# RELATIONSHIP BETWEEN IMMUNOEXPRESSION OF IL-17A, CD68 POSITIVITY AND LYMPHOCYTE INFILTRATION DEGREE IN CHRONIC AUTOIMMUNE THYROIDITIS

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## Introduction

Besides two classical functions, the barrier function and the fence function, tight junctions (Tj) are considered to be a crucial component of innate immunity and their dysfunction could be intimately related to various diseases. Tj proteins ZO-1 and ZO-2 are essential for formation of Tj, and in the absence of ZO, epithelial cells fail to form Tj.

Th17 were reported to be involved in the development of autoimmune orchitis and encephalomyelitis by impairing barrier integrity and by inducing local inflammation. However, the role of their hallmark cytokine IL-17 in autoimmune thyroid disease (AITD) is still debatable. Alterations of follicular tightness through modifications in the expression of junction proteins could be significant in the development of AITD and may be influenced by numerous cytokines including IL-17.

The aim of our study was to estimate immunoexpression of CD68, tight junction protein ZO-1 and IL-17A in the thyroid tissue of patients with Hashimoto's thyroiditis (HT) and Graves' disease (GD) compared to control group.

## Materials and Methods

35 paraffin-embedded thyroid tissue blocks from the adult patients undergoing thyroidectomy were obtained from Pathology Center of Riga East Clinical University Hospital. These 35 cases comprised 18 cases of HT, 7 of GD, and 10 cases of nodular goiter without autoimmune component served as controls. Immunostaining was performed using an anti-ZO-1 and anti-IL-17A antibodies. Macrophages were visualized by CD68 immunohistochemistry. Results were expressed in a semiquantitative manner based on the expression level calculated as the percentage of thyroid follicular cells with positive staining: a) 0, IL-17 staining in <5% of the cells; 1, staining in 5–25% of the cells; 2, staining in 26–50% of the cells; 3, staining in >50% of the cells; b) intact ZO-1 staining was graded - 3, weak immunostaining - 2, very weak or discontinuity of immunostaining - 1, and absent staining overall as 0.

## Results

HT patients demonstrated extensive diffuse inflammatory infiltration (grade 3), lymphoid follicles formation (grade 2) or rarely mild inflammatory infiltration of the thyroid gland without lymphoid follicle formation (grade 1) (Figs. 9 and 10). The lowest expression level of ZO-1 in the follicular epithelial cells was observed in HT patients (Fig. 2), furthermore, it was significantly lower than that in control group and GD patients ($p<0.001$ and $p<0.001$, respectively). A strong positive correlation was observed between the degree of inflammatory cell infiltration and the number of CD68-positive macrophages in HT patients ($r=0.912$, $p=0.0001$) (Fig. 4). In addition, HT patients demonstrated a moderate positive correlation between CD68 and IL-17A immunopositivity (r=0,631, p=0,005) (Fig. 5). All patients demonstrated a negative correlation between ZO-1 expression and inflammatory infiltration degree, CD68 and IL-17A immunoreactivity (r=-0,468, p=0,005, r=-0,434, p=0,009, and r=-0,469, p=0,004, respectively) (Figs. 6-8).

## Figures

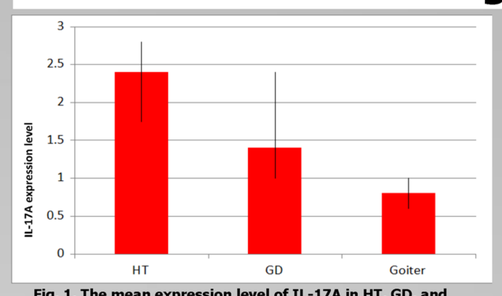



Fig. 1. The mean expression level of IL-17A in HT, GD, and control group.

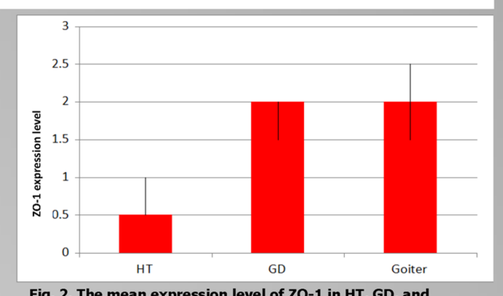



Fig. 2. The mean expression level of ZO-1 in HT, GD, and control group.

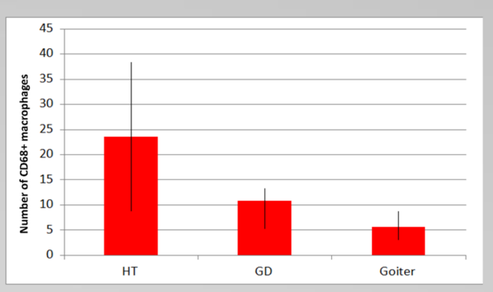



Fig. 3. The number of CD68-positive macrophages within follicular lumen in HT, GD, and control group.

