity of lymphocytic infiltration (42). The autoimmune response to TPO is polyclonal and circulating TPO-Ab are predominantly of the IgG_1 and IgG_4 subclasses with kappa light chain predominance; however, IgG_2 and IgG_3 subclasses and lambda chain-containing autoantibodies have also been detected in the same patients (43-45). TPO and TPO antibodies have been implicated in complement-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity mechanisms involving NK cells (46-51). Moreover, TPO was found to activate the complement cascade in the absence of antibody binding (52). Some TPO autoantibodies have been shown to bind to TPO and inhibit its enzymatic activity *in vitro*, although this finding is controversial (53-55). It has recently been suggested that the effects of TPO-Ab may require the involvement of FcRn, an immunoglobulin receptor expressed on thyrocytes, which is implicated in transcytosis of IgG across epithelia (56).

Thyroid autoimmunization is a T cell-dependent process. The epitopes recognized by T cells are short linear peptides (8-20 residues) produced by the processing of TPO in antigen-presenting cells (APC), which after binding to MHC class II molecules ($CD4^+$ T cells) are recognized by the T cell receptor. Using different methodological approaches (synthetic peptides, bacterially-derived short peptides) several T cell epitopes, located throughout the TPO molecule including the transmembrane domain, have been identified (57-59). Despite considerable experimental data, the identity of the most important, relevant and restricted T cell epitopes of TPO in AITD is still uncertain (60). In addition, the role of T cells in the initiation of AITD has yet to be definitely established. Specific antibodies may affect the T cell response to this enzyme by modulating which TPO peptides are presented to T cell clones by antigen-presenting cells (61).

3.1.3. TPO immunodominant domains

Polyclonal TPO antibodies present in the sera of patients with AITD react with several B cell epitopes located on the surface of hTPO. Autoantibodies mostly recognize conformational epitopes that are dependent on the three-dimensional integrity and folding of the TPO molecule (62-64). In addition, a small minority of TPO-Ab recognize linear epitopes outside the immunodominant region (IDR), including epitopes C2 and C21 (65). These autoepitopes are restricted to the overlapping immunodominant domains A (IDR A) and B (IDR B), as was established by competition experiments between autoantibodies and murine monoclonal anti-hTPO antibodies (66). This finding was subsequently confirmed using human monoclonal antibodies in the form of Fab fragments (67, 68). TPO-reactive Fab fragments were isolated from immunoglobulin gene combinatorial libraries derived from B cells infiltrating thyroid glands or thyroid draining lymph nodes of patients with HT or GD (69-79). Recombinant Fab fragments are similar to Ab present in patients' sera with respect to the IgG class, kappa subclass prevalence and high affinity for the antigen, and therefore are an important tool for the investigation of the autoimmune response to TPO and in IDR mapping studies.

The exact location and structure of the discontinuous IDR of TPO have yet to be determined. Recent convincing data suggest that the IDR of TPO might be restricted to the MPO-like domain (80, 81). The importance of this domain in the proper folding of immunodominant TPO regions was demonstrated by experimental approaches using human Fabs and polyclonal antibodies raised against peptides predicted to be exposed on the surface of hTPO (32, 70). A number of studies have identified fragments involved in the epitope(s) of TPO autoantibodies and support the highly discontinuous nature of the immunodominant region of TPO (82-87). The modeling of TPO structure based on its homology to MPO has allowed the prediction of the potential antigenic surface of TPO. The location of several peptides contributing to epitopes located in the MPO-like domain have been identified: residues 210-225, 353-363, 549-563 599-617, 713-717 (34, 82, 87-89). The participation of the CCP-like and EGF-like domains in the IDR has also been postulated (90). The EGF-like portion of TPO was excluded while the CCP-like module appears to contribute to the IDR in an uncharacterized manner (90). Recent experimental data suggest that the CCP-like TPO module constitutes part of the discontinuous TPO immunodominant region (82, 91). The TPO fragments participating in TPO antibody binding that have been identified to date (residues 210-225, 353-363, 549-563, 599-617, 713-720 and 766-775) are within IDR domains A and B located in the MPO-like and CCP-like modules (Figure 3). Taken together these data demonstrate the discontinuous and complex nature of the TPO IDR. In the native structure of TPO. The MPO- and CCP- like modules appear to be in close proximity and form the surface recognized by TPO-Ab found in the majority of patients with AITD. Following a recent analysis of the TPO IDR using the available human, mouse and rabbit antibodies it was proposed that the IDR (A and B) forms a single complex on TPO centered around residues 599-617 within the MPO-like domain (88, 92). This suggests that native TPO has a very dense folded structure which creates a single highly conformational immunodominant surface against which TPO-Ab are generated. Despite this conclusion, the CCP-like (containing the critical residue Tyr 772) and EGF-like modules, together with the hinge region of TPO are believed to be of importance in the maintenance of a three-dimensional structure required for antibody binding (56, 82, 83). The proportion of TPO autoantibodies directed against different autoepitopic determinants in the IDR suggests that epitopic recognition profiles are unrelated to thyroid status, are conserved over time and appear to be genetically transmitted (93-95). Moreover, there is no difference in the recognition of epitopes by TPO-Ab produced in patients with GD, HT or normal individuals, and as recently demonstrated, the majority of these Ab in the sera of patients with AITD are probably directed against IDR B (96).

