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Role of Pro-Inflammatory and Regulatory Cytokines in Pathogenesis of Graves' Disease in Association with Autoantibody Thyroid and Regulatory FoxP3 T-Cells

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***Abstract*—**Background: Graves' disease (GD) is an autoimmune thyroid disease. Imbalance of Th1/Th2 cells and T-regulatory (Treg)/Th17 cells was thought to play pivotal role in the pathogenesis of GD. Treg FoxP3 produced TGF-β to maintain regulatory function, and Th17 cells produced IL-17 as cytokines that were thought in mediating several autoimmune diseases. The aim of this study is to assess the role of IL-17 and TGF-β in the pathogenesis of GD and to investigate its correlation with Thyroid Stimulating Hormone Receptor Antibody (TRAb) and Treg FoxP3 expression. Method: 30 GD patients and 27 age and sex-matched controls were enrolled in this study. Diagnosis of GD was based on clinical and biochemical of GD. Serum IL-17, TGF-β, TRAb, and FoxP3 were measured by enzyme-linked immunosorbent assay (ELISA). Data were analyzed by using SPSS 21.0 (SPSS Inc.). Spearman rank correlation test was used for assessment of correlation. The statistical significance was accepted as $P<0.05$. Result: There was no significant correlation between IL-17 and TGF-β serum with expression of FoxP3 level in GD, but there was significant correlation between TGF-β and TRAb serum level ($P<0.05$). Serum levels of IL-17 and TGF-β were found to be elevated in patient group compared to control, where mean values of IL-17 were 14.43±2.15 pg/mL and TGF-β were 10.44±3.19 pg/mL in patients group; and in control group, level of IL-17 were 7.1±1.45 pg/mL and TGF-β were 4.95±1.35 pg/mL. Conclusion: Serum Il-17 and TGF-β were elevated in GD patients that reflect the role of inflammatory and regulatory cytokines activation in pathogenesis of GD. There was significant correlation between TGF-β and TRAb, revealing that Treg cytokines may play a role in pathogenesis of GD.

***Keywords*—**IL-17, TGF-β, FoxP3, Graves' disease.

I. Introduction

GD is an autoimmune thyroid disease, which affects 1.2% of western populations (0.5% clinical and 0.7% subclinical) and 0.4% of Indonesian populations [1]. In GD, T-helper cells balance shift may play a key role in pathogenesis of the disease [2]. Evidences indicated that destruction of the balance of Th1/Th2 cells and Treg/Th17 cells could alter the expressions of pro- and anti-inflammatory cytokines resulting in thyroid lymphocytic infiltration and B cell activation, with antibody production against thyroid antibody [3]. This condition may lead to overproduction of TRAb, secretion of thyroid hormone, thus resulting hyperthyroidism and goiter. However, the exact etiology of this disease is not well understood, with the consequence that treatment of GD has not changed over the past 50 years [4].

In a mouse model of GD, Treg cells appear to be crucial in the pathogenesis of GD. The previous studies report an apoptosis induced decrease in the proportion of Treg cells in patients with autoimmune thyroid (AITD). Forkhead box P3 (FoxP3) is a major regulatory factor for Treg cells, and expression of FoxP3 is crucial for Treg development and function in regulating immune system. TGF-β is a cytokine that is induced by Treg cells to inhibit the actions of T and B cells, which act as a regulatory cytokine that may suppress autoimmune diseases [5], [6].

IL-17 is a pro-inflammatory cytokine produced by Th17. Before the discovery of the Th17, as a distinct CD4+ effector population, it was considered that Th1, Th2, and B cells were the main mediators of pathology in autoimmunity, including GD. The discovery of Th17 cell as a T cell subset led to a re-kindling of interest in this cytokine in the context of autoimmunity [7]. The aim of this study is to assess the role of pro-inflammatory cytokine, IL17 and regulatory cytokines, TGF-β in the pathogenesis of GD and to investigate the correlation of these cytokines with expression of Treg FoxP3 and TRAb of GD.

II. Methods

A. Subjects

30 GD patients were recruited from Endocrinology and Diabetes Mellitus Outpatient Department of M. Djamil Hospital in Padang, Indonesia based on clinical and biochemical diagnosis of GD. Patients with history of anti-thyroid drugs medicaments and history of other autoimmune disease were excluded from the study. Age, gender, thyroid function status such as FT4 (free thyroxin), and thyroid stimulating hormone (TSH) were measured and noted as baseline characteristics of this study.

