ISSN: 03875547
Volume 45, Issue 02, April, 2022

Significant variations in the levels of IL33 and IL35 in the serum of an Iraqi patientes with early-onset Hashimoto's thyroiditis reported observed

Khaled Nather Taha[1*], Farhan Abood Risan[2], Abdulameer jasim mohammed[3]

Medical Laboratory Technique Department / College of Health and Medical Technology/Baghdad/ Middle Technical University/Iraq[1]
Medical Technical Institute/Al-Mansour, Middle Technical University/Iraq[2]
Medical Laboratory Technique Department / College of Health and Medical Technology/Baghdad/ Middle Technical University/Iraq[3]

Corresponding Author: 1*

Keywords:
IL-33 and IL-35 cytokines participates in HT development and pathogenesis.

ABSTRACT

Hashimoto's Thyroiditis (HT) are identified by chronic thyroid gland inflammation with uncertain etiologies, is it an autoimmune illness. Interleukin-33 and IL-35 have recently been discovered to play a role in progression of numerous autoimmune disorders. Interleukin-35 (IL-35), a cytokine family member of interleukin-12, has been demonstrated to be an effective immunosuppressive as well as anti-inflammatory action. Treg generate IL-35, which is responsible for Tregs' immunological suppressive role. Increasing IL-35 level through experimental models may play an important function in avoiding Auto - immune illnesses. Furthermore, HT is hypothesized to be a regulatory T cell associated auto-immune disease marked by a lack of self-tolerance. Interleukin-33 (IL-33) has recently been discovered to play a role in progression of numerous autoimmune disorders. In this study, the role of IL-33 and IL-35 in development and pathogenesis of HT are studied. One hundred-twenty subjects enrolled in this study, The levels of IL-33, IL-35 in serum are measured. And the samples divided as 40 newly diagnosed HT patients before treatment classified into (Euthyroid HT, Subclinical HT and Overt HT) based on thyroid function test presentation, 40 non immune hypothyroidism and 40 healthy controls were determined by enzyme-linked immunosorbent assay (ELISA). and Electrochemiluminescence immunoassay (ECLIA) method. The serum IL-33 were significantly elevated in newly diagnosed HT patients, compared to non immunehypothyroidism and healthy control with an significantly decreased in IL -35 in newly diagnosed HT when compare with non- immunehypothyroidism and healthy control.

1. INTRODUCTION

Hashimoto's thyroiditis (HT) is a chronic inflammatory condition that is among common autoimmune

disorder [7]. In iodine-deficient areas of the world, HT is the most common cause of hypothyroidism. It is just a distinct type of AITD, where the thyrocyte is targeted by a number of immune cell with auto antibody mediated immunological mechanisms [7], [12], [17], [23], [44].

Lymphocytic infiltration of the thyroid, anti-thyroid autoAb and thyrocyte death resulting in follicular destruction are all signs of HT [7], [12], [17], [23], [44].

Even though the exact cause of HT isn't known, it's widely thought that the production of some proinflammatory cytokines could be a factor in its development, like rheumatoid arthritis (RA) [3], [40]. As evidence of this, selective blocking of proinflammatory cytokines is one of the best ways to keep pathologic change in the right direction in those illnesses [5]. The proinflammatory cytokine interleukin-1 (IL-1) and its natural antagonist, IL-1 receptor, have been found to be out of balance in several studies, in pathogenesis of HT [30], [42].

Indeed, some IL-1 family members, including interleukin-18 (IL-18), have been linked to HT pathogenesis. IL-18 has been recognized as a key Th1-polarizing cytokine in HT. Previously, it was found that IFN- upregulates IL-18, which may lead to thyroid damage in HT patients [21], [28].

The newest member of the IL-1 family, IL-33 too were considered to involve a function in the of various auto - immune illnesses [25], [47]. Elevated serum IL-33, a disease marker, has been discovered in various autoimmune disorders, including rheumatoid arthritis (RA) [54], Graves' disease (GD) [8] and systemic lupus erythematosus (SLE) [55].

The IL-33 appears to be relevant in a number of chronic inflammatory disorders [31].

IL-33 can induce either immune responses type Th1 or Th2 in immunological disorders, depending on the microenvironment, type of activated cell, and cytokine miliu in injured tissues. Th2 responses are augmented by IL-33, which stimulates the production of IL-5 and IL-13 as Th2 cytokines [31], in comparison to IL-1 and IL-18 It also causes mast cells, natural killer, and natural killer T lymphocytes to secrete proinflammatory Th1 cytokines [38]. IL-33 has emerged as a promising targeted therapy for a variety of illnesses [25], [47]. Using monoclonal antibody against interleukine -33 [27]. In response to an aerosolized ovalbumin challenge, there was a considerable reduction in airway inflammation [36].