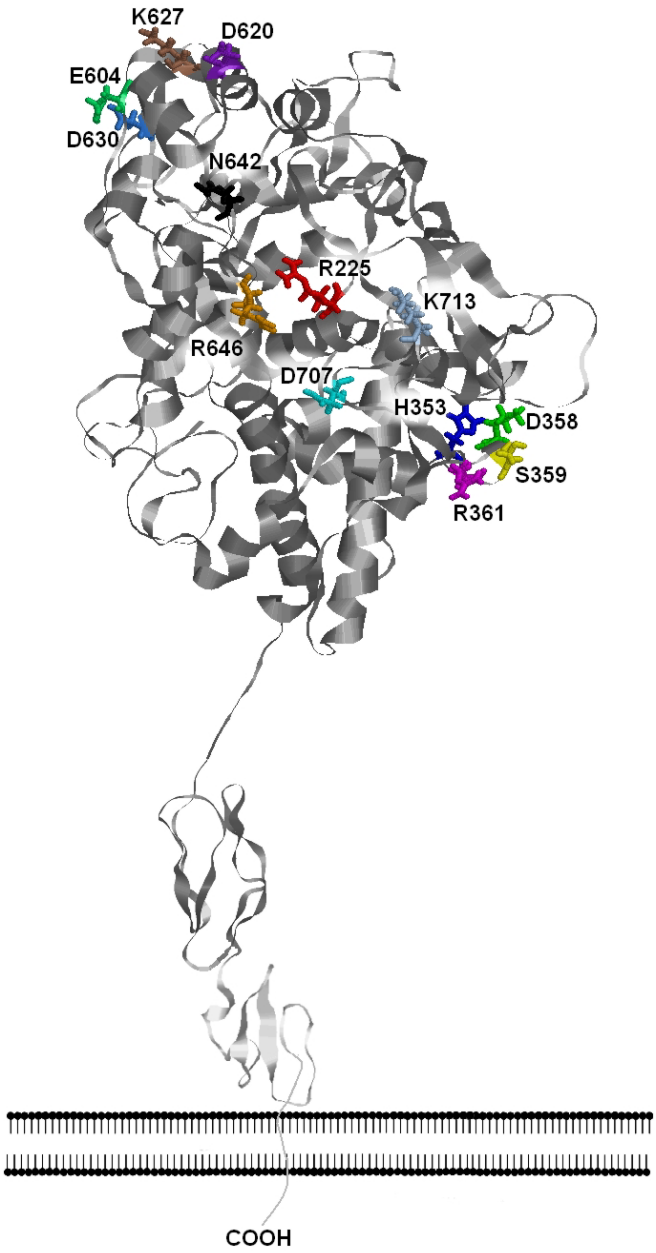

Figure 3. Three dimensional diagram of the structure of hTPO with the location of contact amino acids residues within immunodominant region/s. The residues are shown by coloured sticks: Lys 713 (84), Asn 642 (85), His 353, Asp 358, Ser 359, Arg 361 (86), Arg 225, Lys 627 (88), Glu 604, Asp 620, Asp 630, Arg 646, Asp 707 (92).

