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Association of Th-Tc protein CD28+ and Periodontal Inflammation among Indonesian Women with SLE Disease

Nanda Rachmad Putra Gofur[1], Kusworini Handono[2], Nurdiana[3], Handono Kalim[4], Cesarius Singgih Wahono[4], Sri Poeranto[5], Wisnu Barlianto[6]

[1]Doctoral Program of Medical Science, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.
[2]Dept of Pathology Clinic, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.
[3]Dept of Pharmacology, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.
[4]Dept of Internal Medicine, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.
[5]Dept of Parasitology Clinic, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.
[6]Dept of Pediatrics, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.
Corresponding author: Universitas Brawijaya, E-mail: dr.kusworini@gmail.com

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ABSTRACT

Introduction: Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disorder characterized by secretion of autoantibodies and deposition of immune complexes associated with various clinical manifestations and tissue damage. activates T cell differentiation. T cells experience hyperactivity could produce defects in Th (T helper cell) and Tc (T cytotoxic), also loose CD28+ protein in surface marker. This study aims to associate Th and Tc protein CD28+ and Periodontal Inflammation in SLE disease.

Materials and Methods: Cross-sectional observational analytic study was conducted in 153 SLE patients where clinical examination and laboratory tests were done to assess the activity of the disease, periodontitis and evaluate lymphocytes T CD28 using flow cytometry. Clinical examination of periodontitis was using periodontal index (PI) and gingivitis was using gingival index (GI). PI assessment was done by a WHO Periodontal Examining Probe using Periodontal Index by Russel.

Results: Ratio Th/Tc in the SLE group was lower 0,7% than the control group. The percentage of Th CD28+ was lower 12,65% in SLE than the control group. Also, the percentage of Tc CD28+ was lower 5,28% in SLE than the control group. Then the total percentage CD28+ (both Th and Tc) was lower 23,5%. Confirming Th, Tc, and CD28+ protein between two groups were based on *flow cytometry* analysis. There was a significant strong association ($p<0.01$), Th CD28+ with periodontitis, and also Tc CD28with periodontitis in the SLE group ($p<0.001$). Both Th and Tc were loose CD28+ protein in the SLE group compared to control.

Conclusion: Both Th and Tc were loose CD28+ protein in the SLE group compared to control. There is association Th and Tc protein CD28+ and Periodontal Inflammation in SLE disease.

Keywords: Periodontal Disease, Th, Tc, CD28+, SLE

Correspondence:
Kusworini Handono
Department of Pathology Clinic, Faculty of Medicine, Universitas Brawijaya
Malang, Indonesia
E-mail: dr.kusworini@gmail.com

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INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disorder characterized by secretion of autoantibodies and deposition of immune complexes associated with various clinical manifestations and tissue damage. LES is a type of lupus, besides discoid lupus and drug-induced lupus. The incidence of SLE has continued to increase almost threefold since the last 40 years, mainly due to better diagnosis. The prevalence rate was estimated at 150 per 100,000 people worldwide. SLE was found to be nine times more common in women than men. This disease is found more in African, American, and Asian races compared to Caucasian races. As many as 65% of LES patients are diagnosed in the age range of 16 and 55 years, 20% of cases appear before the age of 16 years, and 15% are found over the age of 55 years[1].

SLE is an autoimmune disease that can be exacerbating and remitting. This disease attacks various organs such as skin, kidney, musculoskeletal, nerve, cardiovascular, and oral cavity. Clinical manifestations of SLE, as many as 50-70% of patient experience disorders of the kidneys. Kidney involvement is a major cause of high morbidity and mortality in this population. Another manifestation in musculoskeletal, arthralgia, joint deformity, temporomandibular joint abnormalities, and periodontal inflammation have been found to occur in LES patients. In Indonesian women, periodontal inflammation in SLE was found 86%[2,3].

