CHAPTER I

1.1. INTRODUCTION

Systemic lupus erythematous (SLE) is a systemic autoimmune disorder caused by autoreactive T and B lymphocytes to their own antigen, leading to heterogeneous chronic inflammation with multiple organ damage.[1]This disorder presents with diverse clinical symptoms and appears to be increasing in prevalence over time.[2] The pathological hallmark of SLE is mediated by alterations in aberrant B-lymphocyte hyperactivity in addition to abnormalities of T –lymphocytes and antigen presenting cells, leading to impaired cell mediated immunity. The dysfunction of the T cells observed in patients with SLE yields in skewed cytokine production resulting an imbalance in CD4+ T cell subsets population which is correlated with the cytokine production profile including the T helper (Th) 1 and Th17 cells. The inappropriate regulation of Th1 and Th17 cells has been suggested as a key event in the pathogenesis of immune diseases.[3–5]Activated Th1 cells produce interferon (IFN)-γ which is responsible for initiating the inflammatory process. Meanwhile, Th17 cells subsequently propagate and expand and promote the massive inflammation initiated by Th1 through the release of interleukin (IL)-17.[5]Therefore, the effort to inhibit the activation of Th1 and Th17 can control the inflammation, leading to improved mileu immunity in autoimmune disorders, including SLE. Mesenchymal stem cells (MSCs) have immunomodulatory properties to control the activation of various immune cells, including the autoreactive subset of T cells and B

cells by releasing anti-inflammatory cytokines such IL-10 and transforming growth beta (TGF)-β.[6,7] This fact suggests that MSCs may control the auto immune disorder, thus becoming a new promising approach for SLE disease.

A previous study has reported that the worldwide current number of SLE cases is 40 to 50 per 100.000 people and that Asian patients present the disease approximately two to three times higher than those of European descents. Women are more frequently affected than men at every age and in every ethnic group at a ratio of 6:1.[2] The evidence of SLE in Indonesia showed a mortality rate around 25% of the total number of patients who were predominantly of productive age.[8]Moreover, SLE can be severe and is linked to increased mortality from various cases, including multi organ failure which is a relevant cause of mortality for those with predispositions secondary to an impaired immune response and with risk factors associated with immunosuppressive treatments in patients with SLE.[9]To date, the clinical trial regarding SLE treatment have progressed using agents controlling B-lymphocyte activation, resulting in only modest benefits.[10,11]Meanwhile, another study reported that anti T-cells and anti-cytokines treatments may offer major efficacy in treating certain autoimmune diseases. However, the effects of treatments in SLE is remain unclear.[11,12] Therefore, the exploration of modality controlling T cells and their cytokines has become a major challenge.

The MSCs are adherent, fibroblast-like and multipotent cells that can differentiate into the osteogenic, chondrogenic, adipogenic and neural cells. These stromal cells are defined by the expression of specific markers including CD44, CD105, CD73 and CD90, and by the absence of hematopoietic markers, such as CD11,CD14,CD34,and CD45, and the lack of costimulatory molecules, such as CD40, CD80, and CD86.[7,13]Several studies have shown that MSCs exert immunosuppressive effects on immune system, including Th1 and Th17 cells (subsets of CD4+ T lymphocytes) by inhibiting excessive inflammatory niche by releasing TGF β, IL-10 and prostaglandin E2 (PGE-2).[7]These superior capacities may hamper the aberrant response of proinflammatory effector cells, including Th1 and Th17 cells, ultimately resulting in normality in the milieu of SLE disorder.

Th1 cells, a CD4+ T-cell subset, are involved in cell-mediated and are one of the causal agents in the pathogenesis of autoimmunity due to their ability to elevate IFN-γ in which triggers inflammation. However, genetically deficient IFN-γ in animal model showed an increase in susceptibility in autoimmunity, suggesting another key factor contributing to the pathogenesis of the disease.[14] Th17 cells, characterized by the production of IL-17 family of cytokines, prompt the production of a milieu cytokines, chemokines and prostaglandin that ultimately results in the stimulation and attraction of neutrophils to the inflammation sites.[3,5,15] Inappropriate regulation of Th17 has been suggested as the key event in the pathogenesis of autoimmune disease.[4,5,14] Consistent with the findings,

higher serum level of IL-17 has been observed in various immune-mediated inflammatory disease, particularly SLE.[4,5]Arelationship exists between Th1 and Th17 in which they both counter-regulate and cooperate in an intertwined and complex mechanism during inflammatory response.[3,14] In line with this, other studies have reported that, in SLE, auto-activation of lymphocytes causes the imbalanced modulation of the helper T CD4+ cells lineage, leading to the emerge of Th1 and Th17 subpopulation.[3,16] The interconnection between Th1 and Th17is due to the imbalanced modulation of regulatory T (Treg) cells and it's one of the major contributors in aggravating SLE.[3] Recent findings show the promising potentials of immunomodulator properties of MSCs which may regulate the deviant responses of proinflammatory effector cells in SLE disorder.[17,18]

