Endokrynologia Polska
DOI: 10.5603/EP.2014.0021
Tom/Volume 65; Numer/Number 2/2014
ISSN 0423–104X

The role of the immune system and cytokines involved in the pathogenesis of autoimmune thyroid disease (AITD)

Rola układu immunologicznego oraz udział cytokin w patomechanizmie autoimmunologicznej choroby tarczycy (AITD)

Hanna Mikoś, Marcin Mikoś, Monika Obara-Moszyńska, Marek Niedziela

II Katedra Pediatrii, Klinika Endokrynologii i Reumatologii Dziecięcej, Uniwersytet Medyczny im. Karola Marcinkowskiego w Poznaniu, Poland

Abstract

Autoimmune thyroid disease (AITD) is the most common organ-specific autoimmune disorder. AITD development occurs due to loss of immune tolerance and reactivity to thyroid autoantigens: thyroid peroxidase (TPO), thyroglobulin (TG) and thyroid stimulating hormone receptor (TSHR). This leads to infiltration of the gland by T cells and B cells that produce antibodies specific for clinical manifestations of hyperthyroidism in Graves' disease (GD) and chronic autoimmune thyroiditis (cAIT). In addition, T cells in Hashimoto's thyroiditis induce apoptosis in thyroid follicular cells, leading ultimately to the destruction of the gland. Cytokines are involved in the pathogenesis of thyroid diseases working in both the immune system and directly targeting the thyroid follicular cells. They are involved in the induction and effector phase of the immune response and inflammation, playing a key role in the pathogenesis of autoimmune thyroid disease. The presence of multiple cytokines has been demonstrated: IL-1α, IL-1b, IL-2, IL-4 , IL-6, IL-8, IL-10, IL-12, IL-13, IL-14, TNF-α and IFN-γ within the inflammatory cells and thyroid follicular cells. Finally, cytokines derived from T cells can directly damage thyroid cells, leading to functional disorders and may also stimulate the production of nitric oxide (NO) and prostaglandin (PG), thus increasing the inflammatory response in AITD.

Immunological mechanisms involved in the pathogenesis of AITD are strongly related to each other, but differences in the image of cAIT and GD phenotype are possibly due to a different type of immune response observed in these two counteracting clinical thyroid diseases. This article describes the potential role of cytokines and immune mechanisms in the pathogenesis of AITD. **(Endokrynol Pol 2014; 65 (2): 150–155)**

Key words: *thyroid; autoimmune thyroid disease; anti-thyroid antibodies; T cells; cytokines*

Streszczenie

Autoimmunologiczna choroba tarczycy (AITD) jest najczęściej spotykaną narządowo-specyficzną chorobą autoimmunologiczną. Rozwój AITD następuje w wyniku utraty tolerancji immunologicznej i reaktywności na autoantygeny tarczycy: peroksydazę tarczycową (TPO), tyreoglobulinę (TG) i receptor tyreotropiny (TSHR). Doprowadza to do infiltracji gruczołu przez limfocyty T oraz limfocyty B, które z kolei produkują charakterystyczne przeciwciała, z kliniczną manifestacją nadczynności tarczycy w chorobie Gravesa-Basedowa (GD) i niedoczynności tarczycy w przewlekłym autoimmunologicznym zapaleniu tarczycy (cAIT). Ponadto, w cAIT limfocyty T indukują apoptozę komórek pęcherzykowych tarczycy, prowadząc w konsekwencji do destrukcji gruczołu.

Cytokiny biorące udział w patomechanizmie chorób tarczycy działają zarówno na układ immunologiczny jak i bezpośrednio na docelowe komórki pęcherzykowe tarczycy. Uczestniczą w indukcji oraz w fazie efektorowej odpowiedzi immunologicznej i zapalnej, odgrywając kluczową rolę w patomechanizmie autoimmunologicznej choroby tarczycy. Zarówno w obrębie komórek zapalnych, jak i komórek pęcherzykowych tarczycy wykazano obecność wielu cytokin: IL-1α, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-14, TNF-α i IFN-γ. Ostatecznie, cytokiny wywodzące się z limfocytów T mogą bezpośrednio uszkadzać komórki tarczycy, prowadząc do zaburzeń funkcji tarczycy oraz mogą również stymulować produkcję tlenku azotu (NO) i prostaglandyn (PG), zwiększając reakcję zapalną w AITD.

