ELUCIDATION OF SERUM LEVELS OF IL-17, IL-23 AND THEIR RECEPTORS IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS: ASSOCIATIONS WITH SEROLOGICAL PARAMETERS AND DISEASE ACTIVITY

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by

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TABLE OF CONTENTS

ACKNOWLEDGEMENT ..... **ii**

TABLE OF CONTENTS ..... **iii**

LIST OF TABLES ..... **vii**

LIST OF FIGURES ..... **viii**

LIST OF ABBREVIATIONS ..... **ix**

LIST OF APPENDICES ..... **xi**

ABSTRAK ..... **xii**

ABSTRACT ..... **xiv**

CHAPTER 1 INTRODUCTION **1**

1.1 Study background 1

1.2 Problem statement 2

1.3 Research question 2

1.4 Objectives of the study 3

1.4.1 General objective 3

1.4.2 Specific objectives 3

1.5 Hypothesis 3

1.6 Importance and benefits of study 4

CHAPTER 2 LITERATURE REVIEW **5**

2.1 Immune response and immunity 5

2.1.1 Innate immunity 5

2.1.2 Adaptive immunity 6

2.1.3 Immunological tolerance 7

2.2 Systemic Lupus Erythematosus (SLE) 8

2.2.1 Epidemiology of SLE 12

2.2.2 Clinical features and manifestations of SLE 12

2.2.3 Laboratory characteristics of SLE 14
2.2.4 Clinical evaluation of SLE disease activity 15
2.3 Cytokines 18
2.3.1 Types of inflammatory cytokines 19
2.3.2 Interleukins 19
2.3.2(a) Interleukin-17 (IL-17 or IL-17A) 19
2.3.2(b) Interleukin-23 (IL-23) 21
2.3.3 IL-23/IL-17 axis in autoimmunity 24
2.3.3(a) IL-23/IL-17 axis in SLE 25
CHAPTER 3 METHODOLOGY 29
3.1 Study area 29
3.2 Study duration 29
3.3 Study design 29
3.4 Study population 29
3.5 Sampling method 30
3.5.1 SLE patients 30
3.5.2 Healthy controls (HCs) 30
3.6 Written consent 31
3.7 Sample size estimation 32
3.8 Demographic data and sample collection 33
3.8.1 Recruitment and demographic data collection 33
3.8.2 Clinical history 33
3.8.3 Blood sample collection 33
3.8.4 Serum collection 33
3.8.5 PBMCs isolation 34
3.8.6 Cell counting using a Hemocytometer 34
3.9 Laboratory tests 35

3.9.1 Flow cytometry analysis (surface staining) for IL-17RA and IL23R 35

3.9.2 ELISA for IL-17 and IL-23 levels 37

3.9.2(a) Human IL-17 ELISA test procedure 37

3.9.2(b) Human IL-23 ELISA test procedure 38

3.9.3 Anti-nuclear antibodies (ANA) 39

3.9.4 Anti-dsDNA 42

3.9.5 C-reactive protein (CRP) 43

3.10 Instruments 45

3.10.1 Fluorochrome-labelled antibodies 45

3.10.2 Chemicals and reagents 46

3.10.3 Kits and consumables 46

3.10.4 Laboratory equipment 48

3.11 Statistical analysis 49

3.12 Flowchart of study 50

CHAPTER 4 RESULTS 51

4.1 Sociodemographic, clinical characteristics and serological parameters of SLE patients and healthy controls 51

4.2 Serological parameters in SLE patients and healthy controls 55

4.3 Assessment of $CD3^+CD4^+$ T helper cells and interleukin receptors levels between SLE patients and healthy controls 57

4.3.1 Flow cytometry analysis on negative cells population 57

4.3.1(a) Determination of negative cells population by unstained controls 57

4.3.1(b) Determination of positive cells population by isotype control as the negative control 57

4.3.2 Flow cytometry analysis on $CD3^+CD4^+$ T helper cells 62

4.3.3 Flow cytometry analysis of IL-17RA and IL-23R levels on $CD3^+CD4^+$ T helper cells 65

4.4 Assessment of interleukin expression levels between SLE patients and healthy controls 66

4.5 Assessment of interleukins and the expression of their receptor among the SLE patients with clinical manifestation and serological parameters 67