3.2. Thyroglobulin (Tg)

3.2.1. Tg protein structure and function

Thyroglobulin (Tg), is a large (660 kDa), soluble, 19S homodimeric glycoprotein of approximately 2768 amino acids that is synthesized by thyroid epithelial cell and secreted into the follicular lumen. It is the most abundant protein of the thyroid gland, representing up to 75-80% of the total protein content of mammalian thyroid (97). Tg functions as a precursor and the storage form of the thyroid hormones triiodothyronine (T_3) and tetraiodothyronine (T_4) (98). These two hormones result from the iodination and coupling of a few specific tyrosine residues within the Tg molecule and the synthesis process depends on the integrity of the Tg structure. The single copy gene encoding Tg maps to chromosome 8q24, is 270 kb long and contains an 8.5 kb coding sequence with 48 exons. The monomeric Tg molecule comprises the 19-residue signal peptide and a 2749-amino acid mature polypeptide containing 67 tyrosyl residues in hTg (99). The polypeptide chain sequence shows a highly organized internal structure with three domains. The N-terminal part (residues 1-1196) contains internal homology, with

repetition of the sequence C-W/Y-C-V-V- ten times in hTg. The central and C-terminal domains together comprise approximately 550 residues and exhibit significant homology to acetylcholinesterase (100-102). The Tg molecule undergoes several posttranslational modifications which contribute to the microheterogeneity of hTg, including glycosylation, iodination, sulfation and phosphorylation (103-107). Tg is highly glycosylated with carbohydrate moieties that are N-linked (types A and B) and hybrid, plus some type C O-linked glycans (108-113). These oligosaccharides seem to be important for a variety of biological processes, such as targeting Tg to the follicular lumen, iodination and hormone synthesis, and Tg immunoreactivity (114-118). Sulfation and phosphorylation of both tyrosine residues of the protein core and the carbohydrate moieties have been reported. The sulfation of tyrosine residues seems to be required for the hormonogenic process, although the functional importance of these modifications remains unclear (119, 120). Tg iodination and coupling are catalyzed by TPO in a process that requires hydrogen peroxide and takes place at the apical pole of the thyrocytes, where the membrane bound TPO and NADPH-dependent oxidases (DUOX1/2) involved in tyrosine iodination are localized (121, 122). The iodotyrosines MIT and DIT are subsequently coupled to form T_3 (MIT + DIT) and T_4 (DIT+DIT). In this process only 25-40 out of 130 tyrosine residues undergo iodination and just a few of them participate in the coupling reaction (123, 124). Four major hormonogenic sites (A, B, C, D) have been identified within the Tg monomer (125, 126). There have been reports suggesting that iodination contributes to thyroglobulin immunogenicity; however available experimental data indicate that this is secondary to the sequence of the molecule (127-132). Tg is synthesized in the endoplasmic reticulum and after maturation is secreted into the follicular lumen. Its transcription is regulated by TSH, insulin and IGF-1 (133, 134). Apart from its role as the matrix for T_3 and T_4 biosynthesis Tg performs an autoregulatory function, acting as a feedback suppressor of transcription factor activity and in consequence decreasing the expression of the major thyroid specific genes (135). This indicates that besides its role in thyroid hormone biosynthesis, Tg can play a regulatory role in the functioning of the thyroid gland. Thyroglobulin is one of the major thyroid autoantigens involved in the pathogenesis of thyroid autoimmunity and is recognized by aAb present in the sera of patients with AITD (136). Tg-aAb are found at high levels in the majority of patients with HT (>90%) and at a low to moderate titer in 40-70% of patients suffering from GD. Tg antibodies are also present in about 20% of clinically euthyroid individuals within the general population (137, 138). The frequency of Tg antibodies is higher in females and in the elderly (139, 140). The pathogenic role of Tg-aAb in AITD is still unclear, although their involvement in cytotoxicity against thyroid follicular cells has been described (47). Moreover, it was reported that the Tg antibodies associated with AITD facilitate the formation of immune complexes, their binding to B cells, and the proliferation of B and hTg-reactive T cells, demonstrating the association between the production of hTg-aAb and AITD progression (141).

Several methodological approaches have been used to characterize the immunological structure/antigenic surface of the hTg molecule. The results of studies with proteolytic, recombinant and chemically synthesized overlapping peptides, CNBr-cleaved hTg, plasmid expression libraries and oxidatively-cleaved hTg strongly suggest that (i) autoantibodies mostly recognize conformational epitopes that are dependant on the three-dimensional structure of Tg and correct folding of the molecule, (ii) some Tg-Ab are bi-specific, reacting with both Tg and acetylcholinesterase, (iii) the majority of epitopes are centered around the C-terminus or encompass both the N- and C-terminal regions of the polypeptide chain, and (iv) hormonogenic determinants are involved in the autoimmune response (Figure 4) (142-146). Probing of the antigenic surface of hTg with a panel of murine monoclonal Tg antibodies revealed six antigenic domains, and autoantibodies from patients with AITD were found to react mainly with domain II located in the middle part of the molecule (147-149).