Mechanizmy immunologiczne zaangażowane w patogenezie AITD są mocno ze sobą powiązane, jakkolwiek skutkują różnicą w obrazie fenotypowym cAIT i GD, prawdopodobnie z powodu swoistego rodzaju odpowiedzi immunologicznej obserwowanej w obu przeciwstawnych klinicznie chorobach tarczycy. W niniejszym artykule przedstawiono potencjalną rolę cytokin i mechanizmów immunologicznych w patogenezie AITD. **(Endokrynol Pol 2014; 65 (2): 150–155)**

Słowa kluczowe: *tarczyca; autoimmunologiczna choroba tarczycy; przeciwciała przeciwtarczycowe; limfocyty T; cytokiny*

The immune system and autoimmune thyroid disease

Autoimmune thyroid dysfunction forms a clinical picture in which Graves' disease (GD), characterised by hyperthyroidism, is at one pole, and chronic autoimmune thyroiditis (cAIT), manifested by hypothyroidism, is at the opposite one.

Antibodies against thyroid antigens are present in both these diseases, but their specific epitopes are dif-

Prof. Marek Niedziela M.D., Ph.D., II Katedra Pediatrii, Klinika Endokrynologii i Reumatologii Dziecięcej, Uniwersytet Medyczny im. Karola Marcinkowskiego w Poznaniu, Poland, Szpitalna St. 27/33, 60–572 Poznań, tel. +48 61 849 14 24, tel./fax: +48 61 848 02 91, tel. kom.: 606 640 392, e-mail: mniedzie@ump.edu.pl

ferent, resulting in different functional antibodies. AITD development occurs due to loss of immune tolerance to autoantigens and reactivity of the thyroid, which leads to infiltration of the gland by T cells and B cells, which in turn produce antibodies specific for clinical manifestations of hyperthyroidism and hypothyroidism, GD and cAIT, respectively. Moreover, T cells in cAIT induce apoptosis in thyroid follicular cells, leading to the destruction of the gland [1, 2].

Antigen-presenting cells (APCs) or accessory cells, belonging to major histocompatibility complex (MHC) class II, especially dendritic cells, accumulate within the thyroid gland. APCs present specific thyroid antigens to lymphocytes within the lymph nodes, which leads to activation and proliferation of autoreactive lymphocytes T and B. In this way, activated antigen-specific T-helper lymphocytes CD4+ induce the formation of cytotoxic CD8+ T cells, and activate B cells, that produce autoantibodies, creating ectopic proliferation centers. Gland infiltration by cytotoxic T cells is primarily responsible for the destruction of thyroid parenchyma [3, 4].

The presence of an immunologically competent cell subset with suppressive activity, preventing a potentially harmful autoimmune response, is due to regulatory T cells CD4+CD25+ (Treg). Lack of T regulatory cells in humans and mice results in the development of a number of systemic autoimmune disorders such as thyroiditis, arthritis, gastritis, multiple sclerosis, inflammation of the ovaries, and others [5, 6].

Currently, there are documented three different populations of CD4+ T lymphocytes: Th1, Th2, and Th17. The development and differentiation of native CD4+ Th cells in populations diagnosing antigen is preceded, in cooperation with MHC class II signalling, by dendritic cells DC, with toll receptors - similar (TLRs) and C-type lectin receptors (CLR). The development of Th1 cells is dependent on IL-12, Th2 cells from IL-4, Th17 cells by TGF-β and IL-6 [7].

Th1 cells produce pro-inflammatory cytokines IL-2, IFN-γ, TNF, IL-1β, which leads to the activation of macrophages and cytotoxic effects. Th2 cells produce IL-4, IL-5, IL-6, IL-10, IL-13, which can inhibit the production of Th1 cytokines, but primarily stimulate B cells to produce antibodies and activation of anti-apoptotic molecules [8].