4.5.1 Associations between interleukins with SLEDAI-2K scores among SLE patients 67

4.5.2 Associations between interleukins with serological parameters among SLE patients 69

4.6 Results summary 72

CHAPTER 5 DISCUSSION 74

5.1 High SLE prevalence in females 74

5.2 Significantly higher activated $CD4^+$ T helper cells in SLE 75

5.3 Emerging roles of cytokine receptors in SLE 76

5.4 Upregulated pro-inflammatory cytokines expression levels 77

5.5 Association of IL-17, IL-23 and their receptors with serological parameters and SLEDAI-2K 78

CHAPTER 6 CONCLUSION AND FUTURE RECOMMENDATIONS 80

REFERENCES 82

APPENDICES

LIST OF PUBLICATIONS

LIST OF TABLES

Page

Table 2.1 Common clinical features of SLE 13

Table 2.2 Laboratory tests for screening and diagnosis of SLE 14

Table 2.3 ACR criteria for the classification of SLE 16

Table 2.4 Sources, production sites and mode of IL-17 production 20

Table 2.5 Sources, production sites and mode of IL-23 production 23

Table 3.1 Inclusion and exclusion criteria of study subjects 31

Table 3.2 Summary of laboratory tests used throughout the study 35

Table 3.3 Summary of fluorochromes used for each specific marker, excitation 36

Table 3.4 Positive or negative ANA results 40

Table 3.5 ANA staining patterns 41

Table 3.6 Test results for CRP 44

Table 3.7 Fluorochrome-labelled antibodies for flow cytometry 45

Table 3.8 Chemicals and reagents 46

Table 3.9 Kits used in this study 46

Table 3.10 Consumables used in this study 47

Table 3.11 Laboratory equipment used in this study 48

Table 4.1 Sociodemographic and clinical characteristics of SLE patients and 53

Table 4.2 Serological parameters in SLE patients and healthy controls 56

Table 4.3 Associations of IL-17, IL-23, IL-17RA and IL-23R with SLEDAI-2K scores 68

Table 4.4 Associations of IL-17, IL-23, IL-17RA and IL-23R with ANA and anti-dsDNA among SLE patients 70

Table 4.5 Asssociations of IL-17, IL-23, IL-17RA and IL-23R with CRP among ... 71

Table 4.6 Summary results on cells and interleukins expression levels 72

Table 4.7 Summary results on the associations of interleukins in 73

LIST OF FIGURES

Page

Figure 2.1 Immuno-pathogenesis of SLE 11

Figure 2.2 IL-23/IL-17 axis in SLE pathogenesis..... 27

Figure 4.1 Age distribution of SLE patients 54

Figure 4.2 Determination of negative cells population by unstained control 58

Figure 4.3 Determination of negative cells population by isotype control 60

Figure 4.4 Increased $CD3^{+}CD4^{+}$ T helper cells in SLE patients compared to 62

Figure 4.5 Gating strategy to determine the IL-17RA and IL-23R expression levels by flow cytometry analysis 63

Figure 4.6 The expression levels of interleukin receptors assessed within..... 65

Figure 4.7 Interleukin expression levels 66

LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
ANA	antinuclear antibodies
APC	antigen presenting cell
BAFF	B-cell activating factor
BILAG	British Isles Lupus Assessment Group Index
BLyS	B lymphocyte stimulator
CRP	c-reactive protein
cSLE	childhood-onset SLE
DC	dendritic cell
dsDNA	double-stranded DNA
ECLAM	European Consensus Lupus Activity Measurements
EDTA	ethylenediamine tetra acetic acid
ELISA	enzyme-linked immunosorbent assay
ENA	extractable nuclear antigen
EULAR	European League Against Rheumatism
FBS	fetal bovine serum
FITC	fluorescein isothiocyanate
HC	healthy control
HCQ	hydroxychloroquine
HEp-2	human epithelium-2
IBD	inflammatory bowel disease
IC	immune complex
IFN	interferon
ILC	innate lymphoid cells
Ig	immunoglobulin
IIFA	Indirect immunofluorescent assay
IL	interleukin
IL-R	interleukin receptor
imDCs	immature myeloid DCs
MAB	monoclonal antibody
MHC II	major histocompatibility complex group II

