

Heterogeneity of tissue IL-17 and tight junction proteins expression demonstrated in patients with autoimmune thyroid diseases

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Abstract

Th17 cells together with their hallmark cytokine interleukin (IL)-17 were identified as crucial contributing factors in the pathogenesis of thyroid autoimmunity. The cytokine-regulated tight junction (Tj) disruption is thought to be essential in the initiation and/or development of several diseases. Still, the role of IL-17 maintaining Tj integrity in autoimmune thyroid diseases (AITDs) has not yet been evaluated. We aimed to investigate integrity of the thyroid follicle by studying immunoreexpression of cellular Tj – zonula occludens (ZO)-1 and claudin-1 proteins coupled to IL-17A and CD68 detection in AITD patients compared with controls.

Thirty-five adult patients undergoing thyroidectomy and presenting 18 cases of Hashimoto thyroiditis (HT), 7 of Graves' disease (GD) as well as 10 subjects of colloid goiter without autoimmune component served as controls were enrolled in this study. An immunohistochemical analysis including IL-17A, ZO-1, claudin-1, and CD68 detection was performed in each case. The correlation of IL-17A with Tj and CD68 in patients with AITD was also analyzed.

Apart from inflammatory cells, we evidenced a stronger expression level of IL17A in the thyroid follicular cells in HT patients when compared with GD or colloid goiter. A significant reduction of ZO-1 immunoreactivity was observed in the thyrocytes in HT patients, whereas no significant differences were found in claudin-1 expression in HT and GD compared with colloid goiter patients. A significantly higher number of thyroid follicles with CD68-positive cells was found in HT patients than that in patients with GD or colloid goiter. In HT patients, the expression of IL-17A in the follicular cells was positively correlated with CD68 immunopositivity, whereas no association with claudin-1 or ZO-1 expression was found. GD patients did not reveal any significant correlation of IL-17A with Tj and CD68.

Strong overexpression of IL-17A observed in the thyroid epithelial cells is associated with the presence of intrafollicular CD68-positive cells in HT patients. We evidenced the changes in molecules of thyrocyte junctional complexes highlighting impairment of the thyroid follicle integrity in HT, but no association with IL-17A was found.

Abbreviations: AITD = autoimmune thyroid diseases, GD = Graves' disease, HT = Hashimoto thyroiditis, IL = interleukin, T helper = Th, Tj = tight junctions, TPO = thyroid peroxidase, TSHR = thyroid-stimulating hormone receptor, ZO = zonula occludens.

Keywords: Graves' disease, Hashimoto thyroiditis, interleukin-17, tight junction protein

1. Introduction

The prevalence and incidence of autoimmune diseases including autoimmune thyroid diseases (AITD) has increased significantly over the last decades.^[1] Thyroid autoimmunity occurs as a result

of immune system impairment leading to the breakdown of self-tolerance to the 3 major thyroid proteins and inflammatory cell, predominantly lymphocytic infiltration of the gland.^[2] It is critical to identify immunological and pathomorphological aspects involved in thyroid autoimmunity. AITD comprise 2 clinically and pathogenetically diverse phenotypes: Hashimoto thyroiditis (HT) and Graves' disease (GD). GD induced by thyroid-stimulating autoantibodies to thyroid stimulating hormone receptor leading to hyperthyroidism is considered to be a T helper type (Th)2-related disease, whereas in HT Th1-driven autoimmune response characterized by the lymphocytic infiltration results in apoptosis of thyrocytes and subsequent hypothyroidism.^[3,4] However, an evident humoral autoimmune response in HT patients and diversity of T cell mediated response and inflammatory infiltration in GD is also demonstrated.^[4,5]

Recently, Th17 cells and their hallmark cytokine (interleukin, IL)-17 have been recognized as crucial contributors to the pathogenesis of thyroid autoimmunity.^[6–8] Production of IL-17A and IL-17F is characteristically attributed to Th17 cells. These ILs can act on a broad range of cells, including epithelial cells, fibroblasts, and macrophages inducing the release of proinflammatory tissue mediators such as interleukins IL-1 β , IL-6, and IL-8, growth and hematopoiesis stimulating factors – tumor necrosis factor- α (TNF- α), granulocyte-macrophage colony-stimulating