Despite GD and HT are both autoimmune thyroid disorders (AITD) with significant similarities, the pathophysiology mechanism are unique. Th2 cells were prevalent during development of GD, whereas HT is associated with classic Th1 illnesses such as RA [16].

A recent study found that people with GD had higher levels of IL-33 in their blood [8], but the important and function of IL-33 in HT is remain unclear.

Considering IL-33 was play role in many auto-immune disorder, study hypothesized that upregulation of IL-33 most likely has a role in the operation of HT [23], [12].

As well as, break of immune-tolerance to thyroid auto antigens is key feature of HT. Reducing the numbers of regulatory T cell is hypothesized to get involved at the start and progression of auto-immune illnesses in thyroid disorders [6]. Interleukin-35, a member of the IL-12 cytokine family, is an important mediator that stimulates T reg proliferation, suppresses CD8+ T cell activity, and reduces inflammation [15], [13], [14].

IL-35 secretion has only been observed in non-stimulated mouse regulatory T cells. [15] and stimulated human regulatory T lymphocyte [10]. In numerous autoimmune models, such as experimental colitis, IL-35 has clear immunosuppressive/anti-inflammatory properties [15], [52], autoimmune diabetes [4] collagen-induced arthritis [34], allergic airway disease [51] and autoimmune encephalomyelitis [13]. The role of IL-35 in HT, is a fascinating subject. Exact mechanism of HT has remained still obscure.

The purpose of this study was to see if circulating levels of IL-35 and IL-33 are connected to HT and any of its components.

2. Method

blood samples are collected from 40 newly diagnosed HT outpatients before start treatment. The presence of goiter, typical U/S, and anti thyroid Ab were used to make the diagnosis of HT (mainly to TPO and Tg). 40 non immune hypothyroidism and 40 healthy volunteers as the control group was made up of people who looked like they were healthy and didn't have any long-term medical problems. They had normal clinical exams and no past of thyroid disease or any other long-term illness, which could affect our results. An Ethics Committee of the Baghdad Teaching Hospital/Medical City gave its approval to this project. Each participant signed a written informed consent form. Overt hypothyroidism was known as low FT4 or FT3 and high TSH in the blood. Subclinical hypothyroidism was defined as high TSH and normal levels of FT4 and FT3 in the blood.

Subjects were excluded if she/he had a history of goiter, chronic respiratory diseases, smoking, neuromuscular disease, narcolepsy, T1DM, malignancy, renal disorder, chronic heart failure, autoimmune hepatitis, multiple sclerosis, rheumatoid arthritis, sedative drug user or alcoholic abuse, pregnancy, other endocrinological diseases, and recent infection.

Serum TSH,T3,T4, FT4 and FT3 levels were evaluated using full automated siemense analyzer (Germany) with electrochemiluminescence immunoassay (ECLIA) method, anti- (TPOAb) and anti- (TgAb) concentration were measured using aeskuliza Elisa kit.

Normal ranges in our laboratory are as follows: TSH=0.27–4.20 μIU/ml, FT4=0.93–1.70 ng/dl, FT3=2.0–4.40 pg/ml, TPOAb <35.0 IU/ml, TgAb <115.0 IU/ml. Enzyme-linked immunoassay (ELISA). Blood samples were centrifuged for 15 min at 3000×g and separated sera were stored at –80 °C. Serum levels of IL-33 and IL-35 are measured using ELISA kit (Sunlong, Wuhan. China).

2.1 Statistical Analysis

Statistical study. The Kolmogorov-Smirnov test was used to check the normality of the parameters. Means and standard deviations (SD), median (range), and one-way analysis of variance were used to look at the differences between the two groups (ANOVAor Kruskal-Wallis test with Mann-Whitney U test. Tukey post hoc test If a significant difference was found after ANOVA among groups. Categorical variables were reported as frequency counts, and the differences were examined using the chi-square test or Fisher exact test. Spearman rank order correlations were used to examine the relationships between IL-35 and IL33 and laboratory indicators, clinical evaluations, and thyroid volume. The statistical analyses were performed using IBM SPSS Statistics version 24, and p0.05 was considered statistically significant.

3. RESULTS

People were put into three groups based on their thyroid hormone levels: In Group 1 (n=40), there were new cases of HT; in Group 2 (n=40), there were new cases of nonimmune hypothyroidism; and in Group 3 (n=40),

there were healthy people who didn't have any thyroid problems in the past or now.

Characteristics of the groups are shown in Table 1. There was no significant difference among groups in terms of age, gender.

