Correlation between the percentage of memory T cells and IFN-γ level in patients with systemic lupus erythematous

Kusworini Handono1*, Fahrina Ulfah2, Hanani Octaviani2, Handono Kalim3
1 Department of Clinical Pathology, Faculty of Medicine-Brawijaya University, Malang, Indonesia
2 Department of Biomedical Science, Faculty of Medicine-Brawijaya University, Malang, Indonesia
3 Rheumatology and Immunology Division, Department of Internal Medicine, Faculty of Medicine-Brawijaya University, Malang, Indonesia
E-mail : dr.kusworini@gmail.com

Abstract. Systemic Lupus Erythematosus (SLE) is a chronic systemic autoimmune disease with diverse clinical and autoantibodies expression. Immune senescence is mostly affect the adaptive immune system, characterized by decrease of naïve T cells and increase of memory T cells. The aim of this study was to analyze the association between percentage of memory helper T lymphocytes (CD4CD45RO+Th), memory cytotoxic T lymphocyte (CD8CD45RO+Tc) and IFN-γ serum levels in patients with SLE. Subjects were 61 female SLE patients, 16-56 years old, from Rheumatology Clinic Dr. Saiful Anwar Hospital Malang (SLICC classification criteria, 2012). Severity of disease activity were assessed using MEX-SLEDAI score, percentage of memory Th cells, memory Tc cells were examined using flow cytometry and IFN-γ serum level were measured by ELISA. The percentages of memory (CD4+CD45RO+) Th cells, memory (CD8+CD45RO+) Tc cells and IFN-γ levels were significantly higher in SLE patients with SLEDAI score > 5 compared with those having SLEDAI score <5 (p = 0.000; p = 0.000; p = 0.032). There were a positive correlation between percentages of memory (CD4+CD45RO+) Th cells (p = 0.003, r = 0.453) and memory (CD8+CD45RO+) Tc cell (p = 0.045, r = 0.284) with IFN-γ serum level. The percentage of memory T cells had a positive association with IFN-γ serum level.

1. Introduction
Systemic Lupus Erythematosus (SLE) is a chronic systemic autoimmune disease with diverse clinical and autoantibodies expression. Chronic inflammation in SLE increase the risk of infections, cancer and degenerative diseases, such as atherosclerosis. The situation is similar with the result of low-level chronic inflammation in the aging process (immune senescence) [1]. Immune senescence is mostly affect the adaptive immune system, characterized by decrease of naïve T cells and increase of memory T cells [2]. Changes in the number of T cells can be identify by several markers such as CD28, C57, CD45RA, CD45O, and KLRG-1 [3]. The decrease of naïve T cells are indicated by lower T cells expressing CD45RA+ and CCR-7, while the increase of memory cells or senescence T cells characterized by the overexpression of CD45RO+, CD28-, CD57+ and KLRG-1+ [4].

Another alteration in immune aging is the increase of inflammatory cytokine production. Hyper-inflammatory state or inflammaging is characterized by an increase in basal circulating levels of pro-inflammatory cytokines related to aged immune system, which has been used as a strong predictor of increased in all-cause mortality risk of the elderly [5]. One of the cytokine changes in inflammaging is IFN-γ. Naïve T cells are tend to have high proliferation ability and low cytokine production while
memory cells or senescence cells are likely to have a lower proliferation ability with increase cytokine production [6].

In previous studies, early onset of immune senescence have been identified in a number of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, idiopathic juvenile arthritis. The aim of our study was to assessed the presence of immune senescence in SLE patients and its relation with IFN-γ cytokine levels.

2. Methods

The study was conducted in Rheumatology Outpatient Clinic, Dr Saiful Anwar Hospital Malang and the Biomedical Laboratory Faculty of Medicine Brawijaya University on October 2017 to February 2018. The subjects were 61 female SLE patients, 16-56 years old, meet the 2012 SLICC criteria. Exclusion criteria were pregnant patients, and patients with severe infections (sepsis). All of the patients were treated with steroids and conventional immune suppressants. Disease activity were assessed using MEX-SLEDAI score. Ethical Clearance for this study has been approved by Dr Saiful Anwar Hospital Ethics Committee.

2.1. PBMC isolation

PBMC were isolated using Lymphoprep and centrifugation (velocity of 1600 rpm, 30 minutes). The formed PBMC layer was taken slowly, washed for 2 times with 10 ml PBS, the supernatant was discarded, centrifuged at room temperature (1200 rpm, 30 minutes).

2.2. Antibody

Monoclonal antibodies used in this study were FITC anti-human CD4 (Biolegend Catalog no. 300506), PerCP anti-human CD8 (Biolegend Catalog no. 344708), PE anti-human CD45RA (Biolegend Catalog no. 322312), and PE anti-human and CD45RO (Biolegend Catalog no. 302908).