MMP	matrix metalloproteinases
Mo-DC	monocyte-derived DC
NET	neutrophil extracellular trap
NF-κB	nuclear factor-kappa B
NK cell	natural killer cell
NO	nitric oxide
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
pDC	plasmacytoid dendritic cell
RA	rheumatoid arthritis
RANKL	receptor activator of nuclear factor kappa-B ligand
RT	room temperature
SLAM	Systemic Lupus Activity Measure
SLE	systemic lupus erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SLEDAI-2K	Systemic Lupus Erythematosus Disease Activity Index 2000
SLICC	Systemic Lupus International Collaborating Clinics
SpA	Spondylarthritis
STAT 3	signal transducer and activator of transcription 3
TCR	T cell receptor
TGF	transforming growth factor
Th	T helper
TNF	tumour necrosis factor
Treg	T regulatory

LIST OF APPENDICES

Appendix A	SLEDAI-2K score calculator
Appendix B	SLEDAI-2K form
Appendix C	Ethics approval (USM/JEPeM/17120680)
Appendix D	Consent form
Appendix E	Cell counting form

PENENTUAN TAHAP SERUM IL-17, IL-23 DAN RESEPTOR MEREKA DALAM PESAKIT SISTEMIK LUPUS ERITEMATOSUS: PERKAITAN DENGAN PARAMETER SEROLOGI DAN AKTIVITI PENYAKIT

ABSTRAK

Interleukin (IL) merupakan kumpulan sitokin, terutamanya yang disintesis oleh pembantu T sel $CD4^+$, dan juga oleh sel-sel endothelial, monosit dan makrofaj. IL terikat pada interleukin reseptor dan bertindak sebagai salah satu faktor penting dalam perkembangan penyakit sistemik lupus eritematosus (SLE). Kajian ini bertujuan untuk menentukan tahap serum IL-17, IL-23 dan reseptor mereka; IL-17RA dan IL-23R pada pesakit SLE berbanding dengan individu sihat. Di samping itu, interleukin dan reseptor mereka telah dikaitkan dengan parameter serologi dan aktiviti penyakit, khususnya SLEDAI-2K skor untuk menyokong hubungan IL-17, IL-23 dan reseptor mereka dalam patogenesis SLE. Seramai 50 pesakit SLE dan 50 individu sihat telah diambil dalam kajian ini. Tahap serum IL-17 dan IL-23 telah dianalisa menggunakan antibodi spesifik 'pre-coated' menggunakan ujian 'Human IL-17/IL-23 ELISA'. Sel darah telah diasingkan menggunakan cara 'Histopaque-1077 density centrifugation' dan ditandakan dengan antibodi spesifik untuk pewarnaan permukaan IL-17RA dan IL-23R, dan tahap mereka ditentukan oleh analisis 'flow cytometry'. Pesakit SLE menunjukkan tahap yang ketara tinggi daripada kedua-dua IL-17RA dan IL-23R (p<0.001) berbanding dengan individu sihat. Tahap signifikasi yang menurun ditunjukkan dalam IL-17 (p<0.001) manakala tiada perbezaan yang signifikan (p=0.73) dalam IL-23 apabila membandingkan dalam pesakit SLE dan individu sihat. Di samping itu, tidak ada korelasi antara tahap interleukin dan SLEDAI-2K dan juga parameter serologi. Menariknya, IL-17RA

mempunyai perkaitan yang ketara dengan antinuklear antibodi (ANA) (p=0.024) dan IL-23R mempunyai hubungkait dengan SLEDAI-2K (p=0.011). Walau bagaimanapun, penurunan tahap serum IL-17 mungkin perlu dikaji dengan lebih mendalam antara perhubungan dengan reseptornya kerana peningkatan tahap ekspresi IL-17RA dijangka mungkin mengambil kebanyakan serum IL-17 yang mengakibatkan tahap pengurangannya. Kesimpulannya, kami mencadangkan bahawa perencatan terapeutik pada IL-17RA dan IL-23R mungkin merupakan pilihan yang berpotensi untuk pesakit SLE.