SLE could increase in the amount of endogenous nucleic acids released by apoptotic cells into the circulation, stimulates the production cytokines and promotes autoimmunity through DC cell hyperactivity. DC cells as antigen-presenting cells (APC) which then activate T cell differentiation. T cells experience hyperactivity could produce defects in Th (T helper cell) and Tc (T cytotoxic), also loose CD28+ protein in surface marker. These protein secreting several cytokines such as interleukin-10 (IL-10) and interleukin-13 (IL -13). All the results of the activation of immune cells namely cytokines are then spread systemically through blood vessels and cause chronic inflammation throughout the body. This situation explains the many clinical manifestations of SLE, especially periodontal inflammation[4,5].

Th (CD4+) lymphocyte cells are lymphocyte cells that play an important role in mediating the immune response by secreting several specific cytokines. CD4 T lymphocyte cells after activation and differentiation have a role in the activation of innate immune system cells, B lymphocytes, cytotoxic T lymphocytes, and a role in suppressing immune reactions. The initial stage of naive CD4 T cell differentiation is the stimulation of antigens through the interaction of TCR and MHC-II-antigen complex receptors presented by APC. The TCR signal thus triggers proliferation and differentiation due to the costimulator protein. There is a decrease in CD4 T cells due to the loss of costimulatory proteins such as CD28 which is expressed when systemic inflammation occured[6,7].

Tc (CD8+) are immune cells that play a role in antigen recognition, intracellular pathogenic clearances, and antitumor immune responses. CD8 + T lymphocytes are activated when TCR recognizes antigens bound to class I MHC peptides that are presented by APC cells in macrophages and dendritic cells. The activation signal in naïve CD8 cells will trigger the proliferation and differentiation of cytotoxic T lymphocytes (CTL) cells which after the effector function is finished will die via the apoptotic pathway. CD8 T lymphocyte cell stimulation triggers cell proliferation and CTL formation. Repeated antigen exposure, causing interference with proliferation and differentiation. This situation explains, as age goes on and in autoimmune an increase in the number of CD8 cells with decreased cytotoxic function[4,6].

Both Th and Tc associate with protein CD28, a type I transmembrane protein weighing 44 kDa, expressed on the surface of CD4 T cells and naive CD8 T cells consisting of a single Ig-V extracellular domain. CD28 is the main costimulatory molecule for T cell activation and is important in the initiation of the immune system of T cells. CD28+ costimulation could prevent activation-induced cell death (AICD) by inhibiting CD95L expression in T cells and by reducing the formation of cell death-inducing signaling complex (DISC). CD28 stimulation increases the expression of c-FLIP protein that competes with Propose 8 to bind to the CD95 and FADD domains. The loss of CD28 causes activation of Procasease 8 and Fas thereby inducing death. Th and Tc that lose the CD28 costimulator molecule will be more susceptible to death and inflammation. This study aims to associate Th and Tc protein CD28+ and Periodontal Inflammation in SLE disease[4,7,8,9].

MATERIALS AND METHODS

The data was taken from observational analytic study with cross-sectional approach. Data was taken on 93 SLE patients and 60 control. Study held from 2017 until 2019 on Rheumatology Department Dr. Saiful Anwar General Hospital Malang, Indonesia. The work has been approved by ethical committees from Dr. Saiful Anwar General Hospital Malang (No. 400/120/K.3/302/2017). In all SLE patients, clinical examination and laboratory tests were conducted to assess the activity of the disease. The severity of SLE measured using MEX-SLEDAI criteria, and T CD28+ ratio using flow cytometry. Inclusion criteria were female subjects with a confirmed diagnose of SLE, could read and write, and had full consciousness. Exclusion criteria were smoking, pregnancy, diabetes, and another systemic disease. For the healthy subject had similar inclusion and exclusion criteria[10].

Clinical examination of periodontitis was using periodontal index (PI) and gingivitis was using gingival index (GI). PI assessment was done by a WHO Periodontal Examining Probe using Periodontal Index by Russel. This index was using the entire surface of the tooth in the oral cavity. Score 0-8 was obtained from clinical examination using the WHO probe. Total score obtained from total findings divided by all teeth in the oral cavity. Gingival Index (GI) was the severity of gingival inflammation using the gingival index by Loe and Sillness. Measurements on 4 sides of the gingiva including distal-vestibular papillae, edge of vestibular gingiva, the mesial-vestibular papillae, and edge of oral gingiva. Gingival index is obtained from total score each teeth divided by four sides then divided by total teeth were evaluated[11].