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factor, and granulocyte colony-stimulating factor (G-CSF), and tissue components degrading enzymes metalloproteinases.^[9–12] Early studies demonstrated that IL-17 stimulates production of other cytokines such as IL-6, IL-8, and G-CSF in nonimmune cells such as connective tissue fibroblasts and epithelial cells via activation of the nuclear factor- κ B (NF- κ B) transcription factor.^[13,14] In addition, IL-17 demonstrates strong synergistic action being combined with other cytokines, such as IL-1 β and TNF α .^[15,16] Previous studies have shown that patients suffering from AITD have enhanced levels of IL-17A and Th17 lymphocytes revealed in blood and colonizing thyroid tissue as well as a marked *in vitro* differentiation of Th17 cells; however, the expression level of IL-17A in the thyroid epithelial cells assessed by immunohistochemistry has not been addressed thoroughly before.

Tight junctions (Tj), also called occluding junctions, are composed of a complex, interwoven network of membrane proteins and glycoproteins. These are distinguished at the apical part of the lateral membranous surface of polarized endothelial and epithelial cells. Tj maintain cell polarity and establish the barrier sealing the paracellular space between neighboring thyrocytes. Numerous studies have demonstrated that claudin family is the key component of Tj, fully performing paracellular barrier function in the absence of occludin.^[17] From the currently known 27 claudins expressed in humans, claudin-1 is considered to be the most important member responsible for the epithelial barrier integrity.^[18] Furthermore, claudin-1 deficiency appeared to cause vital alterations of barrier function leading to the death of claudin-1 deficient mice.^[19] Simultaneously, an essential role of interactions of zonula occludens (ZO)-1 protein and claudin for formation of Tj assembly have been recognized. Numerous studies with ZO-1 and/or ZO-2 knockdown cells demonstrated disruption in both claudin localization and paracellular permeability. Moreover, in the absence of ZO-1, these cells have been shown to exhibit polarity but fail to form Tj.^[20]

Continuing studies have highlighted a broad spectrum of cytokines able to affect the crucial function – barrier function of endothelial and epithelial cells by influencing the structural and functional characteristics of the Tj. Many ILs were found to modulate Tj proteins such ZO-1, occludin, and claudin proteins causing downregulation of these molecules^[21,22]; however, the role of IL-17 in the maintenance of Tj integrity in AITD patients has not yet been evaluated. This study investigates integrity of the thyroid follicle by studying immunoexpression of cellular Tj – ZO-1 and claudin-1 proteins coupled to IL-17A and CD68 detection in AITD patients compared with controls. The correlation of IL-17A with Tj and CD68 in patients with AITD was also assessed, thus making an attempt to determine the possible relationship between IL-17 and Tj integrity.

2. Methods

2.1. Patients

Twenty-five adult AITD patients presenting 18 cases of HT and 7 of GD were enrolled in this study. Ten age and gender-matched cases of colloid goiter without autoimmune component, which served as a control group, were also recruited. The mean age of AITD patients and controls was 49.68 ± 11.01 years (range, 28–68 years; 23 females) and 47.90 ± 11.29 years (range, 33–71 years; 9 females), respectively. AITD were confirmed by the clinical diagnostic criteria of HT and GD, complemented by results of thyroid biochemistry, ultrasound imaging, and histopathology findings. Patients with GD had thyroid hormone levels above the reference range, positive anti-thyroid stimulating hormone receptor (anti-TSHR) antibodies, and

diffuse goiter proved by ultrasound examination. Anti-TSHR antibodies were negative, whereas antibodies to thyroid peroxidase (anti-TPO) and/or thyroglobulin were found at high titers in all HT patients, and thyroid ultrasound examination showed signs of chronic autoimmune thyroiditis. Ten patients with colloid goiter were used as a control group. They were euthyroid and displayed the absence of thyroid antibodies. The current study was approved by the Riga Stradins University Ethics Committee, and all patients gave informed consent.