Hashimoto's thyroiditis (HT), a hyperplastic subtype of cAIT, is predominantly Th1 immune response, favouring the development of cell-mediated immunity, and thyroid follicular cell death by apoptosis [9, 10]. Proapoptotic death ligands and receptors, such as TNF, FasL and TRAIL present on thyroid cells, under physiological conditions are inactive [9]. However, expression of the Fas/FasL-induced

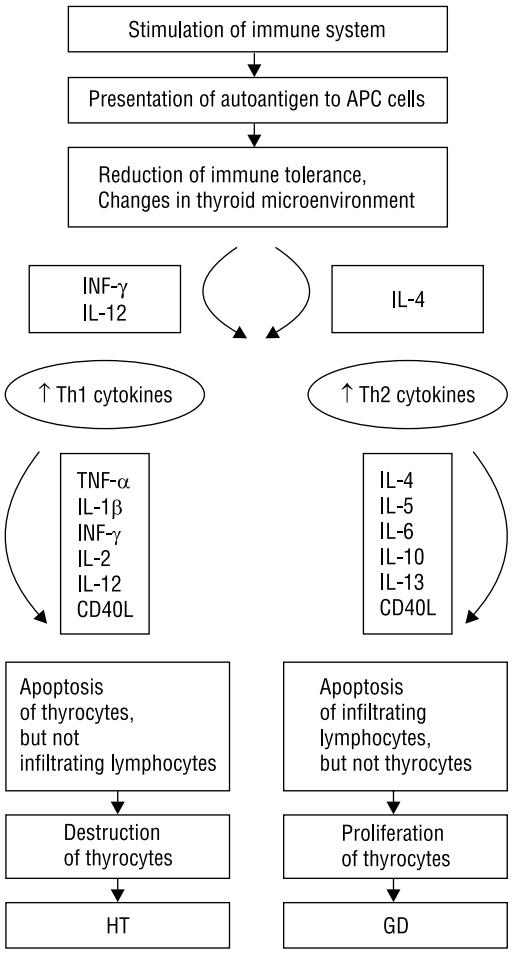

Figure 1. *Pathogenesis of chronic autoimmune thyroiditis (cAIT) and Graves' disease (GD)*

Rycina 1. *Patogeneza przewlekłego autoimmunologicznego zapalenia tarczycy (cAIT) i choroby Gravesa-Basedowa (GD)*

in response to pro-inflammatory Th1 cytokines by infiltrating IFN-γ TNF and IL-1β activates thyroid cell apoptosis [11–13].

The GD promotes Th2 predominantly humoral response, increased production of antibodies by B cells increases concentration of immunoglobulin G (IgG) and the cytokines produced by Th2 cells, which promote humoral response, thus inhibiting the expression of Fas/FasL and results in activation of the anti-apoptotic molecule Bcl-2, that protects thyrocytes from apoptosis, but increases apoptosis of cytotoxic lymphocytes, infiltrating thyroid tissue [10, 12]. Schematic pathogenesis of AITD illustrates the diagram in Figure 1.

A new subtype of Th17 response may also be involved in the pathogenesis of GD [14]. Th17 cells, which have been described recently, secrete proinflammatory cytokines (IL-17, IL-17F, IL-21, IL-22) and play an important role in chronic inflammatory diseases such as asthma and systemic lupus erythematosus [15]. The percentage of Th17 cells in patients

with GD was first described by Nanba et al., showing a higher rate of Th17 lymphocytes in patients not treated with anti-thyroid drugs, compared to remission in patients with GD [16].

Study profile of Th17 cells in HT revealed increased expression of the gene *RORC2* responsible for the differentiation subpopulation Th17 phenotype, and increased number of Th17 cells in peripheral blood and thyroid tissue in patients with HT, without proof of a similar situation in patients with GD [17]. Similar observations have been made by Idzkowska and Bossowski among children with newly diagnosed HT, to yield a statistically significant elevated levels of IL-17 and IL-23, which supports the participation of the Th17 in the pathogenesis of HT [18].

Extracellular mechanism of peripheral tolerance is mediated by regulatory T cells CD4+CD25+, mainly type 1 (Tr1). T regulatory cells were first described in the 1970s and 80s, when they were called suppressor cells. Recent studies have shown the importance of the key T lymphocytes CD4+CD25+ cells in the immune response, in which the suppressor mechanism of action is based on direct effect on thyroid follicular cells. It is assumed now that all T regulatory cells are characterised by expression of the transcription factor Foxp3, which is an essential element in the development and functioning. Regulatory T cells play an important role in disorders of immune tolerance to self antigens. Deficiency and impaired function of these cells may be the cause of autoimmune thyroid disease. Nakano et al. found that the percentage of regulatory T cells within the thyroid lymphocyte cells was lower in patients with autoimmune thyroid disease [19, 20].