T CD28+ cells are several helper T (Th) cell and cytotoxic T cells (Tc). Helper and cytotoxic T cells were collected from PBMC using the flow cytometry method using PE antihuman CD28 (Biolegend Catalog no 301908). PBMCs (Peripheral Blood Mononuclear Cell) cells are formed consisting of T lymphocytes, B lymphocytes, monocytes, and natural killer (NK) cells. 10^3-10^5 PBMC added specific primary antibodies with optimum concentration. Next, incubate for 15-20 minutes in a dark room. Wash twice with at least 2 ml of Cell Staining Buffer by centrifugation at 350xg for 5 minutes. Added secondary antibodies namely fluorochrome-conjugated anti-human immunoglobulin (PE anti-human) and incubation on ice in a dark room for 15-20 minutes, and washed again. Re-suspend pellet cells in 0.5 ml Cell Staining Buffer and add 5µl (0.25µg) Viability Staining Solution to remove dead cells. PBMCs cell readings are obtained by gate lymphocytes. Where the graph has an x-axis that shows cell size, while the y-axis shows the complexity of the cell. Measurements were made at 105 PBMC and the results were obtained in the form of a percentage (%) of cells and analyzed using BD Cell Quest Pro software[12].

Collected data was analyzed using of SPSS version 25 program. The difference of markers on SLE patients was analyzed by Kolmogorov Smirnof for normality test, Spearman/Pearson for correlation test, and One Way ANOVA for compare test $P<0.05$[10].

RESULTS

A total of 153 subjects, SLE subjects 93 patients and 60 control were included in this study. As shown in Table 1, the mean average disease duration and treatment duration was three years. SLE activity disease using MEXICAN SLEDAI was 14,39 within rang 0 to 44. Characteristics of SLE subjects were shown in table 1, contain of drug prescription taken and clinical manifestations.

Table 1: Characteristics of SLE subjects

Clinical Manifestation (Total = 93)	N	Percentage (%)
Lupus Nephritis	46	49,4
Neuro Psychiatric	40	43,0
Vasculitis	41	44,1
Arthritis	68	73,1
Mucocutaneous Manifestation	56	60,2
Fatigue	65	69,8
Fever	48	51,6

Drug Prescription (Total = 93)	N	Percentage (%)
Steroid (methylprednisolone)	90	96,7
Imunosupresan		
- Imuran (azathioprine)	44	47,3
- Sandimun (cyclosporin)	6	6,45

Data analysis from SLE treatment consists of duration of disease, duration of treatment and drug prescription taken was correlate with periodontal inflammation in table 2. There is no significant association between duration of disease, duration of treatment and drug prescription taken in SLE subjects with periodontal inflammation

Table 2: SLE Treatment Characteristic

No.	SLE Treatment	p	r	Coefficient beta
1.	Duration of disease	0,37	0,11	-0,113
2.	Duration of treatment	0,29	0,13	0,280
3.	Drug Prescription	0,62	0,18	0,311

Periodontal inflammation finding all subjects patients are shown in table 3. There is no difference in age between both groups. The mean value of periodontitis, gingivitis, BOP, CAL, and loose teeth in SLE was higher compared to control groups. Moreover, plaque and calculus were higher in control groups than SLE. This condition suggests there is an association in immune response in SLE patients, especially Th, Tc, and protein CD28+.