2.3. Flowcytometry

PBMC pellets were added with cell staining buffer, transferred into eppendorf tubes, and then added with PerCP anti-human CD8, FITC anti-human CD4, and PE anti-human CD45RO. These staining PBMC were stored at room temperature for 20 minutes. PBMC were added with 300 µL of CSB solution and centrifuged (2500 rpm, 3 minutes, 4ºC). The supernatant was discarded, 300 µL of CSB solution was added, and shaken. PBMC solutions are transferred to the cuvette and placed on a flowcytometer device. The number of cells analyzed by BD Cell Quest software. Measurements were made on 10,000 cells and results were obtained in the form of percentages (%) of cells.

2.4. Measurement of IFN-γ serum

IFN-γ serum level was measured by using the ELISA method according to manual procedure of the kit (Elabscience, Cat no E-EL-H0108).

2.5. Data analysis

The collected data were tested with the SPSS for Windows software version 19.0. The differences of memory Th, Tc lymphocytes percentages and IFN-γ serum levels on SLE patients with SLEDAI score <5 and >5 were analyzed by using independent T test. The correlation between percentages of memory T cells and IFN-γ serum level were analyzed by Pearson correlation test.

3. Results

Figure 1. showed means levels of IFN-γ cytokines in the SLEDAI ≤ 5 and SLEDAI> 5 groups. This study found the mean level of IFN-γ cytokines in the SLEDAI ≤ 5 group was 5.39 pg/mL while that in the SLEDAI > 5 group was 7.37 pg/mL. There are significantly different of IFN-γ level between two groups (p = 0.032).

Figure 2 showed the average percentage of CD8+CD45RA+ and CD8+CD45RO+ T cells in both groups. The percentage of memory (CD4+ CD45RO+) Th cells and memory (CD8+CD45RO+) Tc cells in patients with SLEDAI score > 5 was significantly higher (p = 0.001; 0.001) compared with those having SLEDAI score <5.
Our study also found the percentage of memory Th and Tc cells had a significant positive correlation to IFN-γ cytokine levels (p = 0.003, r = 0.453) (p = 0.045, r = 0.284).

4. Discussion

Immune senescence is a term to describe the phenomenon of age-related changes in immune system which characterized by progressive deterioration in immune response both in innate and adaptive compartments which correlated to increase risk of mortality in elderly [7]. Accelerated aging of immune system has been correlated to the development of autoimmune disease, as the presence of autoantibodies such as anti-nuclear antigen can be detected in elderly without causing significant clinical manifestations of autoimmunity [8]. In immune compartment, senescent cell remain metabolically active as they secrete pro-inflammatory cytokine (termed the senescence-associated secretory phenotype or SASP), as the result, accumulation of pro-inflammatory factors in immune senescence often leads to chronic, sterile, low grade inflammation define as inflammaging [9]. Together, they suggested to stand at the origin of morbidity and mortality cause in the elderly, such as risk of chronic inflammatory diseases, cancer, and infection [10]. In this study we confirmed accelerated immune aging process both immune senescent profile and inflammaging, was found in SLE subjects. There was a positive correlation between CD4+ and CD8+ T cells expressing senescence markers of memory T cell (CD45RO+) with increased level of IFN-γ.

Age-related alteration of immune system was mostly found to affect T lymphocyte subsets [11]. The major changes in T cell subsets are the decrease in naïve T cells and the increase in memory T cells which occur in both the CD4+ and in the CD8+ T cell [12]. To distinguish memory and naïve T cell, the expression of the CD45 splicing variants CD45RA and CD45RO has been extensively used [13]. Naïve T cell is defined as T cell expressing the high molecular weight isoform CD45RA, they
are present in very small amount of the elderly immune system [14]. Loss of naïve T cell population occurs gradually as the consequences of chronic antigen stimulation [15]. In elderly, there is 95% decline of TCR repertoire diversity as naïve T cell, the source of TCR diversity, declines. As the result, aged immune system will limit its ability to respond to immune stimulation [15].

ICOS is a ligand that is present in CD4+ and CD8+ T cells. In CD45RA+ T cells, there is no antigen exposure yet so ICOS expression is low. Meanwhile, the activation of MHC class I stimulates CD8+ CD45RA+ T cells to differentiate into CD8+CD45RO+ T cells and express ICOS ligands. With increasing age the proportion of peripheral T cells expressing CD45RO gradually increases until it typically reaches 40–60% of the total repertoire in the adult [16]. The up-regulation of CD45RO on antigen-experienced T cells suggest that CD45R0+ T cells is a population of memory/effector cells [16]. In autoimmune disease, memory T cells are not exhausted as in chronic infection or tumor which make them highly pathogenic. The longevity and active functionality of memory T cells in autoimmune can perpetuate the autoimmune damage [17].