SerumIL-33 levels significantly higher in HT patients compared with the healthy control (P<0.05). The serum level of IL-35 was significantly lower in the Group 1 than in the Group 2 and Group 3.

3.1

3.2 Comparisons of different markers by groups (Hashimoto's, nonimmune hypothyroidism and healthy controls)

An analysis of variance (ANOVA) was conducted to determine whether there were significant differences in IL-35, IL 33pg/ml, by groups.

Table 1 Means, standard deviation, median and interquartile ranges of different markers by the study groups

Markers	Groups	Median (1st Q- 3rd Q) n = 40	P value*	η_p^2
IL 35	Hashimoto	133.60 (105.00- 154.463)	< .0012	0.54
	Non immune hypothyroidism	160.49 (158.44- 181.725)		
	Control	187.15 (165.75- 199.038)		
IL 33	Hashimoto	34.215 (25.17- 45.00)	< .0011	0.18
	Non immune hypothyroidism	26.1 (24.00- 34.250)		
	Control	24.1 (21.51- 32.500)		

*Results for Significance Testing against the levels of groups using F-Tests (ANOVA)

IL-35

There were significant differences in IL-35 among the group, F (2, 117) = 67.20, p < .0012, The eta squared was 0.53 indicating groups explains approximately 53% of the variance in IL-35.. Similarly, the median of IL-35 for NIHT was significantly smaller than for Control, p < .001.

Table ROC curve criteria of IL-33 as discriminating Hashimoto disease from Non-Immune hypothyroidism

(AUC) IL-33	SE	95% CI	P	Youden index J	cutoff	Sensitivity	Specificity
0.694	0.107	0.484 to 0.847	0.0894	0.4377	>32.1	72.39	72.12

AUC = Area under the ROC curve, SE= Standard Error, 95% CI = 95% Confidence interval

Table ROC curve criteria of IL35 as discriminating Hashimoto disease from Non-Immune hypothyroidism

(AUC) IL-35	SE	95% CI	P	Youden index J	cutoff	Sensitivity	Specificity
0.920	0.0712	0.721 to 0.876	<0.0001	0.8231	≤150.12	91.79	79.61

4. DISCUSSION

The IL-1 receptor family appears to play a crucial role in the pathophysiology of HT, according to mounting data. Polymorphisms in the IL-33 and its receptor were found to enhance the risk of AITD in a prior investigation [50]. The IL-33/ST2 pathway has been shown to be active in a variety of autoimmune illnesses, like graves disease [8]. We found a particular dysregulation of IL-33 and its receptor in HT in the current investigation. HT is considered to be Th1-trend disease, meaning it produces more Th1 cytokines like IFN-. In HT thyroids, IL-33 are increased. Increased IL-33 in HT has a specificity that is consistent with RA [46], and Multiple Sclerosis [11], which are characterized by Th1 phenotype. It is therefore conceivable that IL-33 overexpression may represent one of the earlier events in HT pathogenesis that may polarize toward Th1 phenotype, like its role in RA. IL-33 augmented Th2 response by interacting with its receptor ST2L. It attenuated experimental allergic encephalomyelitis (EAE) by suppressing the expression of IFN-γ and IL-17, while increasing Th2-type cytokines of IL-5 and IL-13 production [24].

Furthermore, IL-17 and IFN- were shown to be elevated in HT gland tissues in a prior investigation [41], Increased IL-33 may not inhibit, but it may work in tandem with IFN-gamma and IL-17. In some immunological illnesses, such as collagen-induced arthritis, IL-33 was also thought to be a pro-inflammatory cytokine that might trigger an inflammatory response [36]. IL-33 and IFN- are intimately connected in atopic dermatitis, IL-33 stimulates the release of IFN- by activated T cells, however it does not cause considerable production of IL-5 or IL-13 [22] This is in line with our HT findings. This shows that during the progression of HT, IL-33/ST2 may be in a Th1/Th17 trend. Given the complexity of the HT mechanism, IL-33 may not only correct the Th1/Th2 and Treg/Th17 imbalances, but also engage in those shifts and contribute to the generation of inflammatory cytokines. In HT PBMCs, there were more CD4+ST2L+ T cells, which respond to increased IL-33 and activate the pathway. An key idea in the IL-33/ST2 system that is also shared by other members of the IL-1 family (e.g., IL-1 and IL-18) [1]. The pathophysiology of HT has been associated to imbalances in the IL-1 and IL-18 systems [16], [30], [32]. In this study, we found circulating IL-33 were significantly increased in the HT group, similar to the result in IBD study [37].