Table 3: Periodontal Inflammation in Control and SLE Subjects

Periodontal Inflammation	Control = 60		SLE = 93		
	Mean	SD	Mean	SD	p
Age (years)	32,68	10,225	30,27	10,06	0,65
Periodontal Index	0,25	0,695	1,79	0,63	<0,001*
Gingival Index	0,87	0,53	1,805	1,06	<0,001*
Plaque Index	0,985	0,62	0,73	0,645	<0,001*
Calculus Index	1,18	0,525	0,625	0,515	<0,001*
Bleeding on Probing (%)	23,98	11,78	28,85	13,91	<0,001*
Clinical Attachment Loss (mm)	0,38	0,54	0,595	0,75	<0,001*
Loose teeth	0,25	0,365	0,48	0,915	<0,001*

Data shown in figure 1-4 was a difference average of Th, Tc, and CD28+ protein between the control group and the SLE group. Ratio Th/Tc in the SLE group was lower 0,7% than the control group. The percentage of Th CD28+ was lower 12,65% in SLE than the control group. Also, the percentage of Tc CD28+ was lower 5,28% in SLE than the control group. Then the total percentage CD28+ (both Th and Tc) was lower 23,5%.

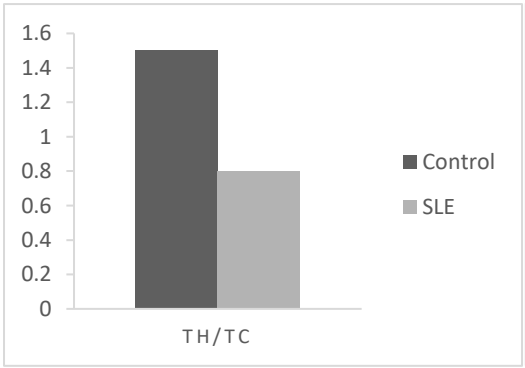

Figure 1: Percentage of Th/TC

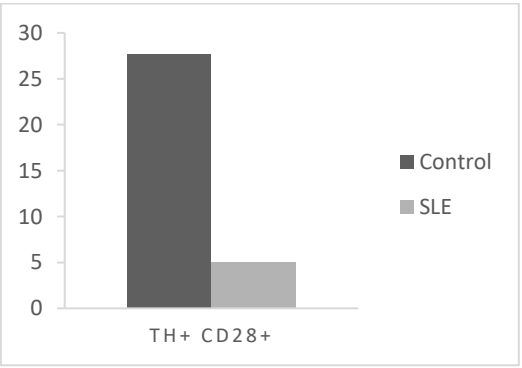

Figure 2: Percentage of Th+ CD28+

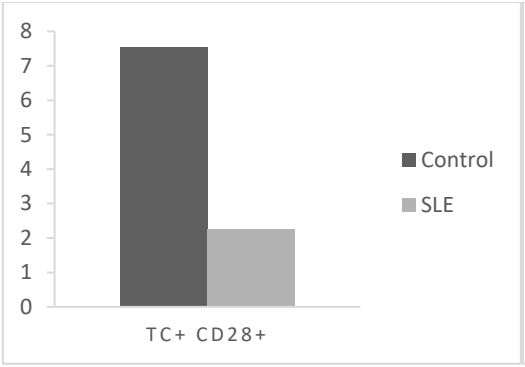

Figure 3: Percentage of Tc+CD28+

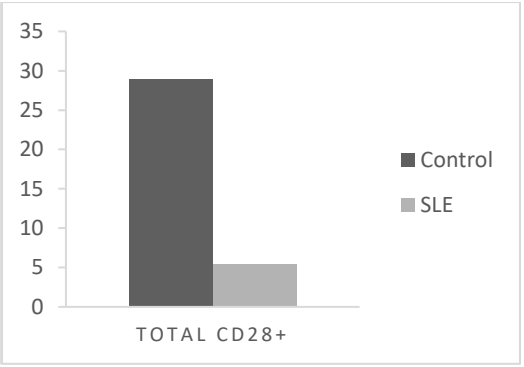

Figure 4: Percentage of Total CD28+

Confirming Th, Tc, and CD28+ protein between two groups were based on *flow cytometry* analysis. There was a significant difference (p<0.01), Th CD28+ between control and SLE group (figure 5), and also Tc CD28+ between control and SLE group (figure 6). Both Th and Tc were loose CD28+ protein in the SLE group compared to control.