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ABSTRACT

Interleukins (ILs) are a group of cytokines, mainly synthesised by $CD4^+$ T helper cells, as well as by endothelial cells, monocytes and macrophages. ILs bind to their interleukin receptors and this is one of the pivotal factors in the development and progression of systemic lupus erythematosus (SLE). This study aimed to determine the serum levels of IL-17, IL-23 and their receptors; IL-17RA and IL-23R, in SLE patients compared with healthy controls. In addition, the associations of these interleukins and their receptors were associated with serological parameters and disease activity, specifically SLEDAI-2K score. SLE patients and healthy controls (n=50 in each group) were recruited in this study. The serum levels of IL-17 and IL-23 were evaluated using pre-coated specific antibody via Human IL-17/IL-23 ELISA test. PBMCs were isolated from the peripheral blood using Histopaque-1077 density centrifugation and stained with fluorochrome-labelled antibodies for staining of surface IL-17RA and IL-23R, and their levels determined by flow cytometry analysis. SLE patients showed significantly elevated levels of IL-17RA and IL-23R ($p<0.001$) compared with healthy controls. Significant downregulation of serum IL-17 ($p<0.001$) while no significant difference ($p=0.73$) in IL-23 levels were observed in SLE patients compared with healthy controls. In addition, no significant associations between IL levels with SLEDAI-2K and serological parameters. Interestingly, IL-17RA levels were significantly associated with antinuclear

antibodies (ANA) (p=0.024) and IL-23R levels were significantly associated with higher SLEDAI-2K scores (p=0.011). However, the decreasing level of serum IL-17 might need further work as the relation with its receptor where the elevated IL-17RA expression is expected to take up most of the circulating serum IL-17 resulting in its reduced levels. In conclusion, we suggest that therapeutic inhibition of IL-17RA and IL-23R represents a potential treatment option for SLE patients.

CHAPTER 1

INTRODUCTION

1.1 Study background

Interleukin (IL) is a form of cytokine that is released by leukocytes only but later found to be generated by many other body cells. They are particularly crucial in arousal of immune responses such as inflammation and also function in cell growth regulation, differentiation, activation and migration (Justiz Vaillant and Qurie, 2019). ILs are a large group of proteins that can induce several cells' and tissues' reactions by binding to strong affinity receptors on the cell surfaces. They may act on the cells in several ways which are autocrine, paracrine and endocrine actions (Abbas et al., 2019).

Acting as a metabolite of Th17, IL-17 has been implicated in the pathogenesis of autoimmune diseases such as SLE (Alessia Alunno et al., 2019). IL-17 is active in epithelial and endothelial cells as well as plays an essential role in releasing IL-6 and other pro-inflammatory cytokines. Other than enhancing the activity of antigen-presenting cells (APCs), IL-17 also stimulated endothelial cell production of chemokines (Dhaouadi et al., 2018; Guerra et al., 2017). Other than IL-17, IL-23 is deduced as one of pro-inflammatory cytokine that takes part in the pathogenesis of SLE. IL-23 is primarily synthesized by macrophage and dendritic cells to operate on T cells that enable the maintenance of IL-17 (Guerra et al., 2017).

In this present study, the levels of IL-17, IL-23 and their receptors; namely IL-17RA and IL-23R were determined in SLE patients and healthy controls associated with serological parameters and SLEDAI-2K score to deduce the role of these interleukins and their receptors in the pathogenesis of SLE.