Serum levels of IL-35 were low in people who had recently been diagnosed with HT. As far as we know, this is the first study in Iraq that looked at IL-35 levels in people who had recently been diagnosed with HT. There is a lot of IL-35 made by Tregs, and it plays a big part in the development of many inflammatory and autoimmune diseases [15], [34], [13], [52], [4], [51].

IL-35 promotes the immune inhibitory function and proliferation of Tregs. It contributes to development of autoimmune thyroiditis by causing loss of immune tolerance depending on the decrease in number of Tregs and impairment of their function [43], [6], [39].

In Apart from low IL-35 levels in the hypothyroidism group, IL-35 levels in the overt hypothyroidism group were found to be lower than those in the subclinical group in the current investigation. There is a link between autoimmune hypothyroidism and other anti-inflammatory cytokines (IL-4, TGF-beta, IL-10) that have comparable qualities to IL-35. TGF-beta levels that are lower have been linked to HT [2], [48], whereas increased levels of TGF-beta and IL-10 have been showed to suppress experimental autoimmune thyroiditis [18], [56]. These investigations corroborated our findings, which revealed that low levels of IL-35 were linked to HT.

The decreased IL-35 levels may contribute to both development of autoimmune thyroiditis and gradual intensification of hypothyroidism by leading to impairment in Treg cell numbers and functions. Besides, IL-35 levels might decrease impairment in Treg cell functions because of impairment in Treg cell functions and

decrease in number of Tregs. The most important mechanism that might explain the physiopathological relation between IL-35 and autoimmune hypothyroidism is the reciprocal interactions between IL-35 and Tregs. The characteristics of thyroid cell destruction for autoimmune thyroiditis can be seen as the consequence of inappropriate expression of Fas or TRAIL death pathway molecules and down-regulation of the apoptosis controlling protein Bcl-2 [19], [45].

IL-35 has recently been found to reduce apoptosis in pancreas cancer cell lines fixed in paraffin by increasing Bcl-2 and decreasing TRAILR1 [33].

Similarly, we assumed that decreased levels of IL-35 could induce apoptosis that may also be involved in the pathogenesis of HT. Reduced antibody titers in the collagen-induced arthritis mice model can be achieved with recombinant IL-35 [26].

Furthermore, IL-35 induced regulatory B cells to decrease autoimmune illness. A reduced IL-35 level was hypothesized to increase the proliferation of TPOA b-producing B cells. There are various flaws in this study. A cross-sectional design meant that we couldn't prove the causal link between IL-35 and HT. To begin with, we failed to assess the level of T-regs in the blood and thyroid tissue. Thirdly, no apoptotic markers (Bcl-2, Fas, TRAIL) were utilized to measure circulating apoptotic activity and thyroid tissue apoptotic activity. This was a significant oversight.

5. CONCLUSION

Our work establishes that IL-33 specifically displaying an increased may have a pivotal role in the pathophysiology of HT. By altering the release of inflammatory cytokines and not just the Th2 response, dysregulated IL-33 may contribute to inflammatory processes in HT.

Our findings suggest that IL-33 might offer some novel targets for therapy of HT, except considered as biomarkers. decreased levels of IL-35 were associated with autoimmune hypothyroidism and its severity. We suggest that IL-35 exerts a protective effect against auto destruction of thyroid tissue. This study was to ascertain whether there could be a role for IL-35 in human autoimmune thyroid disease.

We hypothesize that in this condition the role of IL-35 might be directed to the stimulation of Treg cell proliferation and antibody production rather than to the suppression of the inflammation. Further studies are needed to elucidate a link between IL-35 and autoimmune hypothyroidism.

6. LIST OF ABBREVIATIONS

HT = Hashimoto’s Thyroiditis
IL-33 = Interleukin-33
ELISA = Enzyme-Linked Immunosorbent Assay
RT-PCR = Real-Time Quantitative PCR
CON = Control Group
RA = Rheumatoid Arthritis
IL-1 = Interleukin-1
IL-18 = Interleukin-18
SLE = Systemic Lupus Erythematosus
GD = Graves’ Disease
AITD = Autoimmune Thyroid Diseases
PBMC = Peripheral Blood Mononuclear Cells

PBS = Phosphate-Buffered Saline
TBST = Trisbuffer Saline with 0.1% Tween
HRP = Horseradish Peroxidase
IL1RAcP = IL-1 Receptor Accessory Protein
SIGIRR = Single Ig IL-1-Related Receptor
EAE = Experimental Allergic Encephalomyelitis
IBD = Inflammatory Bowel Disease

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
This project was approved by the Ethics Committee of Baghdad teaching hospital in medical city (NO.2014-06), Baghdad, Iraq.

HUMAN AND ANIMAL RIGHTS
No Animals were used in this study. All the reported experiments involving humans participants are in accordance with the ethical standards of the committee responsible for human experimentation, and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION
Written informed consent was obtained from each participant.