3.2.2. Tg autoantibodies

Autoantibodies against Tg are mostly polyclonal, of the IgG class, with different contributions of the four subclasses (IgG_1<IgG2<IgG3 <IgG4), and with the presence of both kappa and lambda light chains (43, 44). Tg-Ab have a strong affinity for thyroglobulin and their level in some patients can be very high. A variety of studies examining the localization of Tg autoepitopes and their recognition by human polyclonal, murine monoclonal anti-Tg antibodies and recombinant human Fab fragments have clearly demonstrated that the humoral response to hTg is highly restricted to the two immunodominant regions (143, 144, 147, 150-154). Tg-Ab react with restricted epitopes located mainly in the central region and C-terminal end of Tg molecule (144, 153, 155-159). Thyroglobulin antibodies are also present in the sera of normal euthyroid healthy individuals, but these antibodies differ from AITD Tg-aAb because they do not show any restriction of the epitopes recognized, are of low affinity, polyspecific and predominantly of the IgM isotype (137, 138, 147, 160). In addition to the monospecific Tg-aAbs, antibodies with dual specificity for thyroglobulin and thyroid peroxidase so-called TGPO-aAbs are also present in the sera of some patients with autoimmune and non-autoimune thyroperoxidase (161-164). These antibodies are rather polyreactive and may recognize different type of epitopes on Tg and TPO molecules (165).

3.2.3. T cell epitopes

Evidence for the immunopathogenic role of Tg in the development of autoimmune thyroiditis has come from animal models of experimental autoimmune thyroiditis (EAT). EAT exhibits many of the characteristics of HT including mononuclear cell infiltration causing destruction of the thyroid follicles, autoantibody production and an *in vitro* T cell proliferative response to thyroid antigens (132, 166). EAT can be induced in susceptible strains of mice by immunization with autologous or heterologous thyroglobulin and adjuvant (166, 167). A variety of approaches including synthetic peptides and oxidative fragmentation of human thyroglobulin have been used to

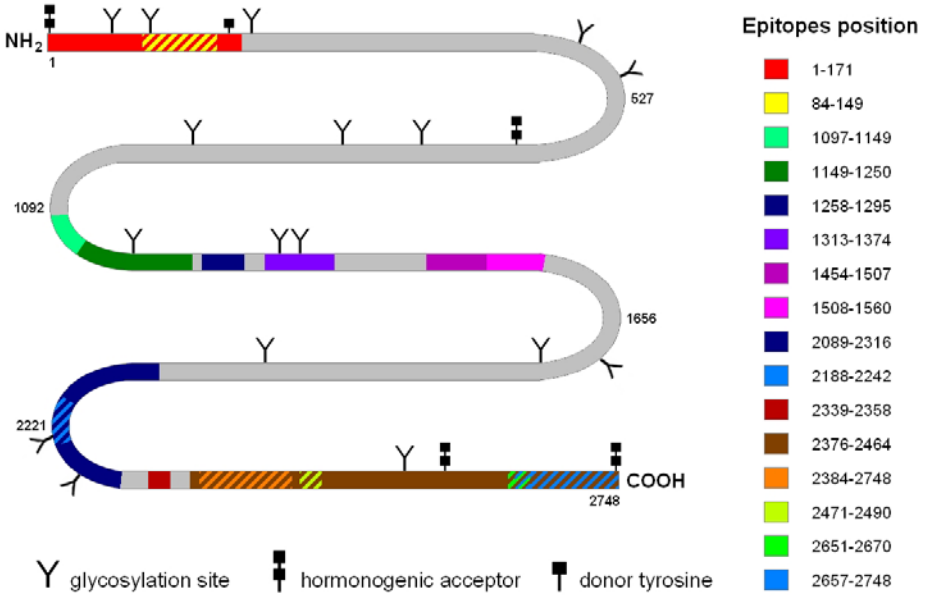

Figure 4. Schematic representation of Tg polypeptide chain showing glycosylation site, hormonogenic acceptor and donor tyrosine residues and the localization of Tg fragments involved in the antibodies binding: 1-171 (143); residues 84-149, 1097-1149, 1258-1295, 1313-1374, 1454-1507, 1508-1560, 2188-2242 (144); residues 2376-2464 (145); residues 1149-1250 (151); residues 2384-2748 (153); residues 2089-2316 (157); residues 2657-2748 (156, 157); residues 2339-2358, 2471-2490, 2651-2670 (158).

identify the pathogenic T cell-dependent epitopes of Tg involved in EAT (153, 168, 169). The oxidative cleavage of human Tg occurring during thyroid hormone synthesis among numerous fragments produced C-terminal P40 peptide containing five T-cell epitopes known to induce EAT in susceptible mice and bearing criptic T-cell epitope prone to induce autoimmune response in an HLA class II background (153-155). Although thirteen thyroidogenic sites containing T cell epitopes have been identified scattered throughout the Tg sequence, none of them are considered immunodominant (170).