1.2 Problem statement

SLE is known as a multifactorial disease, where genetic, endocrine, immunological and environmental factors influence the loss of immunological tolerance against self-antigens. Recently, several studies showed the pathogenic role of T-helper type 17 (Th17) in SLE and the studies of specific therapeutic antibodies targeting IL-17 and IL-23 to aid the treatment of SLE patients. However, the participation of IL-17, IL-23 and their receptors in SLE pathogenesis remain unclear despite several studies has been done. This study aims to determine the levels of IL-17, IL-23 and their receptors in SLE patients and healthy controls. Additionally, this study is to investigate the association of IL-17, IL-23 and their receptors with serological parameters such as C-reactive protein (CRP), antinuclear antibodies (ANA) and anti-double-stranded DNA (anti-dsDNA). This study is also to evaluate the association of IL-17, IL-23 and their receptors with disease activity (Systemic Lupus Erythematosus Disease Activity Index-2000; SLEDAI-2K score).

1.3 Research question

Are pathogenesis of SLE correlated with IL-17, IL-23 and their receptors?

1.4 Objectives of the study

1.4.1 General objective

To determine the levels of IL-17, IL-23 and their receptors in SLE patients and their association with serological parameters and SLEDAI-2K scores.

1.4.2 Specific objectives

1. To determine the correlation of IL-17 and IL-23 levels between SLE patients and healthy controls.
2. To determine the levels of IL-17RA and IL-23R within $CD3^{+}CD4^{+}$ T helper cells between SLE patients and healthy controls.
3. To determine the association of IL-17, IL-23 and their receptors (IL-17RA and IL-23R) levels between SLE patients and serological parameters (ANA and anti-dsDNA autoantibodies).
4. To determine the association of IL-17, IL-23 and their receptors (IL17RA and IL23R) levels with CRP and SLEDAI-2K scores in SLE patients.

1.5 Hypothesis

1. IL-17 and IL-23 are associated with the presence of ANA, anti-dsDNA autoantibodies and CRP in SLE patients.
2. IL-17RA and IL-23R are associated with the presence of ANA, anti-dsDNA autoantibodies and CRP in SLE patients.
3. IL-17, IL-23 and their receptors (IL-17RA and IL-23R) are associated with disease activity based on SLEDAI-2K scores in SLE patients.

1.6 Importance and benefits of study

1. No studies have been conducted in Malaysian or South-East Asian cohort of SLE patients in the determination of IL-17, IL-23 and their receptors (IL-17RA and IL-23R) expression in SLE patients and their association with ANA, anti-dsDNA, CRP and SLEDAI-2K scores.
2. There is no study has been conducted in Malaysia that focusing on specific races; eg. Malay within SLE patients in determining their interleukin receptor levels in SLE patients.
3. Association of ANA, anti-dsDNA and CRP with IL-17, IL-23 and their receptors (IL-17RA and IL-23R) levels might serve as diagnostic markers for SLE patients and/or monitor disease activity in the future.

CHAPTER 2

LITERATURE REVIEW

2.1 Immune response and immunity

Immunity is the capability of multicellular organisms to fight diseases, infections, harmful microorganisms, or other unsolicited biological invasions by having competent biological shield or defense and also possess adequate resistance to bypass autoimmune disease and allergy. The immune response is a mechanism whereby the body recognises and responds against viruses, bacteria and foreign substances. Apart from immunity to pathogenic microorganisms and its products, the immune response also acts as graft rejections, allergies and autoimmunity to self-antigens. The human immune system has two primary constituents, each with different roles and functions; which are innate and adaptive immune responses (Labzin et al., 2018).

2.1.1 Innate immunity

Innate immune response, also called as native immunity symbolizes the primary line of defence against infections and plays diverse functions in health and disease, involving wound healing, tissue repairs and clearance of apoptotic cells and cellular debris. This primary defence response is crucial to promote the outgrowth arousal of adaptive immunity. Innate immunity functions can be classified into four distinct categories: detection, identification and removal of pathogens and contaminated cells, and, fourthly, activation of adaptive mechanism for a broad inflammatory response (Frizinsky et al., 2019).

Acting as a non-specific defense mechanism, innate immunity acts promptly against the antigen's appearance in the body. Major components of innate immunity

including physical barriers (e.g. skin, immune system cells attacking foreign cells, chemicals in the blood), phagocytic leukocytes (e.g. macrophages, neutrophils), antigen-presenting cells (APCs) (e.g. dendritic cells (DCs), natural killer (NK) cells, mast cells and γδ T cells) (Maruyama et al., 2011; Parnham and Rossi, 2019).