AVAILABILITY OF DATA AND MATERIALS
All data generated or analyzed during this study are included in this published article.

FUNDING
No funding for this study

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS
We would like to thank all of the participants who took part in the studies featured in this research.

7. Reference

[1] Akdis, M., Burgler, S., Crameri, R., Eiwegger, T., Fujita, H., Gomez, E., Klunker, S., Meyer, N., O'Mahony, L., Palomares, O., Rhyner, C., Ouaked, N., Schaffartzik, A., Van De Veen, W., Zeller, S., Zimmermann, M., & Akdis, C. A. (2011). Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases. The Journal of Allergy and Clinical Immunology, 127(3), 701–770. https://doi.org/10.1016/j.jaci.2010.11.050

[2] Akinci, B., Comlekci, A., Yener, S., Bayraktar, F., Demir, T., Ozcan, M. A., Yuksel, F., & Yesil, S. (2008). Hashimoto's thyroiditis, but not treatment of hypothyroidism, is associated with altered TGF-β1 levels. Archives of Medical Research, 39(4), 397–401.

[3] Axtell, R. C., Raman, C., & Steinman, L. (2013). Type I interferons: beneficial in Th1 and detrimental in Th17 autoimmunity. Clinical Reviews in Allergy & Immunology, 44(2), 114–120.

[4] Bettini, M., Castellaw, A. H., Lennon, G. P., Burton, A. R., & Vignali, D. A. A. (2012). Prevention of

autoimmune diabetes by ectopic pancreatic β-cell expression of interleukin-35. Diabetes, 61(6), 1519–1526.

[5] Bootz, F., & Neri, D. (2016). Immunocytokines: a novel class of products for the treatment of chronic inflammation and autoimmune conditions. Drug Discovery Today, 21(1), 180–189.

[6] Bossowski, A., Moniuszko, M., Dąbrowska, M., Sawicka, B., Rusak, M., Jeznach, M., Wójtowicz, J., Bodzenta-Lukaszyk, A., & Bossowska, A. (2013). Lower proportions of CD4+CD25(high) and CD4+FoxP3, but not CD4+CD25+CD127(low) FoxP3+ T cell levels in children with autoimmune thyroid diseases. Autoimmunity, 46(3), 222–230. https://doi.org/10.3109/08916934.2012.751981

[7] Caturegli, P., De Remigis, A., & Rose, N. R. (2014). Hashimoto thyroiditis: Clinical and diagnostic criteria. Autoimmunity Reviews, 13(4–5), 391–397. https://doi.org/10.1016/j.autrev.2014.01.007

[8] Celik, H. T., Abusoglu, S., Burnik, S. F., Sezer, S., Serdar, M. A., Ercan, M., Uguz, N., Avcikucuk, M., Ceylan, B., & Yildirimkaya, M. (2013). Increased serum interleukin-33 levels in patients with Graves' disease. Endocrine Regulations, 47(2), 57–64.

[9] Chardès, T., Chapal, N., Bresson, D., Bès, C., Giudicelli, V., Lefranc, M.-P., & Péraldi-Roux, S. (2002). The human anti-thyroid peroxidase autoantibody repertoire in Graves' and Hashimoto's autoimmune thyroid diseases. Immunogenetics, 54(3), 141–157.

[10] Chaturvedi, V., Collison, L. W., Guy, C. S., Workman, C. J., & Vignali, D. A. A. (2011). Cutting edge: Human regulatory T cells require IL-35 to mediate suppression and infectious tolerance. Journal of Immunology (Baltimore, Md. : 1950), 186(12), 6661–6666. https://doi.org/10.4049/jimmunol.1100315

[11] Christophi, G. P., Gruber, R. C., Panos, M., Christophi, R. L., Jubelt, B., & Massa, P. T. (2012). Interleukin-33 upregulation in peripheral leukocytes and CNS of multiple sclerosis patients. Clinical Immunology (Orlando, Fla.), 142(3), 308–319. https://doi.org/10.1016/j.clim.2011.11.007

[12] Cogni, G., & Chiovato, L. (2013). An overview of the pathogenesis of thyroid autoimmunity. Hormones, 12(1), 19–29.

[13] Collison, L. W., Chaturvedi, V., Henderson, A. L., Giacomin, P. R., Guy, C., Bankoti, J., Finkelstein, D., Forbes, K., Workman, C. J., & Brown, S. A. (2010). IL-35-mediated induction of a potent regulatory T cell population. Nature Immunology, 11(12), 1093–1101.