B. Serum

Venous blood samples (5-10 ml) were taken in vacutainer tubes under sterile conditions from patients and control between 09:00–11:00 am. Serum was obtained from freshly

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drawn, rapidly centrifuged samples. Serum was quickly frozen at -20 °C and stored until the end of process.

C. Cytokines, Thyroid Antibody, FoxP3 Detections

Serum of IL-17 and TGF-β were measured by ELISA. These assays detected only human cytokines, and the minimum detectable concentrations in our laboratory were 4.6 pg/mL for IL-17 and 80 pg/mL. Thyroid antibody of GD (TRAb) and FoxP3 were measured by ELISA technique with normal range <1 nmol/L and 3-20 pg/mL, respectively.

D. Statistical Analysis

All data were analyzed by using the statistical package for social science (SPSS) 21.0 for Windows program on the computer. All data were given as mean ± standard deviation (SD). Serum cytokines levels were analyzed by using the normality test. Spearman rank correlation test was used for the assessment of correlation. The statistical significance was accepted as $P<0.05$.

III. Results

A total of 30 patients (20 females, 10 males), of mean age 40.6±14.8 (range: 19-74) years, and 27 age and sex-matched healthy controls were enrolled in this study as described in baseline characteristic (Table I). The diagnosis of GD was confirmed by measurement of serum levels of free T4 (FT4), TSH, and TRAb. Circulating TSH levels were significantly lower, while FT4 levels were significantly higher in patients with GD. In addition, the titers of TRAb were significantly increased in patients with GD.

TABLE I
BASELINE CHARACTERISTIC OF GD PATIENTS (N=30)

Characteristic	Mean	P value	Normal Range
Age (years old)	40.6±14.8	P>0.05	-
Gender (M/F)	20/10	P>0.05	-
FT4 (nmol/L)	74.28±61.1	P<0.05	9-20
TSH (IU/mL)	0.1±0.65	P<0.05	0.25-5
TRAb (pg/mL)	5.63±3.72	P<0.05	< 1
FoxP3 (pg/mL)	23.51±15.7	P<0.05	0.3 – 20

The mean and standard deviation of the serum levels of IL-17 and TGF-β of both groups and statistical results are presented in Fig. 1. All the mean values of cytokines levels of patients were significantly higher than those of controls. The serum cytokines profiles were evaluated in both GD patients and control individuals. Statistical analysis of these cytokines revealed that IL-17 and TGF-β levels were elevated in GD patients compared to controls. The mean values of IL-17 and TGF-β were 14.43±2.15 pg/mL and 10.44±3.19 pg/mL in patient group and 7.1±1.45 pg/mL and 4.95±1.35 pg/mL in control group, respectively.

In another part of the study, the correlation between the cytokines and FoxP3 and thyroid antibody (TRAb) was analyzed (Fig. 2). It was found that there is no relationship between serum cytokine levels and FoxP3, but there is significant correlation between TGF-β with TRAb ($r = 0.81$, $P<0.05$) (Fig. 2 (b)) in these research samples. Moreover, there is significant association which was observed between FoxP3 and thyroid antibody, TRAb in GD patients ($r = 0.03$, $P<0.001$) (Fig. 3).

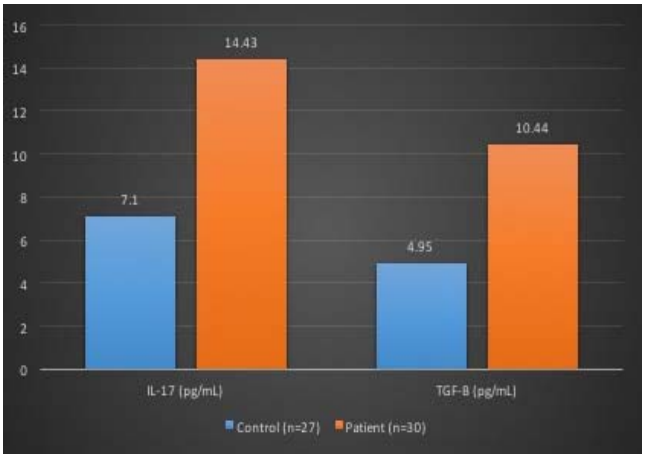

Fig. 1 Result of IL-17 and TGF-β serum levels of control and study group (Control group = blue bar; Patient group = orange bar)

IV. Discussion

GD is a kind of autoimmune receptor disease mediated by autoantibodies against thyrotropin receptor (TRAb). The etiology of GD is not well understood and it appears that different causative factors implicate in the pathogenesis disease [8].

