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Immunophenotype Lymphocyte of Peripheral Blood in Patients with Psoriasis

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ABSTRACT

Introduction: Regulatory T cells (Treg) play a central role in the immunopathogenesis of psoriasis. Immunoregulatory T cells (Tregs) are involved in important homeostatic mechanism for maintaining tolerance and preventing autoimmunity, and autoimmune diseases. The aim of this study was to examine the role of Tregs cells in the pathogenesis of psoriasis, and determine the range value for Treg cells (CD4+ CD25+) in the peripheral blood of patients with psoriasis compared to the severity of disease. **Material and methods:** The study included 51 patients diagnosed with psoriasis and 25 healthy individuals. Phenotype profile of peripheral blood lymphocytes was determined by flow cytometry, and assessment of severity of disease was determined on the basis of PASI score (e.g. Psoriasis Area and Severity Index). **Results:** Proportion of CD4+CD25+T cells in the control group was significantly higher than in the patients with psoriasis [6,4% ±(5,4-7,6) vs. 4,1% (3,1 -5,8)–Mann–Whitney U test, p <0.001]. In the present study we did not find a statistically significant correlation between the levels of CD4+CD25+cells, in patients with psoriasis, compared to the severity of disease–PASI. (i.e. Pearson correlation, r = 0.197, p = 0.194). **Conclusion:** The stratification of patients, according to the severity of the clinical course was not possible on the basis of Treg cells' level. ROC curve analysis of the optimal cutoff (PASI=10) and the CD4+CD25+, which distinguishes between patients and healthy individuals was 5% of CD4+CD25+ of the total number of CD4+ lymphocytes with specificity of 69% and sensitivity of 84%.

Key words: psoriasis, regulatory T cells, CD4 + CD25 +, PASI, flow cytometry.

1. INTRODUCTION

Psoriasis is a chronic–relapsing inflammatory skin disease characterized by inflamed lesions covered with silvery-white scabs of dead skin. Except the skin, the disease affects the scalp and nails, in some patients, as well as joints in the form of psoriatic arthritis (1).

The modern concept of the pathogenesis of psoriasis gives central place to CD4+ T lymphocytes, which have have played all effector mechanisms of the immune system involved in the development of the disease (2). As far as the immunological aspects considered to be signifying an autoimmune disease, the psoriasis is mediated by T–cell immunity. Advances in understanding of the immunological bases of psoriasis and improved insight into the mechanism of the disease results in the concrete benefits for patients, and includes the introduction of new targeted therapies (3).

Phenotype of subpopulation of CD4+T lymphocytes peripheral blood: CD25+Foxp3+ has reduced suppressor function in patients with psorisis.This is connected with the rapid expansion of CD4+ T lymphocyte responses. The presence of non-functional CD4+CD25+ high Treg cells in peripheral blood and tissue leads to a reduced capacity to contain pathogenic T-cells and hyperproliferation of psoriasis plaques, in vivo. Functional studies composed of patients with psoriasis found that Treg cells in peripheral blood and in the skin lesions were of reduced immunoregulatory capacity, suggesting that the deficit of these cells contributes to the pathological process (4).

In patients with psoriasis a satisfactory activation suppressive function of regulatory T cells does not materialize, which in certain conditions can lead to increased proliferation and activation of Th1 and Th17 lymphocytes as well (5).

The aim of this study was to examine the role of Tregs cells in the pathogenesis of psoriasis, and determine the range value for Treg cells (CD4+CD25+) in the peripheral blood of patients with psoriasis compared to the weight of the clinical course of psoriasis.

2. MATERIALS AND METHODS

The study included 51 patients (i.e. 30 men and 21 women, with average age of 56 years). Patients younger than 18 years, patients who were diagnosed with an immunodeficiency or malignancy, as well as the patients who underwent immunosystemic therapy last month were excluded from the study.

A control group of 25 healthy individuals (i.e. 11 men, 14 women, with mean age of 48 years) by age and gender division corresponds to the experimental group. Immunophenotype profile of peripheral blood lymphocytes was determined by the flow cytometry, and severity of disease was determined on the basis of PASI (e.g. Psoriasis Area Severity Index).

Immunophenotyping of cells was carried out by a standard method of sample preparation. After lysis of erythrocytes, the leukocytes of peripheral blood were analyzed for the expression of specific leukocyte markers using a panel of monoclonal antibodies and flow cytometry (flow cytometer–BD FACS Canto II).

Combinations of surface markers that are determined by monoclonal antibody conjugated with FITC (i.e. florescin isothiocyanate), PE (i.e. phycoerythrin) and PerCP (i.e. Peridinin-chlorophyll-protein complex) or APC (i.e. alofikocianin) and subpopulation of lymphocytes also determined some of the combinations are presented on Table 1.