2.1.2 Adaptive immunity

Secondary immune response, mainly known as adaptive immunity, is a specific and acquired response which is established in response to specific foreign antigens exposure. Adaptive immunity can be sub-divided depending on the course immunity acquainted; naturally acquired through liability contact with the disease-causing agent and artificially acquired immunity which evolves through intentional actions such as vaccination. The anatomic sites of adaptive immunity are spleen, lymph nodes and mucosal-associated lymphoid tissue. Adaptive immunity is classified into the humoral immunity (i.e. antibodies by plasma cells) and cell-mediated immunity (i.e. T cell responses) (Pandit, 2017).

Adaptive immunity grows slowly compared to innate immunity where it is distinguished by immunological memory resulting in more severe responses in subsequent exposures to the same antigen which triggered primary immune responses previously (Aribi, 2017). B cells are essential for adaptive immunity because they generate a range of antibodies that help to effectively eliminate antigens, including pathogens. B cells mature into plasma cells to produce immunoglobulins while T cells produce cytokines which can amplify or restrain inflammatory responses (Robert R.R. and David D.C., 2019). In fact, B cells perform an important function in the immune system by secreting cytokines and transmitting antigens (Ma et al., 2019).

Autoimmune diseases induce abnormally low activation or overactive immune system function. Throughout instances of the overactive immune system, the

organisms fight and destroy its tissue such as by invading the joints, causing swelling and inflammation. Apart from B and T cells, neutrophils and macrophages are key components of diseases pathogenesis, since they play a vital role throughout inducing either pro- or anti-inflammatory reactions to the inflammatory site (Navegantes et al., 2017).

2.1.3 Immunological tolerance

Tolerance is the suppression of an inflammatory response to a specific antigen. Immunological tolerance defends against bogus immune response to body constituents; is a condition of unresponsiveness of antigen-specific or tissue-specific immune response caused by prior exposure (Mathis and Benoist, 2010; Wu et al., 2019). One of the key functions of the immune system is to identify self-antigens as non-threatening and produce active responses to the exclusion of non-self-antigens or foreign substances. Thus, the immune system is normally tolerant of self-antigens and typically does not damage owns' cells and organs.

Immunological tolerance involves a network of mechanisms that are central (by clonal deletion) and peripheral tolerance (by clonal ignorance, deletion and anergy) and transplantation tolerance involving suppressor T cells; a type of superior tolerance acting as an alternate mechanism other than clonal deletion and anergy (Wu et al., 2019; Mathis and Benoist, 2010). The immune system typically initiates immune responses promptly the following infection and shuts down immediately upon elimination of foreign invaders to avoid damage caused by excessive immune stimulation, which may lead to self-antigen reactions which trigger autoimmune diseases (Wu et al., 2019).

To date, studies indicated that autophagy as an efficient way to control immune tolerance and evade over-activation of the immune response by attacking

several immune receptor and effector cells (Mocholi et al., 2018). Autophagy plays a vital role in both central and peripheral immunity, helps to prevent the deterioration of immune tolerance. Disorders such as autoimmune disease can arise when tolerance is lost as autoimmunity develops when the T or B cells identify themselves as foreign and strikes own tissues, inducing pathology. The pathological cause may be attributed to antibodies, inflammatory T cells (Th1/Th17) or antigen-antibody complexes (Kavathas P.B. et al., 2019).

2.2 Systemic Lupus Erythematosus (SLE)

SLE is a heterogeneous, multisystem autoimmune disorder that has a vast spectrum of effects on multiorgan and tissue involvements including skin, kidney, lung, brain, heart and joints (Qi et al., 2018; Mohan and Putterman, 2015). Numerous pro- and anti-inflammatory cytokines play pivotal pathogenic roles (Cavaillon, 2001). Thus, the main feature of SLE is the heterogenous autoantibodies group production against autoantigens in cells and the circulatory system (Qi et al., 2018). Essentially, inflammatory cytokines such as transforming growth factor-beta (TGF-β), IL-10, IL-6, interferon-γ (IFN-γ), IFN-*α,* B-cell activating factor (BAFF), IL-17 and IL-23 can be produced by both innate and adaptive immune cells to sustain exquisite immune homeostasis(Cavaillon, 2001). SLE undergoes distinct polymorphism and epigenetic changes caused by complex combination of genetic, epigenetic and environmental factors, leading to gene expression and function alterations in assorted molecules resulting in abnormal responses of T cells (Suarez-Fueyo et al., 2016).