In human and mouse T lymphocytes, CD4+CD25+ represent about 5-10% of the population of CD4+ T lymphocytes. Regulatory T cells mediate suppression mechanisms, by inhibiting IL-2 secretion and the secretion of anti-inflammatory cytokines, such as IL-4, IL-10, and TGF-β. Thus the action of T regulatory lymphocytes inhibits and modulates immune response Th1, Th2 and Th17 [21, 22].

Therefore, the clinical manifestation of autoimmune thyroid phenotype in the direction to GD and cAIT largely depends on the balance of the immune response induced Th1 or Th2 by the APCs, and the cytokine profile, which dominates at this point in thyroid parenchyma. The fact that these two functionally opposite states can develop in the same person at different periods suggests that the Th1/Th2 balance and the related cytokine profile is a dynamic process, evolving under the influence of external factors operating in the local environment of the thyroid gland [23, 24].

The participation of antibodies in the pathogenesis of AITD

Autoimmune thyroid disease is an autoimmune disease that affects approximately 5% of the population and is the most common organ-specific autoimmune disease. Activated B cells secrete antibodies directed against several major thyroid antigens. The occurrence of autoantibodies is characteristic of the disease, although these antibodies are also found in healthy individuals [25].

Anti-thyroid antibodies (ATG, ATPO)

Thyroid peroxidase antibodies (ATPO) are a marker of autoimmune thyroid disease. ATPO are present in nearly all patients with Hashimoto's thyroiditis, in two thirds of patients with postpartum thyroiditis, and in 75% of patients with hyperthyroidism in the course of Graves' disease. Thyroid peroxidase is a key enzyme involved in thyroid hormonogenesis. This antigen was identified in 1959 as the thyroid microsomal antigen. ATPO levels are associated with the expression of MHC on thyrocytes and with degree of infiltration by lymphocytes, which may 'sensitise' and trigger the synthesis of autoantibodies [26, 27].

Antibodies are produced mainly by lymphocytes infiltrating the thyroid gland, and only to a small extent by the local lymph nodes or bone marrow. It turns out that the presence of gammaglobulin antibodies is ascertained in 12–26% of asymptomatic patients with thyroid disease and is a risk factor for the future onset of hypothyroidism. Anti-TPO antibodies, in contrast to anti-thyroglobulin antibodies (ATG), are able to induce the complement system and cellular cytotoxicity. There is a high correlation between the concentration of anti-thyroid peroxidase antibodies and the presence of lymphocytic infiltration of the thyroid gland [28, 29].

ATPO are polyclonal, heterogeneous and directed towards different parts of TPO molecules, most of which recognise epitopes located on the surface of the enzyme TPO. Two major conformational immunomodulatory A and B regions (IDR-A and IDR-B) on the surface of the TPO are of particular importance, since they are in close proximity or even overlap each other [30]. Studies have shown that antibodies to the IDR-B domain are confirmed in most patients with HT and GD [31].

ATPO are also present in 12–16% of euthyroid patients, in 20% of type 1 diabetics and also in other autoimmune diseases: Addison's disease — 50%, rheumatoid arthritis — 30%, Sjogren's syndrome — 17–40%, SLE — 23%, scleroderma — 19%, and fibromyalgia — 24% [27].

In the NHANES III study population, the incidence of ATPO in the United States in healthy Caucasians was 12.3%. It was similar in the population of Mexican

origin — 10.1%, but significantly lower among African-Americans — 4.5%. Female gender is associated with a 2–4-fold higher incidence of ATPO. The percentage of antibodies increases with age for both sexes to almost 30% in chronically ill elderly patients. Antibodies against thyroglobulin are present in 97% of patients with Hashimoto's thyroiditis, and about 50% of cases of Graves' disease and in 10–20% of patients with subacute thyroiditis (de Quervaine's disease)[32].