	FITC Fluorescein Isotiocianat	PE Phycoerythrin	PerCP Peridinin-hlorofil-protein complex	APC Alofikocianin
T1	CD3	CD8	CD45	CD4
T2	CD3	CD16+56	CD45	CD19
T3	CD3	HLA-DR	CD8	CD4
T4	CD3	CD25	CD45	CD4

Table 1. Combinations of surface markers

Statistical analysis was performed using the licensed SPSS statistical software, version 15.0 for Windows. The collected data were analyzed at the level of descriptive statistics, measures of central tendency (e.g. mean and median) and the measures of variability (e.g. standard deviation and standard error). Given the normal distribution of continuous variables, which was proven by the Kolmogorov–Smirnov test, we used a parametric method (e.g. Independent Samples T–test) to calculate the significance of differences between the data for non-parametric data, (e.g. the Mann–Whitney U test).

ROC curve (e.g. Receiver Operating Characteristic) was used to test the diagnostic significance of lymphocyte subpopulations in the detection of symptomatic patients. The optimal cutoff point for mild form of the disease (PASI < 10) and severe (PASI > 10) psoriasis were selected when the sensitivity and specificity was maximal.

All analyses were estimated at the $p < 0.05$ level of statistical significance.

3. RESULTS

The control group had a significantly higher proportion of CD4+CD25+ T cells, as compared to patients with psoriasis (i.e. Mann–Whitney U test, $p < 0.001$). Differences in the mean (median) CD4+CD25+, between groups (exp./cons.) were statistically significant (i.e. U = 238, $p < 0.0005$).

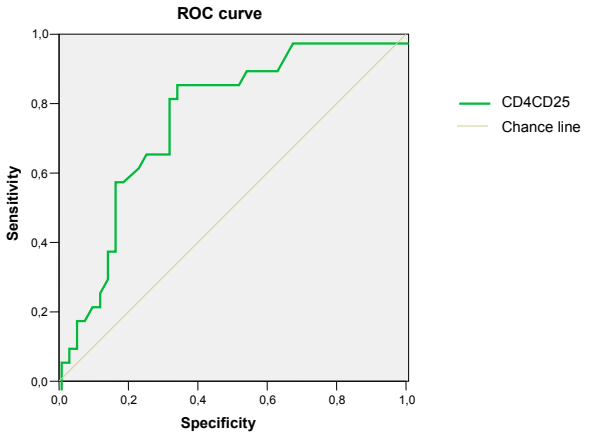

Figure 1. ROC curve (Receiver Operating Characteristic) for T reg lymphocytes

In the present study we did not find a statistically significant correlation between the levels of CD4+CD25+ cells in patients with psoriasis, compared to the clinical picture PASI. (i.e. Pearson correlation, r = 0.197, p = 0.194). The stratification of patients according to the severity of the clinical picture could not be determined based on the level of Treg cells.

We determined the sensitivity and specificity of CD4+CD25+ detection in patients with psoriasis, taking into account the limit values for PASI scores, 10 ROC curve analysis of the optimal cutoff precisely defined, and the CD4+CD25+ that distinguishes these two populations was 5% of CD4+CD25+ from the total number of CD4+ lymphocytes with specificity of 69% and sensitivity of 84% (Table 2).

Values of CD4+CD25 + (exp.) = 4.1 (3.1–5.8) were, on average, less than the value of the CD4+CD25+ (cont.) = 6.4 (5.4 -7.6), the difference was statistically significant (Figure 1).

• By specifying the ROC curve for the CD4 + CD25 + we determined that T reg (CD4 + CD25 +) may differ from the examined control group (psoriasis); (i.e. area = 0.788, $p < 0.0005$). Cut off values for CD4 + CD25 + were 5.0% compared to the total number of CD4 + lymphocytes, with sensitivity of 84%, and specificity of 69%.

• The value of CD4 + CD25 + area under the ROC curve was significantly different from the area under the diagonal which was measured to be (0.788, $p < 0.0005$).

Variable	AUC	Std. Err.	p	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
CD4+CD25+	0,788	0,054	0,000	0,682	0,895

Table 2. AUC–Area Under the Curve for CD4CD25. AUC–Area Under the Curve; p = statistically significant

4. DISCUSSION

The results obtained in our study are consistent with the results of other authors, according to which the psoriasis is a very unpredictable disease. The course of the disease and clinical features are diverse. Start of the disease is possible at any age, from early childhood and later in all periods of life, it can be provoked by various trigger factors in genetically susceptible individuals, but also in those who have inherited inclination towards the disease (6, 7).

Although the pathogenesis of psoriasis is still widely debated, most researchers now agree that the epidermal proliferation in psoriatic lesions tend to be the result of immunologic abnormalities, and the consequences of loss of immune tolerance (8).

Carriers of the immunological tolerance are T regulatory cells, which constitute 5 -10 % of the T helper (CD4+) lymphocytes. They are characterized by medium to high expression of surface recceptora the α-chain of IL–2 (CD25), as well as intracellular transcription factor's (FOXP3 expression) (9). The primary goal of this research are regulatory T cells (Treg), as well as the influence of some immunological parameters in lanced clinical features in patients with psoriasis (PASI score).

Regulatory T–cells are specialized, phenotypic and functional specific subpopulation of T cells that modulate the immune response, thereby maintaining homeostasis and immune tolerance. Better understanding of the immunopathogenesis of psoriasis as well as the relationship of T–regulatory cells with disease activity is crucial in the development of new strategies to control this disease and many other autoimmune and inflammatory processes (10, 11).