Thyroid tissue blocks of 35 thyroidectomy samples (equal to the number of patients enrolled in the study) along with the histopathology reports were obtained from Pathology Center of Riga East Clinical University Hospital, Latvia, between January 2013 and December 2014. All available specimens were analyzed, and a single representative paraffin-embedded block was chosen for immunohistochemical study.

2.2. Immunohistochemistry

Thyroid tissues were formalin-fixed and embedded in paraffin blocks; thereafter, 4 to 5 μ m thick sections were cut from blocks, collected and fixed on slides. The diagnosis was confirmed analyzing the slides stained routinely with hematoxylin and eosin. Immunohistochemical reactions were performed manually on deparaffinized thyroid tissue sections using the following primary antibodies: polyclonal rabbit anti-IL-17A (Biorbyt, Cambridge, UK, dilution 1:300); polyclonal rabbit anti-ZO-1 (Biorbyt, Cambridge, UK, dilution 1:200), and polyclonal rabbit anti-claudin-1 (Biorbyt, Cambridge, UK, dilution 1:100) antibodies. In addition, macrophages were visualized by monoclonal mouse anti-CD68 antibody (DacoCytomation, Glostrup, Denmark, clone PG-M1, dilution 1:100), which recognizes and labels lysosomal membranes and shuttle vesicles travelling between lysosomes and the plasma membrane of these cells. PG-M1 reacts strongly with a fixative-resistant epitope of macrophage-restricted intracytoplasmic molecule.

The results of immunohistochemical reactions were visualized by use of highly sensitive HRP Polymer system – HiDef Detection system manufactured by CellMarque, Rocklin, CA. The antigen sites were visualized applying 3,3'-diaminobenzidine tetrahydrochloride [DAB+Chromogen and DAB+Substrate buffer (CellMarque, Rocklin, CA)], whereas nuclei were stained with Mayer hematoxylin. Finally, the stained sections were mounted in Roti-Histokitt (Carl Roth, Karlsruhe, Germany). Negative immunohistochemical controls were performed either by omitting of the primary antibody or substitution of it with buffer solution. Two certified pathologists with no access to the clinical records and histopathological data contributed to the verification of the immunohistochemistry results. Tissue sections were analyzed applying both low and high power magnification from $\times 100$ up to $\times 400$. Microphotographs were taken with a Leitz DMRB microscope (Leica, Wetzlar, Germany) equipped with a digital camera Leica DFC 450C.

Immunofluorescence staining of IL-17A was performed using goat anti-mouse IgG-FITC: sc-2010 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, dilution 1:300); sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (Thermo Fisher Scientific, Invitrogen, UK, dilution 1:3000) and finally embedded in Prolong Gold with DAPI (Thermo Fisher Scientific, Invitrogen, UK). Digital images were captured using confocal microscope Eclipse Ti-E (Nikon).

Cells were considered immunopositive when labeled by the aforementioned antibodies revealed brown reaction products. IL-

17, claudin-1, and ZO-1 positive structures displayed either cytoplasmic or membranous reaction pattern.

Levels of immunopositivity for IL-17A and claudin-1 were assessed semiquantitatively distinguishing the following grades: negative (0), weak (1), moderate (2), or strong (3), when cells were positive at 0% to 5%, 6% to 25%, 26% to 50%, or >50%, respectively, whereas, for ZO-1 – negative (0), very weak or discontinuous (1), weak (2), and strong or intact (3) following the recommendations of Abd El Atti and Shash.^[23]

CD68 positivity was defined as the percentage of CD68-positive thyroid follicles determined by counting total number of thyroid follicles with CD68-positive cells in 15 representative fields at high magnification (×400).