In a multicentre study performed in 2007–2010 in Poland (PolSenior), in age groups ranging from 65 to 90 years of age, increased ATPO was revealed in 19% of the cohort. Prevalence of ATPO positivity was higher in females than in males (26.6% *v.*15.5%). Among 1,542 subjects with known concentrations of both ATPO and thyroid function, 1,110 were euthyroid (72%), 317 hypothyroid (20.6%), and 115 hyperthyroid (7.4%) [33].

Thyrotropin receptor antibodies (TRAb)

TSH receptor antibodies (TRAb) mimic the function of TSH and cause disease by binding to the TSH receptor and stimulate or inhibit thyroid cells in terms of T3 and T4 production. Patients with AIT may present both stimulating and blocking antibodies. The clinical picture of the disease is a result of the relative strength of each antibody type. While Graves' disease is characterised by the production of stimulating autoantibodies TSHR-TSAb, another group of patients with autoimmune thyroid disease may have autoantibodies that block the activation of TSHR-TBAb [34, 35]

The determination of concentrations of TRAb is helpful in the differential diagnosis of hyperthyroidism, Graves' disease, autoimmune disease aetiology and in predicting relapse after anti-thyroid drug treatment. Patients with high levels of TRAb (> 10 IU/L) have a 8.7 to 31.1 times higher risk of severe course of GO, and are likely to relapse after initial treatment with anti-thyroid drugs [35].

Currently, the detection of TRAb antibodies is widely used in Europe and Japan, the second and third generation of competitive tests TBII were developed for the first time by Smith et al [36]. However, this test does not differentiate the biological activity of the antibodies, TSAb and TBAb, which is possible using a bioassay based on cell culture and recombinant human TSHR, but is limited due to much efforts and costs. Second-generation TBII tests have 100% specificity and 98% diagnostic sensitivity [37–39].

Participation of cytokines in the pathogenesis of autoimmune thyroid disease

Cytokines are glycoproteins released by activated cells from different tissues. They provide a network control system of many processes such as proliferation, differentiation, secretion of biologically important substances. They play a key role in modulating the immune response and influence the balance between maintaining tolerance and initiating autoimmunity. In addition to hormone-like properties, they are involved in the process of haematopoesis, affect the functions of other cells and are mediators of inflammation and immune response, repair processes and wound healing.

Cytokines are involved in the pathogenesis of thyroid diseases, working in both the immune system and directly targeting the thyroid follicular cells. They participate in the induction and effector phase of the immune response and inflammation and play a key role in the pathogenesis of autoimmune thyroid disease, including cAIT and Graves' disease. Within the inflammatory cells and thyroid follicular cells, the presence of multiple cytokines: IL-1α, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-14, TNF-α and IFN-γ has been demonstrated [40, 41].

Cytokines in cytokine network play different functions: some effects are promoted by cytokines and inhibited by others. Cytokine secretion profile can be considered as a pro-inflammatory or anti-inflammatory, pro-apoptotic or anti-apoptotic. Under the influence of chronic antigen challenge, macrophages and CD4+ T cells can be divided into cells Th1 producing cytokines involved in the cellular response (IL-2, IFN-γ, TNF-α, IL-1β), or subpopulations of Th2 lymphocytes, which secrete cytokines associated with humoral response (IL-4, IL-5, IL-6, IL-10, IL-13), and Th17 producing IL-17, IL-21, IL-22. Th3 immune cells produce mainly TGF and play an important role in protecting against the occurrence of autoimmune diseases [42–44]. Despite attempts to classify autoimmune thyroid disease as a classical Th1- or Th2-dependent, there is mixed Th1/Th2 immune response in both HT and in GD [16].

Cytokines are produced by both lymphocytes and thyroid follicular cells. Experiments conducted *in vitro* have confirmed the production of cytokines by the stimulation of thyroid follicular cells by IL-1, IFN-γ and TNF-α, suggesting an increase of activity and an increased infiltration of pro-inflammatory cells *in vivo*. These cytokines increase the expression of adhesion molecules on the surface of thyroid follicular cells, and may also stimulate production of nitric oxide (NO) and prostaglandin (PG), increasing the inflammatory response in AITD. Thyroid tissue cytokines also play a role in antigen presentation to T cells by increasing the expression of MHC class I and II on the surface of the thyroid follicular cells, leading to destruction of the thyroid gland by T cell cytotoxicity. In thyroid ophthalmopathy, pathogenic action of cytokines: IL-β, TNF-α and IFN-γ, exacerbate inflammation and proliferation of fibroblasts, resulting in the accumulation of glycos-

aminoglycans within the orbit. In Graves' disease, IL-1β stimulates the production of hyaluronic acid in thyroid epithelial cells and fibroblasts, thus contributing to the development of the goitre [45–48].