Previous studies reported that MSCs may decrease the level of IFN-γ-secretion of Th1 cells in addition to IL-17-producingTh17 cells which suggests that they may suppress the proinflammatory milieu into an anti-inflammatory milieu in SLE.[19,20] Another study has revealed that the MSCs suppressive effect on Th17 requires IL-25 and IL-25-mediated upregulation of surface PD-L1.[21] Furthermore, MSCs play a role in downregulating Th17 cells in SLE.[16] Given the significant effect of MSCs in modulating Th1 and Th17 cells responses, the possible effect of MSCs in regulating both Th1 and Th17 population from SLE remains unclear. Therefore, we investigate the effect of MSCs in regulating Th1 and Th17 using transwell-cultured

MSC with peripheral blood monoclonal cells (PBMCs) from SLE using ratio dose of 1:1, 1:25 and 1:50.

1.2. RESEARCH PROBLEM

Are the MSC able to regulate the number of active Th1 and Th17 cells in the PBMC from patients with SLE?

1.3. STUDY OBJECTIVE

1. General Purpose

   The purpose of this study is to determine the percentage of Th1 and Th17 cells in the PBMC from patients with SLE transwell-cultured with MSCs

2. Specific Purpose

   The specific objectives of this study are the following:

   a. to determine the percentage of Th1 cells in the PBMC from SLE patients post-MSCs administration with ratio dose of 1:1 compared to the control group

   b. to determine the percentage of Th17 cells in the PBMC from SLE patients post-MSCs administration with ratio dose of 1:1 compared to the control group

   c. to determine the percentage of Th1 cells in the PBMC from SLE patients post-MSCs administration with ratio dose of 1:25 compared to the control group

d. to determine the percentage of Th17 cells in the PBMC from SLE patients post-MSCs administration with ratio dose of 1:25 compared to the control group
e. to determine the percentage of Th1 cells in the PBMC from SLE patients post-MSCs administration with ratio dose of 1:50 compared to the control group
f. to determine the percentage of Th17 cells in the PBMC from SLE patients post-MSCs administration with ratio dose of 1:50 compared to the control group
g. to evaluate the correlation between Th1 and Th17 cells in the PBMC from SLE patients post-MSCs administration compared to the control group

1.4. RESEARCH BENEFICIARIES

This research is expected to be beneficial for academic and researchers because MSCs with the ability to regulate Th1 and Th17 cells might prove to be a foundation for future treatment in patients with SLE. This research is also expected to be beneficial for society because MSCs may be a modality to treat autoimmune disease, particularly SLE.

1.5. RESEARCH ORIGINALITY

Table 1.1. Research Originality

No	Research, Publication, Year	Method	Title	Results
1.	R. Tatara et al Cytotherapy. 2011, 13 (3) :686-694	Experimental in vitro on mice PBMC cocultured with MSCs	Mesenchymal stromal cells inhibit Th17 but not regulatory T-cell differentiation	MSCs inhibit Th17 through PGE2 and IDO but not Treg differentiation.
2.	D. Wang et al Celullar and Molecular Immunology. 2017, 5 : 423-431	Experimental in vivo on SLE patients treated with MSCs	The regulation of the Treg/Th17 balance by mesenchymal stem cells in human systemic lupus erythematosus	MSCs upregulateTreg cells and downregulate Th17 cells via regulation of TGF-b and PGE-2 in SLE patients
3.	W. Wang et al Stem Cell Reports. 2015, 5: 392-404	Experimental in vitro on healthy donor PBMC cocultured with MSCs	Interleukin-25 Mediates Transcriptional Control of PD-L1 via STAT3 in Multipotent Human MesenchymalStromal Cells (hMSCs) to Suppress Th17 Responses	MSCs supress Th17 responses and this required both IL-25 and PD-L1

4.	Luz-Crawford et al PloS ONE. 2012, 7 : e45272	Experimental in vitro on healthy donor PBMC cocultured with MSCs	Mesenchymal Stem Cells Repress Th17 Molecular Program through the PD-1 Pathway	A cell-to cell contact depend mechanism in the selective immunosuppression of MSC on mature Th17 cells through up-regulation of PD-L1.
5.	Rozenberg et al Stem Cells Translational Medicine. 2016, 5:1506-1514	Experimental in vivo on healthy donor PBMC cocultured with MSCs	Human Mesenchymal Stem Cells Impact Th17 and Th1 Responses Through a Prostaglandin E2 and Myeloid-Dependent Mechanism	MSCs inhibit Th1 and induce Th17 responses mediated by PGE-2
6.	Putra et al Open Access Maced J Med Sci. 2018Oct.4;6(10):1779-83.	Experimental in vitro	The Role of TNF-α induced MSCs on Suppressive Inflammation by Increasing TGF-β and IL-10	MSCs increase the level of TGF-beta and IL-10

This study is different with previous researchers who yet to explore MSCs roles on regulating both in SLE patients, we aim to explore the effect of MSCs on

Th1 and Th17 using transwell-culture MSC with PBMC (Peripheral Blood Monoclonal Cells) from SLE with ratio dose 1:1, 1:25 and 1:50 respectively.