Among these factors, autoimmunity has a high possibility of causing GD. Thyroid dysfunction in GD has an impact correlation with autoimmunity. The T-helper cells balance shift may be existed in patients with GD. Several studies showed that the imbalance of Th1/Th2 cells have a key role in pathogenesis of GD, but after discovery of other T cell subsets, such as regulatory T cell and Th17, research about whether those subsets also have a role to the pathogenesis was developing [3], [8].

The first aim of the study is to assess cytokine of regulatory T cell, TGF-β in pathogenesis GD. In the present study, we found increasing of serum TGF-β levels in GD patients compared to control ($P<0.05$). TGF-β were known to be implicated in the induction/maintenance of tolerance and prevention of autoimmunity. TGF-β is also a potent regulator of Teff differentiation, and generally inhibits Th cell functions. TGF-β, through induction of Treg FoxP3 cells, block Th1 cell differentiation by reducing IL-12 receptor β2 (IL-12Rβ2) and T-bet expression, and Th2 cells by inhibiting the expression of GATA-3. In this study, we also found increasing of serum FoxP3 levels that expressed Treg level in ELISA method. Increased number of Treg in thyroid as well in peripheral blood has been reported indicating that Tregs may be deficient in their downregulatory effects in GD. No correlations found between the levels of FoxP3 serum levels and TGF-β serum levels in peripheral blood of GD patient [9]-[11].

