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

Nanda Rachmad Putra Gofur1, Kusworini Handono2, Nurdiviana3, Handono Kalim4, Cesarius Singh Wahan5, Sri Poeranto5, Wisnu Barlianto6

1Department of Pathology Clinic, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.
2Dept. of Pediatrics, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.
3Dept of Pharmacology, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.
4Dept of Internal Medicine, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.
5Dept of Parastitology Clinic, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.
6Dept of Parasitology Clinic, Faculty of Medicine – Universitas Brawijaya, Malang Indonesia.

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

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. 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 6% 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.

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%1-3.

SLE could increase in the amount of endogenous nucleic acids released by apoptotic cells into the circulation, stimulates the production cytokines and promotes autoimmune 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. 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 Rasssel.

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 CD28+ with 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.

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

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Tc (CD8+) are immune cells that play a role in antigen recognition, intracellular pathogenic clearances, and antigens. 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 naive 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.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 IgV 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.4,6

**Materials and Methods**

The data was taken from observational analytic study with cross-sectional approach. Data was taken on 93 SLE patients and 60 controls. 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.

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 Russell. 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 Loes 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 anthuman 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. 10^7-10^8 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 pelleted cells in 0.5 ml Cell Staining Buffer and add 5 μl (0.25μg) Viability Staining Solution to remove dead cells. PBMC 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.11 Collected data was analyzed using SPSS version 25 program. The difference of markers on SLE patients was analyzed by Kolmogorov Smirnoff for normality test, Spearman/Pearson for correlation test, and One Way ANOVA for compare test P<0.05.

**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 shown in table 1, contain of drug prescription taken and clinical manifestations.

<table>
<thead>
<tr>
<th>Clinical Manifestation (Total = 93)</th>
<th>N</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupus Nephritis</td>
<td>46</td>
<td>49.4</td>
</tr>
<tr>
<td>Neuro Psychiatric</td>
<td>40</td>
<td>43.0</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>41</td>
<td>44.1</td>
</tr>
<tr>
<td>Arthritis</td>
<td>68</td>
<td>73.1</td>
</tr>
<tr>
<td>Mucocutaneous Manifestation</td>
<td>56</td>
<td>60.2</td>
</tr>
<tr>
<td>Fatigue</td>
<td>65</td>
<td>69.8</td>
</tr>
<tr>
<td>Fever</td>
<td>48</td>
<td>51.6</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>No.</th>
<th>SLE Treatment</th>
<th>p</th>
<th>r</th>
<th>Coefficient beta</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Duration of disease</td>
<td>0.37</td>
<td>0.11</td>
<td>-0.113</td>
</tr>
<tr>
<td>2.</td>
<td>Duration of treatment</td>
<td>0.29</td>
<td>0.13</td>
<td>0.280</td>
</tr>
<tr>
<td>3.</td>
<td>Drug Prescription</td>
<td>0.62</td>
<td>0.18</td>
<td>0.311</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Periodontal Inflammation</th>
<th>Control = 60</th>
<th>SLE = 93</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.68</td>
<td>10.225</td>
</tr>
<tr>
<td>Periodontal Index</td>
<td>0.25</td>
<td>0.695</td>
</tr>
<tr>
<td>Gingival Index</td>
<td>0.87</td>
<td>0.53</td>
</tr>
<tr>
<td>Plaque Index</td>
<td>0.985</td>
<td>0.62</td>
</tr>
<tr>
<td>Calculus Index</td>
<td>1.18</td>
<td>0.525</td>
</tr>
<tr>
<td>Bleeding on Probing (%)</td>
<td>23.98</td>
<td>11.78</td>
</tr>
<tr>
<td>Clinical Attachment Loss (mm)</td>
<td>0.38</td>
<td>0.54</td>
</tr>
<tr>
<td>Loose teeth</td>
<td>0.25</td>
<td>0.365</td>
</tr>
</tbody>
</table>

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](image1.png)

![Figure 2: Percentage of Th+ CD28+](image2.png)
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.

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>
<thead>
<tr>
<th>CD28+ Protein Association</th>
<th>p</th>
<th>r</th>
<th>Coefficient Beta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th+CD28+ (%)</td>
<td>&lt;0,001*</td>
<td>-0,363</td>
<td>-0,218</td>
</tr>
<tr>
<td>Tc+CD28+ (%)</td>
<td>&lt;0,001*</td>
<td>-0,315</td>
<td>-0,330</td>
</tr>
<tr>
<td>Total CD28+ (%)</td>
<td>&lt;0,001*</td>
<td>-0,926</td>
<td>-0,624</td>
</tr>
</tbody>
</table>

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
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 inflammation18,19.

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 proliferation20,21.

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 TA80) 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 associated 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 periodontitis22,23.

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.
REFERENCES
We would like to thank all support for this study.

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. Saeful 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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REFERENCES