On the basis of thyroid tissue immunohistochemistry, the presence of IFN-γ in cells infiltrating the thyroid gland, IL-1 in endothelial thyroid cells, and increased the amount of IL-1, IL-6 and TNF-α in thyroid follicular cells was revealed. In cell cultures, IL-1 and IL-6 increase the proliferation of the thyroid follicular cells, but also have inhibitory effects on thyrocytes during stimulation of these cells by TSH [49].

Cytokines in many cases exhibit a pleiotropic action and often may function as both an immunostimulatory and an immunosuppressive agent, depending on the type and location of the target. For example, the cytokine IL-2, IFN-γ and TNF-α activity may exhibit both inhibitory and excitatory function in autoimmune diseases [50].

IFN-γ and TNF-α inhibit the growth and proliferation of the thyroid follicular cells, but do not affect the viability of cells [49]. Pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α are generally associated with the stimulation of inflammation and autoimmunity, and may also stimulate production of T regulatory lymphocytes, inhibiting development of autoimmune diseases [51].

Cytokines enhance the inflammatory response by stimulation of both T and B lymphocytes, resulting in the production of antibodies and thyroid tissue damage by apoptosis, in particular in HT [52]. Cytokines can modulate the growth and function of thyroid follicular cells themselves in AITD, playing an important role in the extra-thyroid AITD complications, particularly ophthalmopathy. Exogenous administration of cytokines such as IFN-α is associated with the appearance of autoimmune thyroid disorders and the emergence of antibodies ATPO and ATG [40]. Krupinska et al. reported an elevated concentration of antithyroid antibodies in 17.14% of patients, with no signs of AIT at baseline, detected during therapy with interferon, after six and 12 months of treatment. After six months of treatment in this group of patients, 11 had hypothyroidsm and six had hyperthyroidism [53].

Conclusions

The role of cytokines in the development of autoimmune thyroid disease and their participation in the processes leading to the development of hypothyroidism in chronic autoimmune thyroiditis and hyperthyroidism in Graves' disease is still not clear. The demonstration of immune cells and anti-thyroid antibodies within the thyroid gland, and the determination of the levels of cytokines in peripheral blood, provide information about their involvement in the pathogenesis of AITD. These observations serve to confirm the effect of cytokines and their important role in maintaining the balance between health and disease.