2.3. Statistical analysis

Immunohistochemical results were expressed as medians and interquartile range. Mann–Whitney test was applied to examine differences between 2 groups and Kruskal–Wallis H test – for more than 2 study groups. To assess the potential correlations between estimated variables, Spearman rank correlation test was carried out. A 2-tailed significance level of .05 was applied. Statistical testing was performed using SPSS (version 20.0, IBM, Armonk, NY).

3. Results

Microscopic examination of slides processed for routine histopathology revealed widespread mild-to-extensive inflammatory infiltration of the thyroid gland by lymphocytes, macrophages, and plasma cells in AITD. Intraepithelial lymphocytes were also seen. HT patients demonstrated extensive diffuse inflammatory infiltration (7; 39%), lymphoid follicles formation (6; 33%), or rarely mild inflammation of the thyroid gland without lymphoid follicle formation (5; 28%). Some HT patients revealed a marked stromal fibrosis qualified as interfollicular presented with the deposition of increasingly accumulated collagenous fibers and lymphoplasmacytic infiltration in the interfollicular area, thus separating and distorting the thyroid follicles. Still, some tissue blocks obtained from HT patients revealed extensive destruction of thyroid parenchyma with minimal residual follicles. Simultaneously, the vast majority of GD patients presented with mild inflammatory cell infiltration and diffuse hyperplasia of the follicular cells.

The results describing immunohistochemical expression of the antigens tested in the given study and applied to the study groups are summarized in Table 1. In HT patients, the expression of IL-17A was detected both in the inflammatory lymphocytic infiltrates and follicular epithelial cells confirmed by immunohistochemistry and immunofluorescence (Fig. 1A). Notably, thyroid epithelial cells located close to lymphocytes or surrounded by inflammatory cells had a strongly marked IL-17A immunopositivity. Moreover, these thyroid follicles revealed atrophic