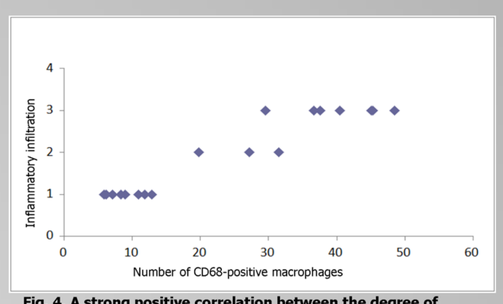



Fig. 4. A strong positive correlation between the degree of inflammatory cell infiltration and the number of CD68-positive macrophages in HT (r = 0.912, p = 0.0001).

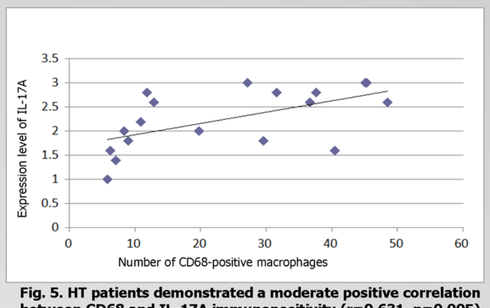



Fig. 5. HT patients demonstrated a moderate positive correlation between CD68 and IL-17A immunopositivity (r=0,631, p=0,005).

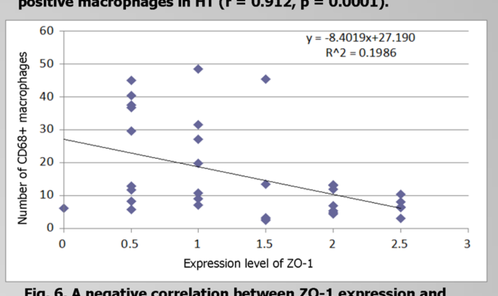



Fig. 6. A negative correlation between ZO-1 expression and and the number of CD68-positive macrophages was found in all patients (r=-0,434, p=0,009).

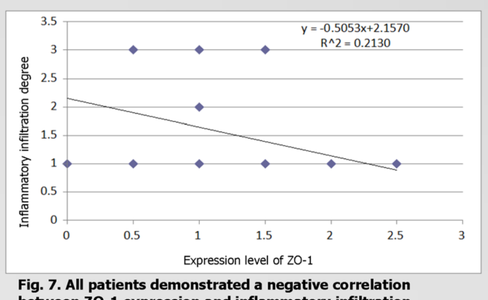



Fig. 7. All patients demonstrated a negative correlation between ZO-1 expression and inflammatory infiltration degree (r=-0,468, p=0,005).

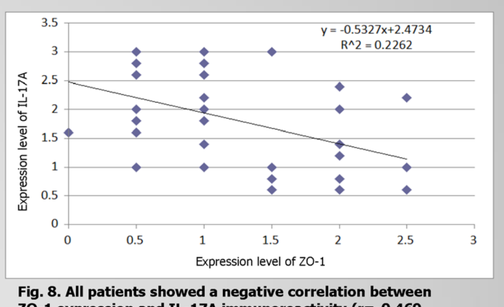



Fig. 8. All patients showed a negative correlation between ZO-1 expression and IL-17A immunoreactivity (r=-0,469, p=0,004).

## Conclusion

Underexpression of ZO-1 is associated with overexpression of IL-17A, CD68 and enhanced inflammatory infiltration degree in AITD leading to tight junction disruption and may indicate the involvement of IL-17 in thyroid follicular barrier impairment in HT patients.

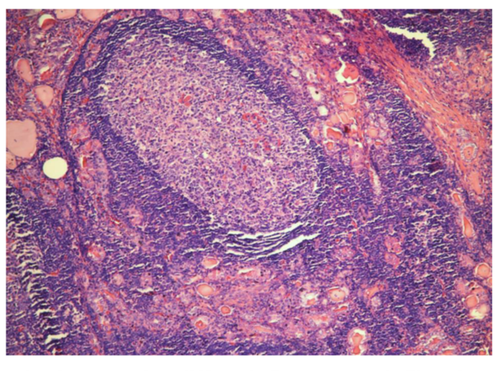


Fig. 9. Extensive diffuse inflammatory infiltration, parenchymal atrophy and fibrosis (H&E x100).

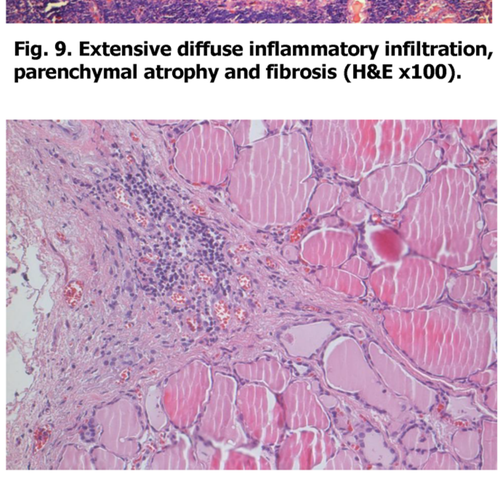


Fig. 10. Mild inflammatory infiltration and fibrosis of the thyroid gland (H&E x 250).