Immuno-pathogenesis of SLE is complex where the progression of disease affected by immune responses and components. Both immune responses are impaired in SLE patients resulting in self-tolerance deficit and production of autoantibodies,

associated with vital immunological alterations in SLE patients like T cell function, T regulatory (Treg) cells, cytokine production, epigenetic regulation as well as the balance of Th17 cells (Tsokos, 2011). Aetiology of SLE is multifactorial and incorporates contribution from genetic, epigenetic, stochastic factors, hormonal, environmental and immuno-regulatory factors, which hinges on tolerance deficit and maintained lifelong autoantibodies production (Tsokos et al., 2016). Main SLE immuno-pathogenesis is self-tolerance breakdown where both innate and adaptive immunity is interlinked with each other through complements, immune complexes, cytokines and kinases of intracellular machinery (Pan et al., 2019). Immuno-pathogenesis of SLE can be divided into two, which are dysregulation of DCs and neutrophils in innate immunity and both T and B cells impairment in adaptive immunity.

In addition, to exhibit bizarre secretion of cytokine and cell signal transduction, T cells can also lead to improper recruitment and activation of B cells and DCs in inflammatory (Comte et al., 2015). Frauwirth et al., (2002) discovered T cells metabolism as activation of lymphocytes trigger cells growth, proliferation and differentiation which expand metabolic demand. A cellular metabolism disruption in SLE was first reported in T cell mitochondria, which give rise to the expanding of mitochondrial mass and $CD4^+$ T cells in SLE (Frauwirth et al., 2002). A subtype of $CD4^+$ T cells, T helper cells impairment is approximately associated with the manifestation and development of SLE, thus Th1/Th2 cells imbalance is deliberated as a vital part for SLE immuno-pathogenesis. Meanwhile, a subset of effector $CD4^+$ T cells; Th17 cells are recognized with the capability to produce IL-17A, IL-17F and IL-22, mediate inflammatory responses and engage in autoimmune diseases development. Beyond dysregulation of DCs, neutrophils and T cells as well as their

signalling abnormalities, a peculiar tolerance of central and peripheral B cells resulting a diverse of autoantibodies production by a vast amount of autoreactive B cells, leading to SLE immuno-pathogenesis (Morel, 2017).

SLE has correlated with B cell polyclonal activation and autoantibodies formation that induces complex immune inflammation (Zamora et al., 2019). The immuno-pathogenesis of SLE was presented in Figure 2.1. The immature myeloid DCs (imDCs) undergo maturation and expressed abundance amount of costimulatory molecules (CD86, CD40 and MHC ll) and high levels of IL-6 and IL-12p70 which in turn mature DCs activates Th1 and Th2 cells by binding CD86 and CD40 to their ligand. Apart from inhibiting Treg proliferation, high production of IL-6 helps promotes Th17 differentiation which then IL-23 promotes the production of IL-17. Plasmacytoid dendritic cells (pDC) produced a high amount of IFN-α promoting B cell activation and B-T cells interaction enhancing the autoantibodies production. T cell leads to improper recruitment and activation of B cell in inflammation. Autoantibodies produced involved in forming autoantibodies immune complexes (ICs) which leads to inflammation. The improper activation of neutrophils released proteases which leads to tissue damage in SLE, while increases neutrophil extracellular trap (NET) released involved in pDCs activation in SLE, associated in inflammation. Sources: (Wu et al., 2016; Pan et al., 2019; van der Linden et al., 2018; Bruschi et al., 2019).