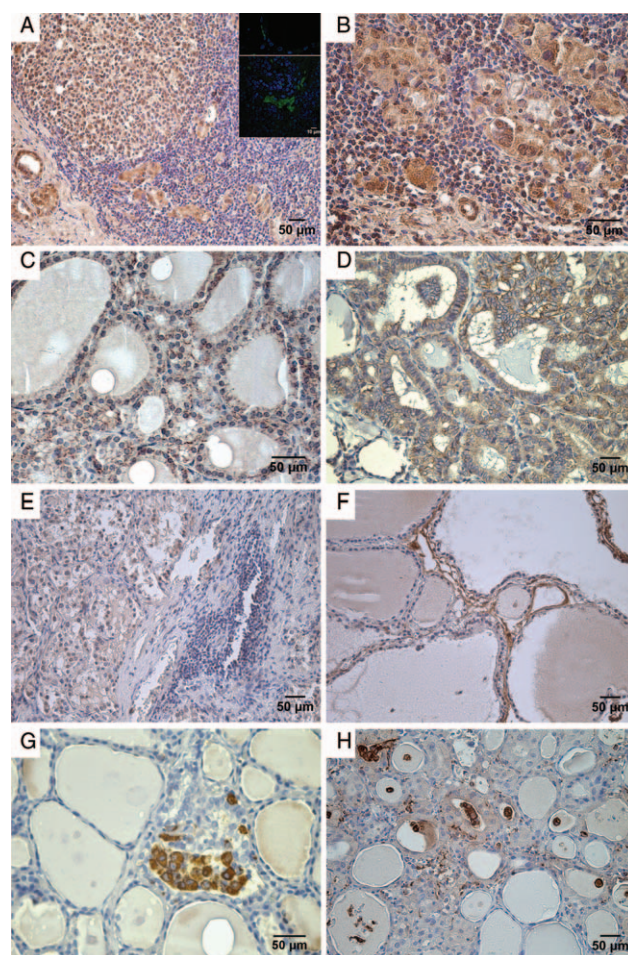


Figure 1. Immunohistochemistry. (A) IL-17A positivity demonstrated in the remnants of thyroid follicles and lymphoid follicles as well as in some inflammatory cells in HT patient (x200). Confocal microscopy, representative images of IL-17A positive thyrocytes, and lymphocytes: green staining shows IL-17A specific staining, blue staining shows nuclei, inserts (x1000). (B) Destructive IL-17A positive thyroid follicles surrounded by inflammatory cells in HT patient (x400). (C) Moderate circumferential membranous claudin-1 immunostaining revealed in GD patient (x400). (D) Strong membranous claudin-1 expression in the thyroid follicular cells in HT patient (x250). (E) Weak ZO-1 immunoreactivity in GD patient (x250). (F) Colloid goiter evidencing weak to moderate and moderate positive ZO-1 expression in the thyrocytes and vascular endothelium, respectively (x250). (G) Cluster of CD68-positive cells demonstrated within the thyroid follicular cells in HT patient (x400). (H) Occasional intrafollicular CD68-positive cells individually distributed within the lumen of thyroid follicles in HT patient (x250). GD=Graves’ disease, HT=Hashimoto thyroiditis, IL=interleukin, ZO=zonula occludens.

Table 1
Immunohistochemical expression patterns of tested antigens in patients with autoimmune thyroid diseases and colloid goiter.

	IL-17A median (IQR)	ZO-1 median (IQR)	Claudin-1 median (IQR)	CD68 median (IQR)
Hashimoto thyroiditis	2.4 (1.8–2.8)	0.5 (0.5–1)	1.5 (1–2.2)	23.5 (8.9–38.3)
Graves’ disease	1.4 (1–2.4)	2 (1.5–2)	1.2 (1–1.4)	10.8 (5.4–13.2)
Colloid goiter	0.8 (0.6–1)	2 (1.5–2.5)	1.6 (1.2–2.0)	5.6 (3.1–8.7)

IL = interleukin, IQR = interquartile range, ZO = zonula occludens.

changes with destruction of epithelial cells and nuclear polymorphism (Fig. 1B). By contrast, the tissues obtained from ordinary colloid goiter and GD patients demonstrated mostly the weak or almost negative expression of IL-17A. Only in 1 case of GD and 1 case of colloid goiter the grade of IL-17A positivity was ≥ 3 and ≥ 2 , respectively. The highest expression level of IL-17A in the follicular epithelial cells was observed in HT patients: 11 out of 18 cases demonstrated IL-17A staining grade of 2 to 3. Furthermore, in HT and GD patients, it was significantly higher than colloid goiter ($P < .001$; $P = .007$, respectively) (Fig. 2A).

When studying the occurrence and localization of Tj proteins, and more specifically claudin-1 by the use of immunohistochemistry, we found that it displayed a circumferential and