[14] Collison, L. W., & Vignali, D. A. A. (2008). Interleukin-35: odd one out or part of the family? Immunological Reviews, 226(1), 248–262.

[15] Collison, L. W., Workman, C. J., Kuo, T. T., Boyd, K., Wang, Y., Vignali, K. M., Cross, R., Sehy, D., Blumberg, R. S., & Vignali, D. A. A. (2007). The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature, 450(7169), 566–569.

[16] Drugarin, D., Negru, S., Koreck, A., Zosin, I., & Cristea, C. (2000). The pattern of a T(H)1 cytokine in autoimmune thyroiditis. Immunology Letters, 71(2), 73–77. https://doi.org/10.1016/s0165-2478(99)00156-x

[17] Effraimidis, G., & Wiersinga, W. M. (2014). Mechanisms in endocrinology: autoimmune thyroid

disease: old and new players. European Journal of Endocrinology, 170(6), R241-52.

[18] Gangi, E., Vasu, C., Cheatem, D., & Prabhakar, B. S. (2005). IL-10-producing CD4+CD25+ regulatory T cells play a critical role in granulocyte-macrophage colony-stimulating factor-induced suppression of experimental autoimmune thyroiditis. Journal of Immunology (Baltimore, Md. : 1950), 174(11), 7006–7013. https://doi.org/10.4049/jimmunol.174.11.7006

[19] Giordano, C., Richiusa, P., Bagnasco, M., Pizzolanti, G., Di Blasi, F., Sbriglia, M. S., Mattina, A., Pesce, G., Montagna, P., & Capone, F. (2001). Differential regulation of Fas-mediated apoptosis in both thyrocyte and lymphocyte cellular compartments correlates with opposite phenotypic manifestations of autoimmune thyroid disease. Thyroid, 11(3), 233–244.

[20] Hong, Y.-S., Moon, S.-J., Joo, Y.-B., Jeon, C.-H., Cho, M.-L., Ju, J. H., Oh, H.-J., Heo, Y.-J., Park, S.-H., Kim, H.-Y., & Min, J.-K. (2011). Measurement of interleukin-33 (IL-33) and IL-33 receptors (sST2 and ST2L) in patients with rheumatoid arthritis. Journal of Korean Medical Science, 26(9), 1132–1139. https://doi.org/10.3346/jkms.2011.26.9.1132

[21] Huang, C.-Y., Ting, W.-H., Lo, F.-S., Wu, Y.-L., Chang, T.-Y., Chan, H.-W., Lin, W.-S., Chen, W.-F., Lien, Y.-P., & Lee, Y.-J. (2013). The IL18 gene and Hashimoto thyroiditis in children. Human Immunology, 74(1), 120–124. https://doi.org/10.1016/j.humimm.2012.10.005

[22] Imai, Y., Yasuda, K., Sakaguchi, Y., Haneda, T., Mizutani, H., Yoshimoto, T., Nakanishi, K., & Yamanishi, K. (2013). Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. Proceedings of the National Academy of Sciences of the United States of America, 110(34), 13921–13926. https://doi.org/10.1073/pnas.1307321110

[23] Jacobson, E. M., & Tomer, Y. (2007). The genetic basis of thyroid autoimmunity. Thyroid, 17(10), 949–961.

[24] Jiang, H.-R., Milovanović, M., Allan, D., Niedbala, W., Besnard, A.-G., Fukada, S. Y., Alves-Filho, J. C., Togbe, D., Goodyear, C. S., Linington, C., Xu, D., Lukic, M. L., & Liew, F. Y. (2012). IL-33 attenuates EAE by suppressing IL-17 and IFN-γ production and inducing alternatively activated macrophages. European Journal of Immunology, 42(7), 1804–1814. https://doi.org/10.1002/eji.201141947

[25] Kakkar, R., & Lee, R. T. (2008). The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nature Reviews. Drug Discovery, 7(10), 827–840. https://doi.org/10.1038/nrd2660

[26] Kochetkova, I., Golden, S., Holderness, K., Callis, G., & Pascual, D. W. (2010). IL-35 stimulation of CD39+ regulatory T cells confers protection against collagen II-induced arthritis via the production of IL-10. The Journal of Immunology, 184(12), 7144–7153.

[27] Lee, H. Y., Rhee, C. K., Kang, J. Y., Byun, J. H., Choi, J. Y., Kim, S. J., Kim, Y. K., Kwon, S. S., & Lee, S. Y. (2014). Blockade of IL-33/ST2 ameliorates airway inflammation in a murine model of allergic asthma. Experimental Lung Research, 40(2), 66–76.