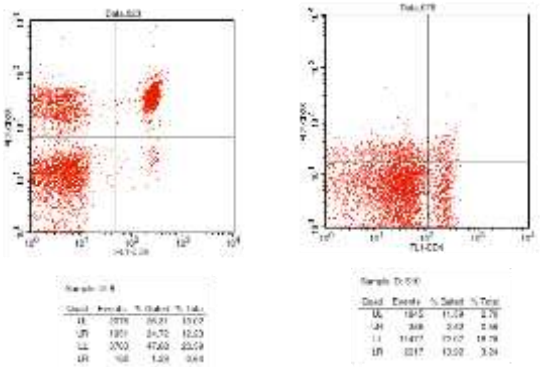

Figure 5: Th Difference in Control (Left) and SLE (Right)

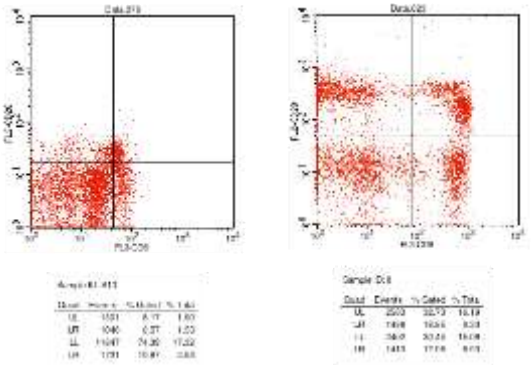

Figure 6: Tc Difference in Control (Left) and SLE (Right)

Association between Th, Tc, and CD28+ with periodontal inflammation were assessed using Pearson correlation test and the results were shown in Table 4. It can be seen that there was a significant (p<0.001) and a strong negative correlation between Tc/Th and Total CD28+ protein with periodontal inflammation.

Table 4: Periodontal Inflammation in Control and SLE Subjects

CD28+ Protein Association	p	r	Coefficient Beta
Th+CD28+ (%)	<0,001*	-0,363	-0,218
Tc+CD28+ (%)	<0,001*	-0,315	-0,330
Total CD28+ (%)	<0,001*	-0,926	-0,624

The Test Result of Direct Effect and Indirect Effect were assessed using linear and multivariate regression tests in table 4. The results of data analysis of the direct and indirect relationship between research variables will explain how the role of intervening variables (Th, Tc, and CD28+ protein) could induce periodontal inflammation in SLE patients. Based on the output data in table 5, it can be seen that the

direct impact was low, but the indirect relationship has a greater effect on both Th/Tc and protein CD28+.

Table 5: Periodontal Inflammation in Control and SLE Subjects

Variable	p	Direct impact	Indirect impact
Th+ CD28+ (%)	<0,001	9,9%	59%
Tc+ CD28+ (%)	<0,001	2,7%	38%
Total CD28+ (%)	<0,001	10,7%	66%

DISCUSSION

Our previous study also showed that periodontal inflammation was found in 88.53% of LES patients and 13.11% of healthy subjects. It was further shown that there was a correlation between the severity of periodontitis and SLE[3]. SLE patients are suspected to have an immune defect that triggers periodontitis. The loss of immune tolerance in SLE is marked by the hyperactivity of the antigens themselves and the clonal expansion of T lymphocytes cells, including T helper (Th) and T cytotoxic (Tc). Continual exposure to antigens will induce Clonal Exhausting and this condition is thought to trigger immune cell aging. T cell aging is characterized by loose of CD28+. Loose of CD28+ protein could induce cytokines as interleukin and TNF. This condition causes a continuous inflammation that triggers an increase in cell apoptosis and chronic inflammation in various tissues including periodontal tissue[13,14].

T cells differentiate into several subtypes depending on cell surface receptors, antigen exposure, and cytokine induction. Early differentiation during antigen introduction will trigger the response of naïve T cells to mature T cells. As a result of continuous stimulation, the number of Tc will increase, but subsequently will decrease Th. Thus, a decrease in both Tc, Th, and CD28+ protein results in decreased immune system function in the defense against bacteria. Loose CD28+ proteins in the Th-Tc surface so that it starts an inflammatory response. Cells release vasoactive amines and TNFα, which increases vascular permeability and expression of adhesion molecules such as cell-1 (ICAM-1) and P-selectin adhesion molecules on the surface of endothelial cells. This process triggers migration increasing Tc into periodontal tissue and releases cytotoxic activation, which contributes to soft tissue degradation. Lymphocytes and macrophages will also further migrate to the tissue. Macrophages will differentiate into mature osteoclasts and degrade alveolar bone[15,16].