Positive correlation had been found between IFN-γ level with memory (CD4+CD45RO+) and (CD8+CD45RO+) T cells. In SLE, the presence of autoantigen presented by APC cells will stimulate the differentiation of naïve CD8+ T cells that express CD45RA+ to CD8+ T memory cells that express CD45RO+. Proportion of antigen-stimulated interferon-gamma (IFN-γ) producing cells from elderly people, was lower than in young people. However, it has been shown that the cumulative IFN-γ production was higher in the elderly due to greater absolute numbers of senescent cells [18]. In immune aging process, there is accumulation of end stage memory T cell releasing large amount of pro inflammatory cytokines including IFN-γ contribute to persistent low-grade inflammatory state is commonly observed in old age [19]. The increase of IFN-γ level in SLE patient in our study suggested that inflammaging process is also found in SLE and contributes to premature aging of immune system.

The findings of this study will provide a new information regarding to the pathogenesis of SLE, especially the role of immune-aging in the progressivevity of SLE. However, there are still some limitations from this study. This study used a cross-sectional design, however there was no follow up of the effect of increase percentages in T cell senescence markers to increase risk of morbidity or mortality within few years. We need further study in larger populations, and in the wider range of age to confirm these initial findings.

5. Conclusion
In conclusion, our study had confirmed the presence of T cell senescence especially memory T cell in SLE patients. T cell senescence was also correlated with increased proinflammatory cytokine production. Thus, these findings indicate that T cell senescence can be used as a new marker to monitor and to predict the progressivity of SLE. Therefore, investigations of T cell senescence profile in SLE patients have implications for better understanding in SLE clinical manifestations and development of monitoring or treatment strategies of SLE.

6. References
[1] Montoya-Ortiz G 2013 Immunosenescence, aging, and systemic lupus erythematos Autoimmune Dis. 2013
[2] Ventura M T, Casciaro M, Gangemi S and Buquicchio R 2017 Immunosenescence in aging: between immune cells depletion and cytokines up-regulation Clin. Mol. Allergy 15 21
[3] Laderoute M P 2015 A new paradigm about HERV-K102 particle production and blocked release to explain cortisol mediated immunosenescence and age-associated risk of chronic disease Discov. Med. 20 379–91
[4] Mahnke Y D, Brodie T M, Sallusto F, Roederer M and Lugli E 2013 The who’s who of T-cell differentiation: human memory T-cell subsets Eur. J. Immunol. 43 2797–809
[5] Xu W and Larbi A 2017 Markers of T cell senescence in humans Int. J. Mol. Sci. 18 1742
[6] Petri M, Orbai A, Alarcón G S, Gordon C, Merrill J T, Fortin P R, Bruce I N, Isenberg D, Wallace D J and Nived O 2012 Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus Arthritis Rheum. 64 2677–86
[7] Moreau J-F, Pradeu T, Grignolino A, Nardini C, Castiglione F, Tieri P, Capri M, Salvioli S, Taupin J-L and Garagnani P 2017 The emerging role of ECM crosslinking in T cell mobility
as a hallmark of immunosenescence in humans Ageing Res. Rev. 35 322–35

[8] Thewissen M, Somers V, Venken K, Linsen L, Van Paassen P, Geusens P, Damoiseaux J and Stinissen P 2007 Analyses of immunosenescent markers in patients with autoimmune disease Clin. Immunol. 123 209–18

[9] Franceschi C and Campisi J 2014 Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases Journals Gerontol. Ser. A Biomed. Sci. Med. Sci. 69 S4–9

[10] Fulop T, Larbi A, Dupuis G, Le Page A, Frost E H, Cohen A A, Witkowski J M and Franceschi C 2018 Immunosenescence and inflamm-aging as two sides of the same coin: friends or foes? Front. Immunol. 8 1960

[11] Fülöp T, Larbi A, Exterman M, Solana R, Dupuis G, Kotb R, Derhovanassian E and Pawelec G 2014 Aging, Immunosenescence, and Cancer Inflammation, Advancing Age and Nutrition (Elsevier) pp 55–69

[12] Le Page A, Dupuis G, Larbi A, Witkowski J M and Fülöp T 2018 Signal transduction changes in CD4+ and CD8+ T cell subpopulations with aging Exp. Gerontol. 105 128–39

[13] Kovaivu R D and Grubeck-Loebenstein B 2006 Age-associated changes within CD4+ T cells Immunol. Lett. 107 8–14

[14] Rheinländer A, Schraven B and Bommhardt U 2018 CD45 in human physiology and clinical medicine Immunol. Lett.

[15] Hakim F T and Gress R E 2007 Immunosenescence: deficits in adaptive immunity in the elderly Tissue Antigens 70 179–89

[16] McNeill L, Cassady R L, Sarkardei S, Cooper J C, Morgan G and Alexander D R 2004 CD45 isoforms in T cell signalling and development Immunol. Lett. 92 125–34

[17] Devarajan P and Chen Z 2013 Autoimmune effector memory T cells: the bad and the good Immunol. Res. 57 12–22

[18] Turner J E 2016 Is immunosenescence influenced by our lifetime “dose” of exercise? Biogerontology 17 581–602

[19] Müller L and Pawelec G 2014 Aging and immunity–impact of behavioral intervention Brain. Behav. Immun. 39 8–22