3.3. Na^+/I^- symporter (NIS)

3.3.1. NIS protein structure and function

Iodide (I^-) is a scarce environmental microelement that is a vital component of thyroid hormones. The iodide-containing thyroid hormones T_3 and T_4 are crucial for normal development and the correct functioning of numerous metabolic pathways in probably all adult tissues. The trapping I^- from the blood and concentration in the thyroid gland is a prerequisite for and first step of hormonogenesis. The functional units of the thyroid gland where thyroid hormone synthesis takes place are the follicles consisting of a single layer of epithelial cells surrounding the follicular lumen. The thyroid gland is able to concentrate iodide by a factor 20-40 compared to the circulation (171). The I^- actively transported against its electrochemical gradient across the basolateral plasma membrane into the cytoplasm, is then translocated across the apical plasma membrane into the colloidal lumen, the main component of which is the thyroglobulin: the matrix and store of thyroid hormones. I^- reaches the apical pole of the cell/colloid interface, the site where biosynthesis primarily occurs (172). Cellular uptake of I^- is mediated by Na^+/I^- symporter (NIS), a plasma membrane glycoprotein which transports two Na^+ ions per each I^- ion. The Na^+ gradient that provides the driving force for this process is maintained by the Na^+/K^+-ATPase activity (Figure 1) (173). The molecular characterization of NIS began in 1996 when cDNAs encoding the rat and human proteins were isolated (174, 175). The human NIS gene, located on chromosome 19 at position 19p12-13.2, consists of 15 exons and codes for a glycoprotein of 643 amino acids with a molecular mass of approximately 70-90kDa (176). NIS (SLC5A5) belongs to the sodium-dependent transporter family 5A. The secondary structure model for family members predicts that NIS is an integral membrane protein (13 transmembrane domains) with the amino-terminus facing the extracellular milieu and carboxyl-terminus facing the cytoplasm (Figure 5) (173). The NIS protein has three N-linked glycosylation sites, but glycosylation is not essential for proper NIS function, stability and targeting (177, 178). Although the NIS protein shows significant selectivity for iodide, other monovalent anions with an ionic radius similar to that of I^- such as CLO_3^-, SCN^-, $SeCN^-$, and NO_3^- are readily transported by NIS (179).

Thyroid stimulating hormone (TSH) is the essential regulator of thyroid cells proliferation, differentiation and function (180). Numerous studies using different experimental approaches have demonstrated the role of TSH in the activation of the cyclic adenosine monophosphate (cAMP) pathway, which is the most important regulator of NIS gene and protein expression, and I^- uptake (181, 182). Many other factors including insulin and IGF, EGF, IL-1,IL-6, IFN gamma, TNF, TGF

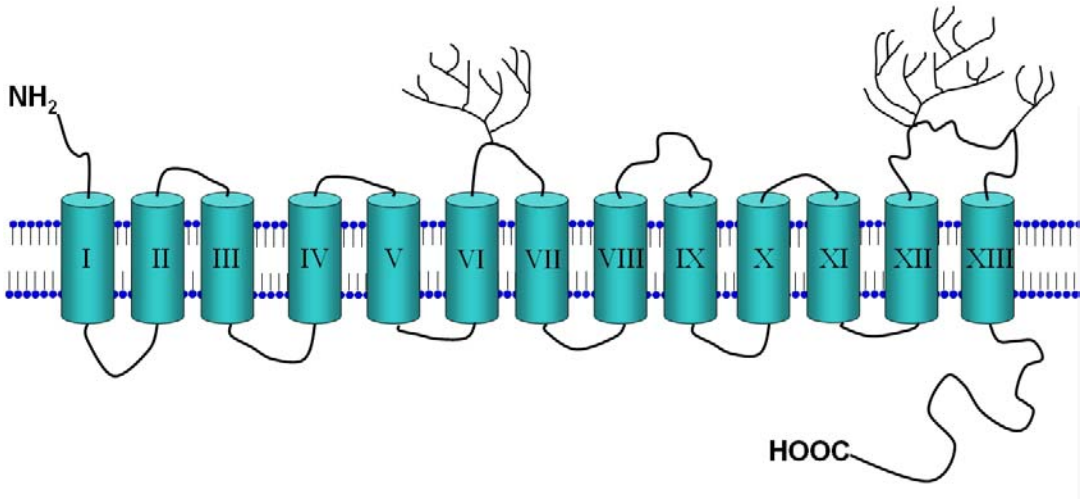

Figure 5. Schematic model of Na^+/I^- symporter.