References

1. Weetman AP. Autoimmune thyroid disease: propagation and progression. Eur J Endocrinol 2003; 148: 1–9.
2. Collins J, Gough S. Autoimmunity in thyroid disease. Eur J Nucl Med Mol Imaging 2002; 29 (Suppl. 2): 417–424.
3. Crivellato E, Vacca A, Ribatti D. Setting the stage: an anatomist's view of the immune system. Trends Immunol 2004; 25: 210–217.
4. Jacobson EM, Huber A, Tomer Y. The HLA gene complex in thyroid autoimmunity: from epidemiology to etiology. J Autoimmun 2008; 30: 58–62.
5. Sakaguchi S, Yamaguchi T, Nomura T et al. Regulatory T cells and immune tolerance. Cell 2008; 133: 775–787.
6. Shevach EM. Regulatory T cells in autoimmmunity. Annu Rev Immunol 2000; 18: 423–449.
7. Weaver CT, Harrington LE, Mangan PR et al. Th17: an effector CD4 T cell lineage with regulatory T cell ties. Immunity 2006; 24: 677–688.
8. Berger A. Th1 and Th2 responses: what are they? BMJ 2000; 321: 424.
9. Wang SH, Baker JR. The role of apoptosis in thyroid autoimmunity. Thyroid 2007; 17: 975–979.
10. Salmaso C, Bagnasco M, Pesce G, Montagna P et al. Regulation of apoptosis in endocrine autoimmunity: insights from Hashimoto's thyroiditis and Graves' disease. Ann N Y Acad Sci 2002; 966: 496–501.
11. Giordano C, Stassi G, De Maria R et al. Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. Science 1997; 275: 960–963.
12. Bretz JD, Baker JR Jr. Apoptosis and autoimmune thyroid disease: following a TRAIL to thyroid destruction? Clin Endocrinol (Oxf) 2001; 55: 1–11.
13. Giordano C, Richiusa P, Bagnasco M et al. Differential regulation of Fas-mediated apoptosis in both thyrocyte and lymphocyte cellular compartments correlates with opposite phenotypic manifestations of autoimmune thyroid disease. Thyroid 2001; 11: 233–244.
14. Nagayama Y. Graves' animal models of Graves' hyperthyroidism. Thyroid 2007; 17: 981–988.
15. Traves SL, Donnelly LE. Th17 cells in airway diseases. Curr Mol Med 2008; 8: 416–426.
16. Nanba T, Watanabe M, Inoue N et al. Increases of the Th1/Th2 cell ratio in severe Hashimoto's disease and in the proportion of Th17 cells in intractable Graves' disease. Thyroid 2009; 19: 495–501.
17. Figueroa-Vega N, Alfonso-Perez M, Benedicto I et al. Increased circulating pro-inflammatory cytokines and Th17 lymphocytes in Hashimoto's thyroiditis. J Clin Endocrinol Metab 2010; 95: 953–962.
18. Idźkowska E, Bossowski A, Noiszewska K et al. Analiza stężenia IL-17 i IL-23 w dynamice chorób autoimmunizacyjnych tarczycy u młodocianych. Endokrynologia Pediatryczna 2012; 3: 17–26.
19. Sakaguchi S: Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 2005; 6: 345–352.
20. Nakano A, Watanabe M, Iida T et al. Apoptosis-induced decrease of intrathyroidal CD4(+)CD25(+) regulatory T cells in autoimmune thyroid diseases. Thyroid 2007; 17: 25–31.
21. Fu S, Yopp AC, Mao X et al. CD4+ CD25+ CD62+ T-regulatory cell subset has optimal suppressive and proliferative potential. Am J Transplant 2004; 4: 65–78.
22. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003; 299: 1057–1061.
23. Tsatsoulis A. The role of stress in the clinical expression of thyroid autoimmunity. Ann N Y Acad Sci 2006; 1088: 382–395.
24. Phenekos C, Vryonidou A, Gritzapis AD et al. Th1 and Th2 serum cytokine profiles characterize patients with Hashimoto's thyroiditis (Th1) and Graves' disease (Th2). Neuroimmunomodulation 2004; 11: 209–213.
25. Caturegli P. Autoimmune hypophysitis: an underestimated disease in search of its autoantigen(s). J Clin Endocrinol Metab 2007; 92: 2038–2040.
26. Belyavin G, Trotter WR. Investigations of thyroid antigens reacting with Hashimoto sera; evidence for an antigen other than thyroglobulin. Lancet 1959; 1: 648–652.
27. Prummel MF, Wiersinga WM. Thyroid peroxidase autoantibodies in euthyroid subjects. Best Pract Res Clin Endocrinol Metab 2005; 19: 1–15.
28. Fink H, Hintze G. Autoimmune thyroiditis (Hashimoto's thyroiditis): current diagnostics and therapy. Med Klin (Munich) 2010; 105: 485–493.
29. Czarnocka B, Janota-Bzowski M, McIntosh RS et al. Immunoglobulin G kappa antithyroid peroxidase antibodies in Hashimoto's thyroiditis: epitope-mapping analysis. J Clin Endocrinol Metab 1997; 82: 2639–2644.