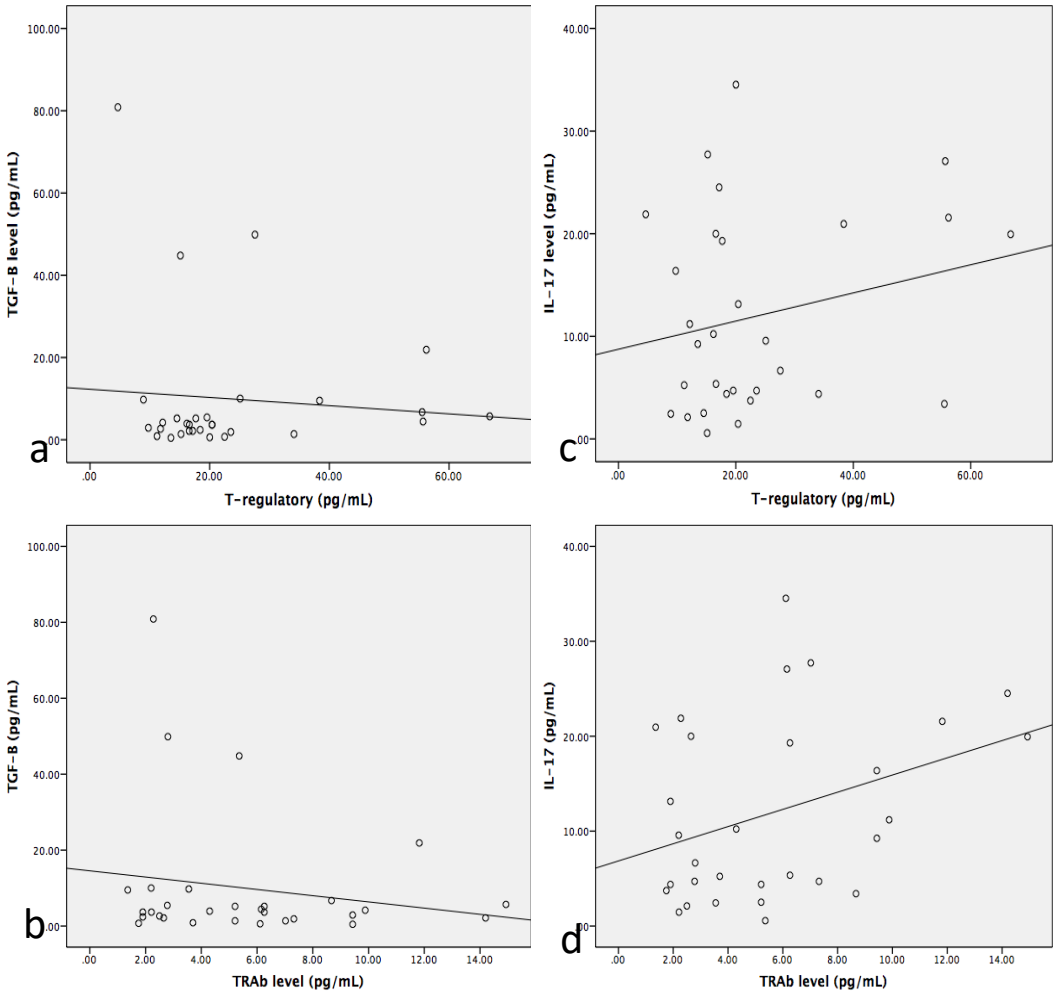

Fig. 2 Correlation of TGF-β and IL-17 with thyroid antibody (TRAb) and Treg cell using FOXP3 marker

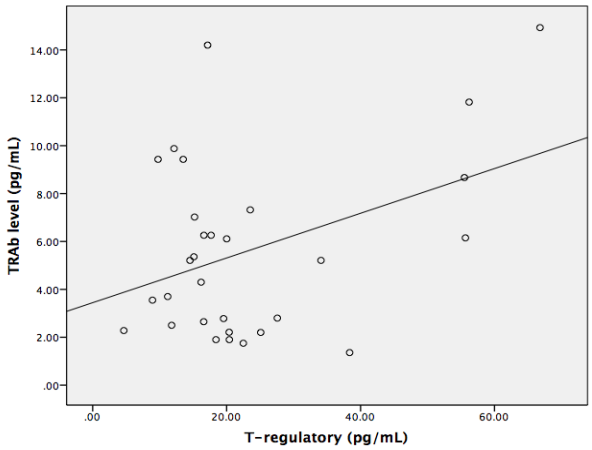

Fig. 3 Correlation between thyroid antibody (TRAb) and Treg cell in GD patients

Second, we are trying to assess whether there is correlation between IL-17 and FoxP3 serum levels. As mentioned before, IL-17 is a pro-inflammatory cytokine that produced by Th17, a new subset of CD4+ T cell that together with Th1, Th2 play essential role in immune system, especially in autoimmune disease [12]. In our research, there is no correlation between IL-17 and FoxP3, but we found the increased level of IL-17 serum in GD patients. The increased serum of IL-17 was also found in [12] and [13] that investigated IL-17 serum in patients with GD and it was dependent on disease activity and severity. However, Zheng et al. reported increased expression of IL-17 mRNA also in euthyroid patients with GD and it was upregulated after stimulation of IL-23 [14]. Shi et al. suggested that Th17 plays a central role in autoimmune thyroid disease, especially in Hashimoto's thyroiditis rather than GD [15].

Third, we investigate correlation between these cytokines with thyroid antibody of GD, TRAb. On this research, we only found significant correlation between TGF-β and TRAb, but there is no correlation with IL-17. From this result, we can conclude that the role of regulatory cytokine, as described in TGF-β that is produced by Treg, has a key role in immune pathogenesis of GD. As seen in Fig. 3, the higher levels of

autoantibody thyroid are produced in GD. TGF-β is an essential cytokine for Treg development and function. Tregs have a central role in prevention of GD development, and a major mechanism applied to delay GD onset by Treg is TGF-β secretion. Functional defect related to signaling pathways leading to TGF-β production in these cells or other TGF-β sources in GD may be the reason for the dysfunction of the cytokine [16], [17]. Furthermore, we found the significant correlation between TRAb serum level and FoxP3 serum level, that is consistent with correlation of TGF-β cytokine with autoantibody as described previously. These research works strengthen the possibility role of Treg in GD, specifically in immune regulatory function, whereas GD is an autoimmune disease stimulated by autoantibodies of TSHR, that is induced by B cell overproduction because of imbalanced of Th1/Th2, which is normally regulated by Treg. For the further research, it is also important to investigate the role of Th17 in the other T cell subset, such as Th1, Th2, and Treg, to recognize more about role of T cell subsets in GD pathogenesis.