beta and iodide itself also affect the I^- uptake (183, 184). Small to moderate doses of I^- do not influence the uptake of radioiodide; however, when the I^- dose becomes very high, iodide organification (i.e. incorporation into certain tyrosine residues of the thyroglobulin molecule to form MIT and DIT) is blocked (185). This acute effect is transient, as the thyroid gland restarts normal hormone production once it has adapted to prolonged iodide excess. Several studies have investigated the mechanisms underlying this escape effect and the combined experimental data indicate that exposure to high doses of I^- causes down-regulation of NIS protein expression, resulting in decreased intrathyroidal I^-, thus permitting resumption of iodide organification (173, 182, 186). In the normal thyroid, NIS protein is expressed heterogeneously at the basolateral membranes of a minority of follicular cells (187). The thyroid tissues in Graves' disease show high levels of NIS protein confined to the basal pole of the vast majority of thyrocytes, while the NIS expression pattern in cases of autoimmune thyroiditis appears to be similar to that observed in normal thyroid glands (187, 188). The three dimensional organization of the thyroid cells into follicles is an essential factor in the control of Na^+/I^- symporter expression (189).

3.3.2. NIS antibodies

Taking into account the key role of NIS in the functioning of the thyroid gland, its potential as a novel putative thyroid autoantigen in the pathogenesis of thyroid autoimmunity has been examined. Several studies have attempted to detect the presence of antibodies against NIS (8, 190, 191). The first data demonstrating the inhibition of I^- uptake by antibodies present in the sera of patient with AITD was reported even before the cloning of the NIS cDNA (192). This study found that one out of 147 serum samples from patients with AITD could inhibit iodide uptake by cultured dog thyrocytes in a specific manner, and it was hypothesized that antibodies directed against NIS might be responsible for the decreased I^- transporting activity. Although positive, this result suggested that such sera are rare in cases of AITD. Subsequently, numerous studies have used different methodologies to screen the sera of patients with GD and HT for the presence of autoantibodies reacting with recombinant rat NIS protein. Antibodies binding to the rat NIS protein were detected in 84% of individuals with GD and 15% of Hashimoto's thyroiditis cases, but only a small number of patient was tested (8, 193). In another study, IgG preparations from sera of AITD patients were tested using an ELISA method with a panel of synthetic peptides spanning the extracellular sequence and putative intracellular loops of the rat NIS (191). Antigenic epitopes predicted from the current NIS secondary structure model were mapped to the 8^{th}, 12^{th}, 13^{th} and 14^{th} extramembranous domains, and the sera of the majority of GD and a minority of HT patients contained antibodies that recognized corresponding synthetic peptides. In contrast, none of the control IgG preparations displayed any reactivity against NIS peptides (191). The presence of IgG with NIS reactivity suggests that these autoantibodies might affect thyroid function by inhibiting the uptake of I^-, thus playing a role in hypothyroidism. Iodide transport inhibitory activity was found in the sera of 11% of patients with HT, and IgG from these sera tested by immunoblotting reacted with a protein of approximately 80kDa that co-migrated with a band recognized by rabbit anti-NIS antibody (8). However, this inhibition of I^- uptake was also observed with the sera of some control subjects (which showed no immunoreactive band), but this activity was lost after the sera were dialyzed. Another study involving the expression of a truncated form of hNIS in a stable CHO-NIS cell line demonstrated iodide uptake inhibition activity in ~31% of GD sera (194). In a further study using an *in vitro* transcription and translation method, 22% of GD and 24% of HT sera contained NIS binding antibodies, and of these aAb-positive sera, 73% of GD and 43% of HT samples were also found to inhibit iodide uptake (190). Although these data suggested that antibodies modulating NIS physiological activity, and hence influencing the thyroid gland function, are present in various proportions of AITD sera, confirmation of this hypothesis awaits the testing of sera from a large number of patients. Using a similar sensitive and quantitative binding