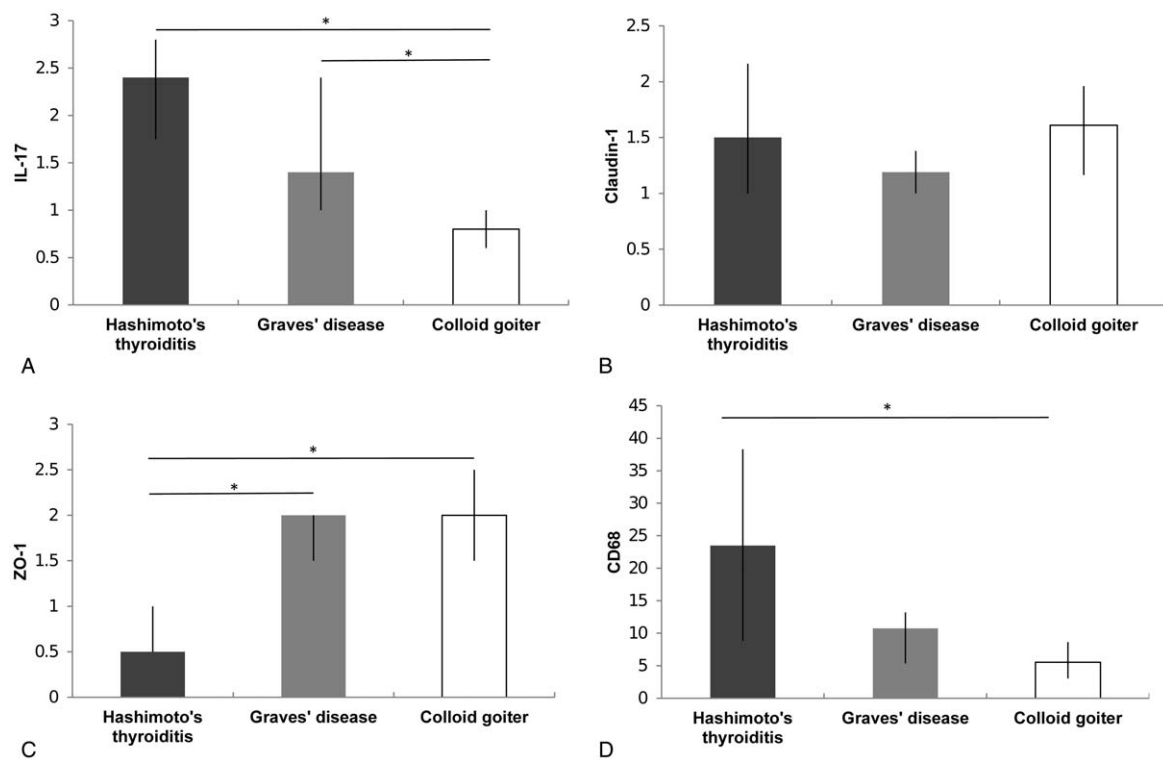


Figure 2. Comparison of immunohistochemical results between Hashimoto thyroiditis (n=18), Graves' disease (n=7), and colloid goiter (n=10) patients. (A) represents expression levels of IL-17A, (B) the number of CD68-positive thyroid follicles, (C) claudin-1 expression levels, and (D) immunopositivity levels of ZO-1 in AITD and colloid goiter patients. Comparisons were made by Mann-Whitney *U* test. Data are shown as median (interquartile range); AITD=autoimmune thyroid diseases, IL=interleukin, ZO=zonula occludens. **P* < .05.

membranous pattern related to the thyroid epithelial cells (Fig. 1C, D). The expression of claudin-1 was greatly varying from being almost nil in one-third of HT cases (6; 33%), but still being heavily expressed in others (5; 28%). There were no significant differences found in claudin-1 expression levels between patients with AITD and colloid goiter (Fig. 2B). However, the number of claudin-1 positive follicles in HT patients was significantly higher than that demonstrated in colloid goiter (*P* = .03 and data not shown).

Regarding the second Tj protein – ZO-1 in the thyroid tissue samples studied, HT patients demonstrated predominantly negative and very weak or discontinuous ZO-1 expression patterns. In 10 HT cases (56%), ZO-1 staining score was 0 to 1, whereas only 1 specimen (14%) showed such staining score in GD group. Immunohistochemical analysis revealed a significant reduction of ZO-1 expression level in the follicular epithelial cells in HT patients when compared with colloid goiter and GD patients (*P* < .001 for both) (Fig. 1E, F, 2C). ZO-1 immunopositivity did not differ significantly between these 2 last groups of patients (*P* = .47).

CD68-positive macrophages of both intra- and extrafollicular localization were observed in all tissue samples. The cells were irregularly shaped and displayed a vacuolated cytoplasmic appearance being arranged either in clusters or individually distributed within the thyroid follicles (Fig. 1G, H). Large clusters of intrafollicular CD68-positive cells were found exclusively in HT patients, whereas occasional CD68 positive cells – mostly in GD and colloid goiter. Lymphocytes, along with macrophages, were also seen within the follicles. A significantly higher number

of thyroid follicles with CD68-positive cells was found in HT patients than that in patients with GD or colloid goiter (*P* = .05 and *P* = .001, respectively) (Fig. 2D). CD68 positivity observed in GD patients was higher than colloid goiter, but the difference was not significant (*P* = .06).