Our study showed that Th/Tc ratio <1 was found in the SLE group compare to normal. There is a strong relationship between the decrease in Th/Tc cell ratio and the severity of periodontitis. This result was following the study before, irreversible disorder of self-immunological tolerance to endogenous antigens is a characteristic feature of autoimmune disease. As a result of this immune dysfunction, there was abnormal Th/Tc. However, this abnormal ratio does not always appear in all autoimmune diseases, decreasing Th/Tc cell ratio consistently seen in SLE. The lower this immune risk phenotype reflects inflammation associated with pathology especially in periodontal[17].

Th and Tc are 2 phenotypes of T lymphocytes, characterized by different surface markers and functions. These cells are present in and circulate blood, also cell ratio between 1.5-2.5 is generally considered normal. The normal ratio can be reversed or <1 through apoptosis or death of circulating Th and Tc expansion, or a combination of the two phenomena. An inverse ratio is associated with changes in immune function, immune aging, and chronic inflammation. Chronic inflammation could occur in any organ, our case was periodontal inflammation[18,19].

CD28 protein molecules play a role in intracellular biochemistry including phosphorylation and transcription signaling, metabolism, and production of cytokines, chemokines, and survival signals that are important for the expansion and differentiation of T cells. At least CD28 expression decreases the induction of tolerance to specific antigens and plays a role in the development of autoimmune diseases and grafts. organ. Binding with the CD28 receptor on T cells provides a second critical signal along with T cell receptor ligation (TCR) for naive T cell activation. Loose CD28 in both Th and Tc co-stimulation has various effects on T cell function, including biochemical events at immunological synapses defect, downstream phosphorylation and other post-translational modifications, transcription changes, and cytoskeletal remodeling failure. At the most basic level, loose of CD28 protein reduce the level of glycolytic cells, blocking cells to produce the energy needed for growth and proliferation[20,21].

Decreased CD28 causes reduced Treg cell production. A decrease in CD28 ligation causes no bond with Foxp3 + due to TGF-β stimulation. CD28 signals via TGN1412 (also known as TAB08) result in decreased Treg cell proliferation. This decrease in CD28 directly through miR17-92, expressing Bcl xL resulting in a decrease in IL-10 production by Treg cells. A decrease in IL-10 causes excessive inflammation when an infection occurs. The results of this study indicated important variables such as Th, Tc, and CD28+ proteins associate with periodontal inflammation in SLE patients. There is also a difference percentage Th, Tc, and CD28+ between control and SLE group. It also could have a direct and indirect impact on periodontal inflammation. High inflammation in periodontal tissue resulting in degradation collagen and bone resorption, called periodontitis[21,22,23].

CONCLUSION

There was a significant and strong negative correlation between Tc/Th and Total CD28+ protein with periodontal inflammation. Both Th and Tc were loose CD28+ protein in the SLE group compared to control. There is association Th and Tc protein CD28+ and Periodontal Inflammation in SLE disease.

SOURCE OF FUNDING

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CONFLICT OF INTEREST

The authors declare that we have no competing and conflict of interests.

ETHICAL APPROVAL

The work has been approved by ethical committees from Dr. Saiful Anwar General Hospital Malang (No. 400/120/K.3/302/2017).

CONSENT

All subjects were required to sign informed consent and willing to be part of this study.

AUTHOR CONTRIBUTION

All authors participated in conceiving and designing the study, reviewing the literature, and collecting and analyze the data. Authors testify that all persons designated as authors qualify for authorship and have checked the article for plagiarism. If plagiarism is detected, all authors will be held equally responsible and will bear the resulting sanctions imposed by the journal thereafter. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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