V. Conclusion

Serum IL-17 and TGF-β which were elevated in GD patients reflect the role of pro-inflammatory and regulatory cytokine activation in pathogenesis of GD. There is no correlation between pro-inflammatory or regulatory cytokine, IL17 and TGF-β, respectively, with regulatory FOXP3 T cell in GD patients. Otherwise, we found the significant correlation between TGF-β with TRAb, which described that there is a role of regulatory T cell in pathogenesis of GD.

References

[1] Zhao S, Pan C, Cao H, Han B, Shi JY, Liang J Et al. Association of the CTLA4 gene with Graves' disease in the Chinese Han population. Plos ONE. 2010;5(3):1-10.
[2] Wang H, Zhao S, Tang X, Li Jingyuan, Zou P Et al. Changes of regulatory T cells in Graves disease. Journal of Huazhong University of Science and Technology. 2006;26(5):545-547.
[3] Li X, Qi Y, Ma X, Huang F, Guo H, Jiang X. Chemokine (C-C Motif) ligand 20, a potential biomarker for Graves' disease, is regulated by osteopontin. Plos ONE. 2013;8(5):1-8.
[4] Zha B, Wang L, Liu X, Liu J, Chen Z, Xu J. Decrease in proportion of CD19+CD24hiCD27+B cells and impairment of their suppressive function in Graves' disease. Plos ONE. 2012;7(11):1-10.
[5] Inoue N, Watanabe M, Morita M, Tomizawa R, Akamizu T, Tatsumi K. Association of functional polymorphisms related to the transcriptional level of FoxP3 with prognosis of autoimmune thyroid diseases. Clinical and Experimental Immunology. 2010;162:402-6.
[6] Jiang W, Zheng L, Xu L, Zhang Y, Liu X, Hu L, Wang X. Association between FOXP3, FOXE1 gene polymorphisms and risk of differentiated thyroid cancer in Chinese Han population. Mol Biol. 2015;4(3):1-5.
[7] Onishi RM, Gaffen SL. Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. Immunology. 2010;129:311-21.
[8] Esfahanian F, Naimi E, Doroodgar F, Jadali Z. Th1/Th2 cytokines in patients with Graves' disease with or without ophtalmopathy. Iran J Allergy Asthma Immunol. 2013;12(8):168-75.
[9] Marazuela M. Garcia-Lopez MA, Figueroa-Vega N, de la FH, varado-Sanchez B, Monsivais Urenda A, et al. Regulatory T cells in human autoimmune thyroid disease. J Clin Endocrinol Metab. 2006;91(9):3639-46.
[10] Morshed SA, LAtif R, Davies TF. Delienating the autoimmune mechanisms in Graves' disease. Immunol Res. 2012;54:191-203.
[11] Ganesh BB, Bhattacharya P, Gopisetty A, Prabhakar BS. Role of cytokines in the pathogenesis and suppression of thyroid autoimmunity. Journal of Interferon and Cytokine Research. 2011;31(10)1-13.
[12] Kim SE, Yoon JS, Kim KH, Lee SY. Increased serum interleukin-17 in Graves' ophtalmopathy. Graefes Arch Clin Exp Ophtalmol. 2012;250(10):1521-6.
[13] Nanba T, Watanabe M, Inoue N, Iwatani Y. Increased of Th1/Th2 cell ratio in severe Hashimoto's disease and in the proportion of Th17 cells in intractable GD. Thyroid. 2009;19(5):459-501.
[14] Zheng L, Ye P, Liu C. The role of the IL-23/IL17 axis in the pathogenesis of Graves' disease. Endocrin J. 2013;60:591-7.
[15] Shi Y, Wang H, Su Z. Differentiation imbalance of Th1/Th17 in peripheral blood mononuclear cells might contribute to pathogenesis of Hashimoto's thyroiditis. Scand J Immunol. 2010;72:250-55.
[16] Tabarkiewicz J, Pogoda K, Karczmarczyk A, Pozarowski P, Giannopoulus K. The role of IL-17 and Th17 lymphocytes in autoimmune diseases. Archivum Immunologiae et Therapiae Experimentalis. 2015;63(6):435-49.
[17] Roohi A, Tabrizi M, Abbasi F, Ataie-Jafari A, Nikbin B, Larijani B. Serum IL-17, IL-23, and TGF-β levels in type 1 and type 2 diabetic patients and age-matched healthy controls. BioMed Research International. 2014;1-8