In HT patients, the expression of IL-17A in the follicular cells was positively correlated with intrafollicular CD68 immunopositivity (*r* = 0.631, *P* = .005). When analyzing the correlation between IL-17A and Tj proteins expression, no significant relationship between IL-17A and ZO-1 or claudin-1 was found in HT patients (*r* = 0.267, *P* = .28 and *r* = 0.194, *P* = .44, respectively). GD patients did not reveal any significant correlation of IL-17A with Tj – ZO-1 and claudin-1 (*r* = -0.199, *P* = .67 and *r* = 0.734, *P* = .06, respectively) and CD68 (*r* = 0.631, *P* = .13).

4. Discussion

Although Th1-driven autoimmune response has long been considered as dominant for HT development, recent studies are suggesting an evident participation of Th17 cells in AITD, particularly HT pathogenesis. In our study, we identified higher expression levels of tissue IL-17A assessed by immunohistochemistry in thyroid samples from HT patients compared with GD and colloid goiter. Similarly, Li et al^[24] detected that HT patients exhibited increased levels of thyroid tissue infiltrating Th17 cells as well as higher IL-17 levels in sera than controls and patients with other thyroid pathologies. In addition, an association between thyroid IL-17 expression and fibrosis evidenced within the gland stroma was demonstrated in patients suffering from

HT.^[24] Figueroa-Vega et al^[6] evidenced increased levels of IL-22 and IL-17 both in colonizing thyroid tissue and peripheral blood from HT patients, as well as high levels of Th17 differentiation affecting cytokines, such as IL-6, IL-15, and IL-23. Finally, Konca Degertekin et al^[25] found that HT patients displaying normal thyroid function had higher circulating levels of IL-17 and IL-23 than HT patients suffering from hypothyroidism suggesting that hypothyroidism may have an inhibitory effect on cytokine production. Overall, these findings provide strong supportive evidence of Th17 cell involvement in the development of HT.

Participation of Th17 lymphocytes and their produced cytokines in GD pathogenesis is less understood; however, Peng et al^[26] indicated significantly enhanced levels of both Th22 and Th17 cells revealed in peripheral blood, paralleled by higher detectable concentrations of IL-22 and IL-17 in blood plasma of Chinese patients with newly diagnosed GD compared with controls. We also found a difference in IL-17A expression between GD and colloid goiter. Still, it seems the association between Th17 lymphocytes and development of GD is less obvious than that of HT.

IL-17A positive cells were found within inflammatory cell infiltrates invading the thyroid gland. Apart from inflammatory cells, the given study demonstrated a strongly marked expression of IL-17 in the thyroid follicular cells surrounded by lymphocytes or located close to them. Various cell types, including epithelial, Paneth cells and smooth muscular cells, neutrophils and mast cells, natural killer cells as well as $\gamma\delta$ and $\alpha\beta$ T lymphocytes have been recognized to express IL-17 from A to F.^[27] Previous studies have revealed that thyroid follicular cells expressing MHC class II molecules colocalized with B7.1 antigens and interacting with CD4⁺ T cells allow the thyrocyte to act as an antigen-presenting cell initiating and/or maintaining the autoimmune process.^[28] Therefore, thyrocytes may have a much larger role to play in the pathogenesis of AITD than initially thought.

A vast majority of intrafollicular CD68-positive cells were observed in patients with HT being indicative of their migration to the thyroid follicular lumen. It is worth noting that these patients displayed a strong positive correlation between CD68 and IL-17A immunopositivity as well. Although we have not found any significant correlation between IL-17 and Tj proteins expression, accumulation of macrophages in the follicular lumen can be interpreted as an impairment of thyroid epithelium integrity. It has been reported previously that anti-TPO antibodies caused damage of cultured thyrocytes performed upon binding to TPO located on the apical cell membrane via both antibody (ADCC) and complement-dependent cytotoxicity; moreover, the authors demonstrated that monocytes via their Fc γ RI are pivotal players in ADCC mediated by thyroid antibodies.^[29] Thus, understanding and describing the role of intrafollicular macrophages in HT pathogenesis is of great interest.