[28] Liu, Z., Wang, H., Xiao, W., Wang, C., Liu, G., & Hong, T. (2010). Thyrocyte interleukin-18 expression is up-regulated by interferon-γ and may contribute to thyroid destruction in Hashimoto's thyroiditis.

International Journal of Experimental Pathology, 91(5), 420–425.

[29] Masterson, J. C., Capocelli, K. E., Hosford, L., Biette, K., McNamee, E. N., de Zoeten, E. F., Harris, R., Fernando, S. D., Jedlicka, P., Protheroe, C., Lee, J. J., & Furuta, G. T. (2015). Eosinophils and IL-33 Perpetuate Chronic Inflammation and Fibrosis in a Pediatric Population with Stricturing Crohn's Ileitis. Inflammatory Bowel Diseases, 21(10), 2429–2440. https://doi.org/10.1097/MIB.0000000000000512

[30] Mikos, H., Mikos, M., Rabska-Pietrzak, B., & Niedziela, M. (2014). The clinical role of serum concentrations of selected cytokines: IL-1β, TNF-α and IL-6 in diagnosis of autoimmune thyroid disease (AITD) in children. Autoimmunity, 47(7), 466–472. https://doi.org/10.3109/08916934.2014.914175

[31] Milovanovic, M., Volarevic, V., Radosavljevic, G., Jovanovic, I., Pejnovic, N., Arsenijevic, N., & Lukic, M. L. (2012). IL-33/ST2 axis in inflammation and immunopathology. Immunologic Research, 52(1–2), 89–99. https://doi.org/10.1007/s12026-012-8283-9

[32] Mok, M. Y., Huang, F. P., Ip, W. K., Lo, Y., Wong, F. Y., Chan, E. Y. T., Lam, K. F., & Xu, D. (2010). Serum levels of IL-33 and soluble ST2 and their association with disease activity in systemic lupus erythematosus. Rheumatology (Oxford, England), 49(3), 520–527. https://doi.org/10.1093/rheumatology/kep402

[33] Nicholl, M. B., Ledgewood, C. L., Chen, X., Bai, Q., Qin, C., Cook, K. M., Herrick, E. J., Diaz-Arias, A., Moore, B. J., & Fang, Y. (2014). IL-35 promotes pancreas cancer growth through enhancement of proliferation and inhibition of apoptosis: evidence for a role as an autocrine growth factor. Cytokine, 70(2), 126–133.

[34] Niedbala, W., Wei, X., Cai, B., Hueber, A. J., Leung, B. P., McInnes, I. B., & Liew, F. Y. (2007). IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. European Journal of Immunology, 37(11), 3021–3029.

[35] Olson, B. M., Sullivan, J., & Burlingham, W. (2013). Interleukin 35: a key mediator of suppression and the propagation of infectious tolerance. Frontiers in Immunology, 4, 315.

[36] Palmer, G., Talabot-Ayer, D., Lamacchia, C., Toy, D., Seemayer, C. A., Viatte, S., Finckh, A., Smith, D. E., & Gabay, C. (2009). Inhibition of interleukin-33 signaling attenuates the severity of experimental arthritis. Arthritis and Rheumatism, 60(3), 738–749. https://doi.org/10.1002/art.24305

[37] Pastorelli, L., Garg, R. R., Hoang, S. B., Spina, L., Mattioli, B., Scarpa, M., Fiocchi, C., Vecchi, M., & Pizarro, T. T. (2010). Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis. Proceedings of the National Academy of Sciences, 107(17), 8017–8022.

[38] Pei, C., Barbour, M., Fairlie-Clarke, K. J., Allan, D., Mu, R., & Jiang, H. (2014). Emerging role of interleukin-33 in autoimmune diseases. Immunology, 141(1), 9–17.

[39] Pellerin, L., Jenks, J. A., Bégin, P., Bacchetta, R., & Nadeau, K. C. (2014). Regulatory T cells and their roles in immune dysregulation and allergy. Immunologic Research, 58(2–3), 358–368.

[40] Phenekos, C., Vryonidou, A., Gritzapis, A. D., Baxevanis, C. N., Goula, M., & Papamichail, M. (2004). Th1 and Th2 serum cytokine profiles characterize patients with Hashimoto's thyroiditis (Th1) and Graves' disease (Th2). Neuroimmunomodulation, 11(4), 209–213. https://doi.org/10.1159/000078438

[41] Qin, Q., Liu, P., Liu, L., Wang, R., Yan, N., Yang, J., Wang, X., Pandey, M., & Zhang, J. (2012). The increased but non-predominant expression of Th17- and Th1-specific cytokines in Hashimoto's thyroiditis but not in Graves' disease. Brazilian Journal of Medical and Biological Research = Revista Brasileira de Pesquisas Medicas e Biologicas, 45(12), 1202–1208. https://doi.org/10.1590/s0100-879x2012007500168

[42] Rebuffat, S. A., Kammoun-Krichen, M., Charfeddine, I., Ayadi, H., Bougacha-Elleuch, N., & Peraldi-Roux, S. (2013). IL-1β and TSH disturb thyroid epithelium integrity in autoimmune thyroid diseases. Immunobiology, 218(3), 285–291.