30. Berthold H, Steffens U, Northemann W. Human thyroid peroxidase: autoantibody recognition depends on the natural conformation. J Clin Lab Anal 1993; 7: 401–404.
31. Jastrzebska-Bohaterewicz E, Gardas A. Proportion of antibodies to the A and B immunodominant regions of thyroid peroxidase in Graves and Hashimoto disease. Autoimmunity 2004; 37: 211–216.
32. Hollowell JG, Staehling NW, Flanders WD et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 2002; 87: 489–499.
33. Bar-Andziak E, Milewicz A, Jedrzejuk D et al. Thyroid dysfunction and thyroid autoimmunity in a large unselected population of elderly subjects in Poland - the 'PolSenior' multicentre crossover study. Endokrynol Pol 2012; 63: 346–355.
34. Quadbeck B, Hoermann R, Hahn S, et al. Binding, stimulating and blocking TSH receptor antibodies to the thyrotropin receptor as predictors of relapse of Graves' disease after withdrawal of antithyroid treatment. Horm Metab Res 2005; 37: 745–750.
35. Eckstein AK, Plicht M, Lax H et al. Thyrotropin receptor autoantibodies are independent risk factors for Graves' ophthalmopathy and help to predict severity and outcome of the disease. J Clin Endocrinol Metab 2006; 91: 3464–3470.
36. Rees Smith B, McLachlan SM, Furmaniak J. Autoantibodies to the thyrotropin receptor. Endocr Rev 1988; 9: 106–121.
37. Kamath C, Adlan MA, Premawardhana LD. The role of thyrotrophin receptor antibody assays in graves' disease. J Thyroid Res 2012; 2012: 525936.
38. Tozzoli R, Bagnasco M, Giavarina D et al. TSH receptor autoantibody immunoassay in patients with Graves' disease: improvement of diagnostic accuracy over different generations of methods. Systematic review and meta-analysis. Autoimmun Rev 2012; 12: 107–113.
39. Zophel K, Roggenbuck D, Schott M. Clinical review about TRAb assay's history. Autoimmun Rev 2010; 9: 695–700.
40. Ajjan RA, Weetman AP. Cytokines in thyroid autoimmunity. Autoimmunity 2003; 36: 351–359.
41. Ajjan RA, Watson PF, Weetman AP. Detection of IL-12, IL-13, and IL-15 messenger ribonucleic acid in the thyroid of patients with autoimmune thyroid disease. J Clin Endocrinol Metab 1997; 82: 666–669.
42. Prud'homme GJ, Piccirillo CA. The inhibitory effects of transforming growth factor-beta-1 (TGF-beta1) in autoimmune diseases. J Autoimmun 2000; 14: 23–42.
43. Ajjan RA, Watson PF, Weetman AP. Cytokines and thyroid function. Adv Neuroimmunol 1996; 6: 359–386.
44. Pedro AB, Romaldini JH, Takei K. Changes of serum cytokines in hyperthyroid Graves' disease patients at diagnosis and during methimazole treatment. Neuroimmunomodulation 2011; 18: 45–51.
45. Natt N, Bahn RS. Cytokines in the evolution of Graves' ophthalmopathy. Autoimmunity 1997; 26: 129–136.
46. Rasmussen AK. Cytokine actions on the thyroid gland. Dan Med Bull 2000; 47: 94–114.
47. Hunt PJ, Marshall SE, Weetman AP. Cytokine gene polymorphisms in autoimmune thyroid disease. J Clin Endocrinol Metab 2000; 85: 1984–1988.
48. Gianoukakis AG, Khadavi N, Smith TJ. Cytokines, Graves' disease, and thyroid-associated ophthalmopathy. Thyroid 2008; 18: 953–958.
49. Ajjan RA, Watson PF, McIntosh RS et al. Intrathyroidal cytokine gene expression in Hashimoto's thyroiditis. Clin Exp Immunol 1996; 105: 523–528.
50. O'Shea JJ, Visconti R. Type 1 IFNs and regulation of TH1 responses: enigmas both resolved and emerge. Nat Immunol 2000; 1: 17–19.
51. Nakagawa T, Tsuruoka M, Ogura H et al. IL-6 positively regulates Foxp3+CD8+ T cells in vivo. Int Immunol 2010; 22: 129–139.
52. Palazzo FF, Hammond LJ, Goode AW et al. Death of the autoimmune thyrocyte: is it pushed or does it jump? Thyroid 2000; 10: 561–572.
53. Krupinska J, Wawrzynowicz-Syczewska M, Urbanowicz W et al. The influence of interferon alpha on the induction of autoimmune thyroiditis in patients treated for chronic viral hepatitis type C. Endokrynol Pol 2011; 62: 517–522.