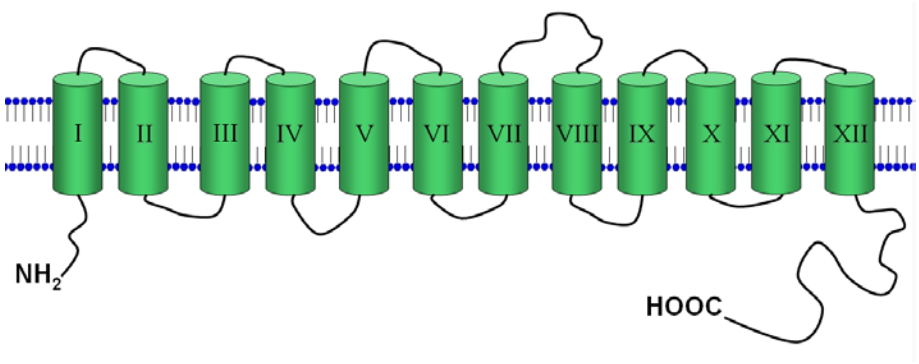

Figure 6. Schematic model of pendrin representing an intrinsic membrane protein with 12 transmembrane domains.

assay for the screening of NIS antibodies in a large series of serum samples from GD (177), and HT (72) patients, and 165 healthy individuals, NIS antibodies were detected in only 10.7% of patients with GD and 20% of those with HT (195). When more stringent cut-off criteria were applied (99.4th percentile of normal controls instead 95.2th percentile), the presence of NIS aAb was found in only 5.6% patients with GD and 6.9% of those with HT. To further evaluate the role of NIS as an autoantigen, COS-7 cell line stably expressing functional hNIS was established, which permitted the screening of a large panel of sera (514) from normal controls and patients with AITD, non-autoimmune thyroid diseases, and non-thyroid autoimmune diseases for the presence of aAb with functional activity (196). Reduced iodide uptake was observed following treatment of the cells with the sera of 14 patients. However, this inhibitory activity was lost when the IgG preparations or sera were tested following dialysis, indicating that some dialyzable serum factor was responsible for the observed reduction in I^- uptake, and that functional anti-NIS antibodies capable of modulating iodide trapping are very rare in AITD. The presence of I^- uptake inhibiting activity related to some unknown serum factor(s) in sera of patients with GD and HT was subsequently confirmed by others (197). Several at least partially overlapping NIS antibody binding domains have been identified on hNIS protein, mapping mainly to the extracellular parts of the molecule. However, no correlation between specific epitope recognition and autoimmune thyroid disease was demonstrated (198). In a very recent study using immunoblotting to screen sera, NIS antibodies were found in 38% of patients with GD and 27% of those with HT (9).

Although contradictory, the results summarized above suggest that NIS antibodies are present in a proportion of sera from patients with AITD. Their clinical and pathogenic importance for thyroid function in thyroid autoimmunity remains to be determined and they do not offer any apparent diagnostic benefit. In conclusion, hNIS does not appear to be a major functionally relevant antigen in humoral thyroid autoimmunity.

3.4. Pendrin

3.4.1. Pendrin protein structure and function

The iodide actively transported into follicular cells is released at the apical end of the thyrocytes into the follicular space, where is oxidized by TPO in the presence of H_2O_2. The oxidized form of iodide is rapidly organified in a mechanism catalyzed by TPO. Pendrin, composed of 780 amino acids, is a highly hydrophobic transmembrane glycoprotein localized at the apical pole of the thyrocytes facing colloidal lumen (199-202). The PDS/*SLC26A4* gene *(Pendred Syndrome Gene)* encoding pendrin is located on chromosome 7q22-31 and contains 21 exons that form an open reading frame ~2,4 kbp (203, 204). The pendrin gene was originally identified when the mutation causing Pendred syndrome was mapped (203). The protein is a member of the solute carrier family 26A or multifunctional anion exchanger family, which contains several transporters exchanging various anions (205). The secondary structure model of pendrin predicts that the protein has 12 transmembrane domains, with both the N- and C-termini located in the cytoplasm of the follicular cells (Figure 6) (199, 206). Functional studies have demonstrated that pendrin can mediate iodide transport in thyroid cells (207, 208). The localization of pendrin at the apical end of thyrocytes, its ability to mediate the iodide translocation across the apical membrane, plus the defective I^- organification detected in patients with Pendred syndrome indicate that this protein could be involved in mediating the efflux of iodide from thyrocytes into the follicular lumen through an iodide-chloride transport exchange (206-210). The occurrence and level of pendrin expression and iodide efflux are regulated by thyroid transcription factor 1, TSH and thyroglobulin, while iodide itself does not have a major effect on *SLC26A4* gene expression (199, 211-215). While experimental evidence has confirmed that pendrin is an apical transporter of iodide, electrophysiological studies have suggested the existence of other iodide channels that could also be involved in I^- efflux (216, 217). Thus, it is assumed that besides pendrin, other yet to be identified apically-located proteins may be involved in the translocation of iodide from the cell to the colloidal lumen of the thyroid follicle. The role of the pendrin gene and protein in the development and maintenance of thyroid autoimmunity is uncertain. In a case-control study comparing four microsatellite markers it was found that the pendrin gene could be linked to AITD as a new susceptibility gene with varying contribution to Graves' disease and Hashimoto's thyroiditis (218). The antigenicity of the pendrin protein was not examined until a recent study in which it was