[43] Shevach, E. M. (2009). Mechanisms of foxp3+ T regulatory cell-mediated suppression. Immunity, 30(5), 636–645.

[44] Stathatos, N., & Daniels, G. H. (2012). Autoimmune thyroid disease. Current Opinion in Rheumatology, 24(1), 70–75. https://doi.org/10.1097/BOR.0b013e32834ddb27

[45] Stojanović, J., Srefanović, D., Vulović, D., Puskas, L., & Marković, L. (2009). [Role of apoptosis in pathogenesis of thyroiditis]. Medicinski pregled, 62(1–2), 49–52. https://doi.org/10.2298/mpns0902049s

[46] Talabot-Ayer, D., McKee, T., Gindre, P., Bas, S., Baeten, D. L., Gabay, C., & Palmer, G. (2012). Distinct serum and synovial fluid interleukin (IL)-33 levels in rheumatoid arthritis, psoriatic arthritis and osteoarthritis. Joint Bone Spine, 79(1), 32–37.

[47] Theoharides, T. C., Petra, A. I., Taracanova, A., Panagiotidou, S., & Conti, P. (2015). Targeting IL-33 in autoimmunity and inflammation. Journal of Pharmacology and Experimental Therapeutics, 354(1), 24–31.

[48] Vural, P., Degirmencioglu, S., Erden, S., & Gelincik, A. (2009). The relationship between transforming growth factor-beta1, vascular endothelial growth factor, nitric oxide and Hashimoto's thyroiditis. International Immunopharmacology, 9(2), 212–215. https://doi.org/10.1016/j.intimp.2008.11.003

[49] Wang, R.-X., Yu, C.-R., Dambuza, I. M., Mahdi, R. M., Dolinska, M. B., Sergeev, Y. V, Wingfield, P. T., Kim, S.-H., & Egwuagu, C. E. (2014). Interleukin-35 induces regulatory B cells that suppress autoimmune disease. Nature Medicine, 20(6), 633–641.

[50] Wang, X., Zhu, Y. F., Li, D. M., Qin, Q., Wang, Q., Muhali, F. S., Jiang, W. J., & Zhang, J. A. (2016). Polymorphisms of ST 2-IL 18R1-IL 18 RAP gene cluster: a new risk for autoimmune thyroid diseases. International Journal of Immunogenetics, 43(1), 18–24.

[51] Whitehead, G. S., Wilson, R. H., Nakano, K., Burch, L. H., Nakano, H., & Cook, D. N. (2012). IL-35 production by inducible costimulator (ICOS)–positive regulatory T cells reverses established IL-17–dependent allergic airways disease. Journal of Allergy and Clinical Immunology, 129(1), 207–215.

[52] Wirtz, S., Billmeier, U., Mchedlidze, T., Blumberg, R. S., & Neurath, M. F. (2011). Interleukin-35 mediates mucosal immune responses that protect against T-cell–dependent colitis. Gastroenterology, 141(5),

1875–1886.

[53] Xiangyang, Z., Lutian, Y., Lin, Z., Liping, X., Hui, S., & Jing, L. (2012). Increased levels of interleukin-33 associated with bone erosion and interstitial lung diseases in patients with rheumatoid arthritis. Cytokine, 58(1), 6–9. https://doi.org/10.1016/j.cyto.2011.12.010

[54] Xu, W.-D., Zhang, M., Zhang, Y.-J., & Ye, D.-Q. (2013). IL-33 in rheumatoid arthritis: potential role in pathogenesis and therapy. Human Immunology, 74(9), 1057–1060.

[55] Yang, Z., Liang, Y., Xi, W., Li, C., & Zhong, R. (2011). Association of increased serum IL-33 levels with clinical and laboratory characteristics of systemic lupus erythematosus in Chinese population. Clinical and Experimental Medicine, 11(2), 75–80.

[56] Yu, S., Fang, Y., Sharp, G. C., & Braley-Mullen, H. (2010). Transgenic expression of TGF-beta on thyrocytes inhibits development of spontaneous autoimmune thyroiditis and increases regulatory T cells in thyroids of NOD.H-2h4 mice. Journal of Immunology (Baltimore, Md. : 1950), 184(9), 5352–5359. https://doi.org/10.4049/jimmunol.0903620