Due to the structural peculiarities of the wall of thyroid follicle, the pass for anti-TPO antibodies attempting to get access to the molecule expressed on the apical pole of thyrocyte facing colloid and nonaccessible to immune cells is still poorly understood. Some studies have assumed that TPO might be translocated by lateral diffusion to the basolateral pole of the thyrocytes and recognized by macrophages and/or dendritic cells.^[30] Another explanation of the disturbance of follicular integrity highlights modifications of Tj proteins enabling the migration of antigen-presenting cells across thyroid follicular epithelium.^[31]

In this study, we evaluated Tj ZO-1 and claudin-1 protein immunoreactivity in thyroid tissues obtained from patients with AITD and colloid goiter. A significant reduction in ZO-1 expression noted in our study in HT patients correlates with the disturbed integrity of thyroid epithelium that appears in HT. By contrast, a higher number of claudin-1 positive follicles in HT patients was demonstrated in HT when compared with colloid goiter. The aforementioned differences in claudin-1 and ZO-1 expression observed between HT and GD patients are in agreement with a study describing under-expression of ZO-1 in thyroid tissues of HT patients, although claudin-1 appeared to be more strongly expressed.^[31] We speculate that increased claudin-1 immunoreactivity in HT patients might be interpreted as a cell survival and/or compensatory mechanism.

Several paracrine factors including cytokines and growth factors have been recognized as ones affecting the distribution of Tj and regulating the epithelial barrier function.^[32] IL-17A was shown to downregulate expression of occludin thus having a disruptive effect on the hematoencephalic and testicular barrier.^[21,22] However, whether the elevated IL-17 in HT affects Tj integrity and thereby alters the barrier function of thyroid follicular cells is unknown. A distinguishable negative effect of IL-1 α on Tj and a loss of epithelial cell integrity have been described previously in cultured thyroid cells.^[33] Recently, Rebuffat et al^[31] showed that Tj protein – ZO-1, claudin-1, and JAM-A protein organization and expression as well as localization in the thyroid tissue of patients suffering from AITD are modified by proinflammatory cytokine IL-1 β thus affecting thyroid epithelium integrity. Zhang et al^[34] explored the signaling pathway that links IL-17 with Tj in salivary gland cell culture and postulated that IL-17 could directly modulate Tj protein expression and distribution through the NF- κ B signaling pathway. To evaluate whether there is a relationship between IL-17 and Tj disruption, we did correlation analysis between the levels of IL-17 and Tj proteins expression in AITD patients, but no significant association with IL-17A was found.

We recognize limitations of our study, including a small number of thyroid tissue sections, semiquantitative measures of immunoexpression, and the lack of data regarding the expression of Treg cytokines, as nowadays both T cell types should be investigated as important players in AITD pathogenesis. The given study was retrospective in nature and thus was based on analysis of archived paraffin-embedded tissues. Because of methodological limitations, our knowledge about cytokine-regulated Tj disruption remains insufficient and interpretation of our findings regarding the association of IL-17 and Tj should be cautious. Therefore, further prospective studies based on larger number of samples and methods estimating causal effect between IL-17 and Tj disruption are required. A growing number of evidence including this deciphering autoimmune disease demonstrates involvement of Tj barrier disruption and increased paracellular permeability.^[35] Therefore, we believe that a proper understanding of the pathogenic role of IL-17 and the imbalance in the expression and distribution of Tj in AITD may contribute to the development of new therapeutic approaches in the future.

5. Conclusion

Our study evidenced a strong IL-17A overexpression in the thyroid epithelial cells associated with the presence of intra-follicular CD68-positive cells in HT patients, suggesting that the

role of affected thyrocytes and antigen-presenting cells in thyroid autoimmunity might extend far deeper than initially thought. Simultaneously, we evidenced the changes in molecules of thyrocyte junctional complexes highlighting impairment of the thyroid follicle integrity in HT, but no significant association with IL-17A was found.

Author contributions

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