shown to be a novel antigen in AITD (9). Using an immunoblotting method to screen sera it was found that 74% of patients with GD and in 97.5% of those with HT were positive for anti-pendrin antibodies, but none of the control sera from healthy individuals showed immunoreactivity to this protein (9). The occurrence of antibodies to pendrin correlated with the presence of TPO, NIS and Tg Ab, but not with TSH-R Ab, and appeared to be as reliable as antibodies to TPO and Tg in the diagnosis of AITD. Despite their high frequency in AITD patients, the clinical relevance and pathogenic role of anti-pendrin antibodies in thyroid autoimmunity is currently unknown and requires further study.

4. SUMMARY AND CONCLUSIONS

The thyroid autoantigens TPO, Tg, NIS, and pendrin are very important proteins in the physiological function of the thyroid gland. Thyroperoxidase is the primary enzyme involved in thyroid hormone biosynthesis and one of the main target antigens in thyroid autoimmunity. Autoantibodies to TPO are the most significant marker of AITD, although their role in the development of thyroid autoimmunity is still controversial. In recent years considerable efforts have been made to identify and characterize the immunodominant TPO domain and more precisely the amino acid residues recognized and contacted by TPO antibodies. Recent and future data on the thyroperoxidase IDR may assist the development of new approaches to the therapeutic modulation of immune responses in thyroid autoimmunity. Thyroglobulin is the prohormone and store for thyroid hormones T_3 and T_4. Despite numerous studies, the recognition of thyroglobulin by immune system has yet to be fully characterized. Although the restricted nature of the autoimmune response to Tg has been verified, the diversity of pathogenic epitopes recognized by autoantibodies and their localization within the Tg molecule need further investigation. Similarly, the T cell response to Tg in patients with AITD and their epitopes require systematic analysis. The hormonogenesis is an oxidative process generating free radicals and the iodide and its excess has been linked with the occurrence of the thyroid autoimmunization in the clinical and experimental studies suggesting their possible contribution to the autoimmune response to both autoantigens Tg and TPO which are directly involved in the thyroid hormone synthesis. Thus the role and mechanism/s underlying above association yet unknown need to be precisely elucidate.

The presence and functional role of NIS antibodies is still uncertain. The NIS protein seems to be an autoantigen only in minority of patients with AITD, and the employment of various methodological strategies is necessary to definitely determine their occurrence, functional effect and pathological significance in the development and maintenance of these diseases. An autoimmune response against pendrin is found in the majority of patients with AITD, although its importance is unknown.

The mechanisms involved in the production of a pathological autoimmune response to thyroid antigens are still largely uncharacterized. The presence of autoantibodies in the sera of patients with AITD is one of the characteristics of autoimmune thyroid disease and an excellent marker of thyroid autoimmunity. However, their full patho-physiological importance has yet to be determined.

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Abbreviations: AITD: autoimmune thyroid diseases, GD: Graves' disease, HT: Hashimoto's thyroiditis, TPO: thyroperoxidase, Tg: thyroglobulin, NIS: Na^+/I^- symporter, sodium-iodide symporter, T_3: triiodothyronine, T_4: tetraiodothyronine/thyroxine, MPO: myeloperoxidase, CCP: complement control protein, EGF: epidermal growth factor, FcRn: neonatal Fc receptor, APC: antigen-presenting cells, IDR: immunodominant region, MIT: monoiodotyrosine, DIT: diiodotyrosine, CNBr: cyanogen bromide, EAT:experimental autoimmune thyroiditis, TSH: thyroid stimulating hormone, TSH-R: TSH receptor,

Key Words: Autoimmune thyroid disease, Antigen, Autoantibodies, Epitopes, Thyroperoxidase, Thyroglobulin, Sodium iodide symporter, Pendrin, Review

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