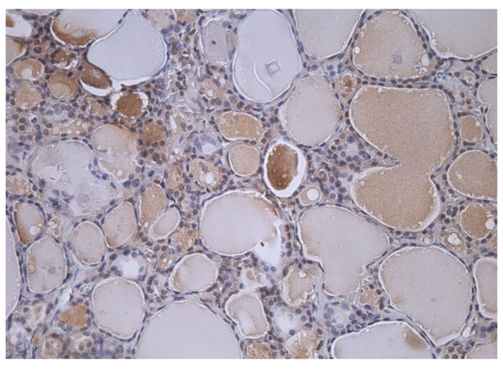
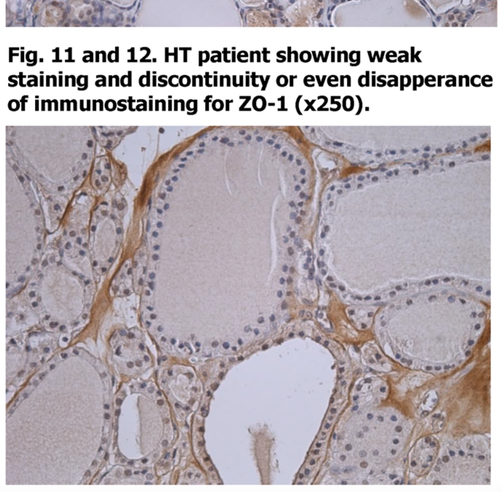


Fig. 11 and 12. HT patient showing weak staining and discontinuity or even disapperance of immunostaining for ZO-1 (x250).



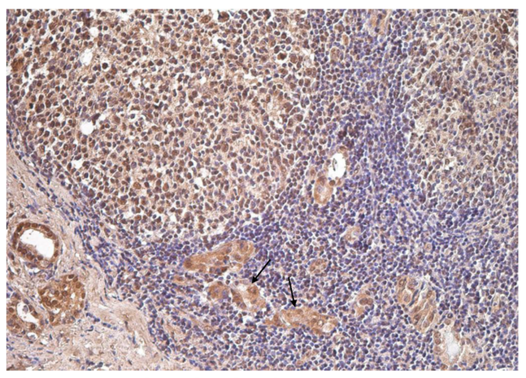


Fig. 13. HT patient showing destroyed IL-17 positive thyroid follicles and extensive inflammatory infiltration (x200).

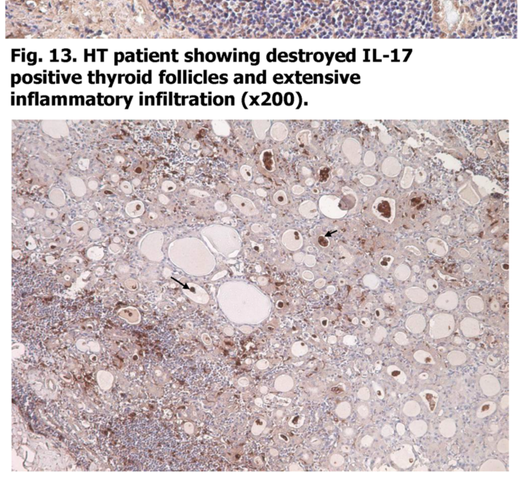


Fig. 14. CD68 positive macrophages individually dispersed within the thyroid follicles of HT (×100).

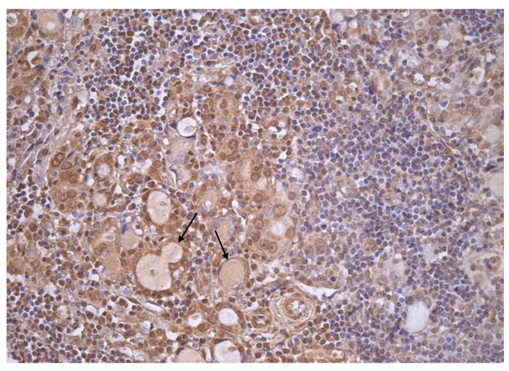


Fig. 15. The expression of IL-17A was detected both in the lymphocyte-infiltrated thyroid tissues and in the follicular epithelial thyroid cells of HT patients (x200).

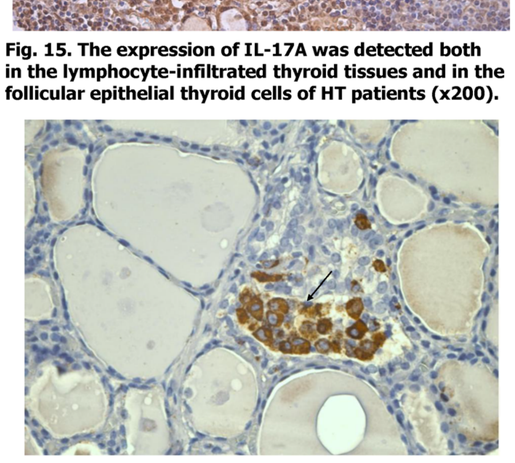


Fig. 16. Cluster of CD68 positive macrophages within the thyroid follicle of HT patient (×400).

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