According to the reports of other authors, it was found that Treg frequency in the peripheral blood of patients suffering from psoriasis can be compared with these of healthy controls (12). Our results, in contrast to the results of Chen et al., as well as Zhang et al., show that there is a significant difference in the percentage of Treg cells. In our research experimental group compared to the control group. The measured percentage refers to the total number of CD4 + lymphocytes (13, 14). Sugiyama et al. found that Treg cells in patients with psoriasis showed impaired suppressor activity that is not associated with a decrease in their number in the peripheral blood. Autoimmune disease concept was explained that the reduction of activity of Treg cells may ultimately affect the failure of regulation of autoreactive T cells resulting in consequent hyperproliferation in patients with psoriasis (4).

Confirmation of the theory of the strong influence of Treg cells in the immunopathogenesis of psoriasis was proven by numerous authors who were researching the effects of systemic and other therapies. Specifically, Quaglino et al. showed that treatment with biologics (i.e. infliximab, etanercept, efalizumab) was able to regulate the expression of regulatory T lymphocytes (CD4CD-25brightFoxP3) and that this increase is associated with achieving the desired clinical response (15).

The introduction of additional markers (e.g. Foxp3), which are now used for the identification of regulatory T cells and the differentiation of effector CD4 + T cells is likely to contribute to a better discrimination of cells within the activated T lymphocytes and better diagnostic differentiation between the patients.

Psoriasis, as the result of an imbalance between Treg and effector T cells has recently been described by several authors. Their results show that the number and/or function of Treg cells can be reduced with consequent proliferation of pathogenic T cells in this disease (4, 18).

5. CONCLUSION

Treg cells represent a scientific challenge and provide promise for the immunotherapy of autoimmune diseases based on their manipulation. Significantly lower values of the relative number of regulatory T cells (CD4 + CD25 +) in patients with psoriasis compared to healthy controls indicate a strong immune influence in the pathogenesis of this disease. Monitoring subpopulation of T cells in peripheral blood by flow cytometry provides insight into the abnormal T cell function in psoriasis and may also be a diagnostic option for monitoring the activities of these diseases, as well as for the efficiency and effectiveness of the evaluation therapy.

CONFLICT OF INTEREST: NONE DECLARED

REFERENCES

1. Myers WA, Gottlieb AB, Mease P. Psoriasis and psoriatic arthritis: clinical features and disease mechanisms. Clin Dermatol. 2006; 24: 438-447.
2. Griffiths CE. The immunological basis of psoriasis. J Eur Acad Dermatol Venereol. 2003; 17: 1-5.
3. Nestle F, Kaplan D, Barker J. Mechanisms of disease: Psoriasis. N Engl J Med. 2009; 361: 496-509.
4. Sugiyama H G R. Dysfunctional blood and target tissue CD4+CD25 high regulatory T Cells in psoriasis: Mechanism underlying unrestrained pathogenic effector T cell proliferation. J immunol, 2005; 164-173.
5. Lowes MA, Kikuchi T, Fuentes-Duculan J, et al. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. J Invest Dermatol. 2008; 128:1207-1211.
6. Langley RG, Krueger GG, Griffiths CE. Psoriasis: epidemiology, clinical features, and quality of life. Ann Rheum Dis. 2005; 64: 8-25.
7. Naldi, L, Gambini D. The clinical spectrum of psoriasis. 2007; Clin Dermatol. 25(6): 510-518.
8. Sweeney CM, Tobin AM, Kirby B. Innate immunity in the pathogenesis of psoriasis, Arch Dermatol Res. 2011; 303(10): 691-705.
9. Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG , Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity. 2005; 22(3): 329-341.
10. Langewouters AMG, van Erp PEJ, de Jong EMGJ. et al. Lymphocyte subsets in peripheral blood of patients with moderate-to-severe versus mild plaque psoriasis. Arch Dermatol Res. 2008; 300: 107-113.
11. Takahashi T, Sakaguchi S. Naturally arising CD25+CD4+ regulatory T cells in maintaining immunologic self-tolerance and preventing autoimmune disease. Curr Mol Med. 2003; 3(8): 693-706.
12. Stephens GL, Andersson J, Shevach EM. Distinct subsets of FoxP3+ regulatory T cells participate in the control of immune responses. J Immunol 2007; 178: 6901-6911.
13. Chen L, Shen Z, Wang G, Fan P, Liu Y. Dynamic frekvencija of CD4+ CD25+Foxp3+Treg cells in Psoriasis vulgaris. J Dermatol Sci 2008; 51(3): 200–203.
14. Zhang L, Yang XQ, Cheng J, Hui RS, Gao TW . Increased Th17 cells are accompanied by FoxP3(+) Treg cell accumulation and correlated with psoriasis disease severity. Clin Immunol 2010; 135(1): 108–117.
15. Quaglino P, Bergallo M, Ponti R., et al. Th1, Th2, Th17 and regulatory T cell pattern in psoriatic patients: modulation of cytokines and gene targets induced by etanercept treatment and correlation with clinical response. Dermatology 2011; 223(1): 57–67.