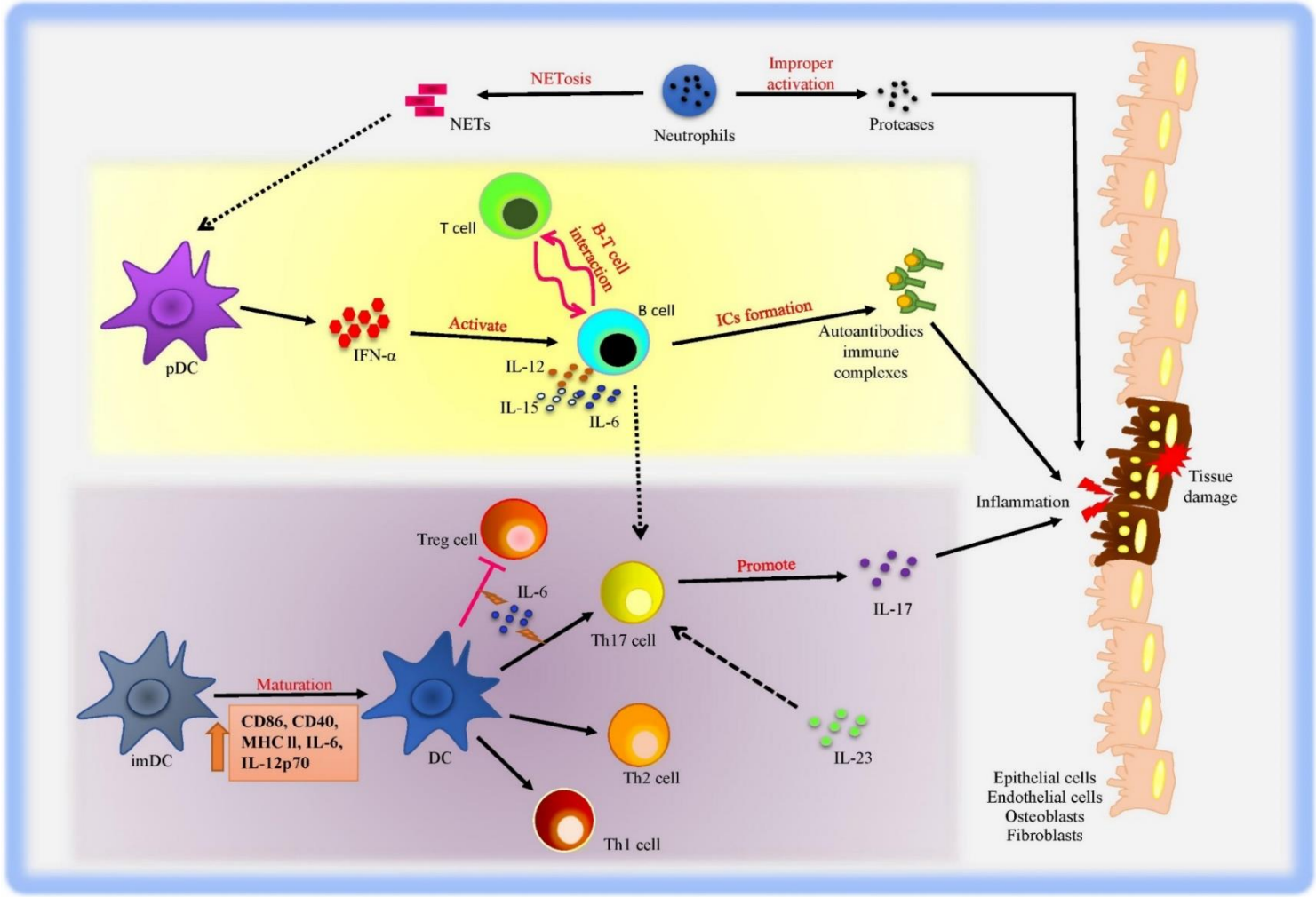

Figure 2.1 Immuno-pathogenesis of SLE

2.2.1 Epidemiology of SLE

SLE affects women more frequently and the incidence peaks between 15 and 44 years old (Fatoye et al., 2018; Rees et al., 2017; Carter et al., 2016). The prevalence of SLE is predicted 91.9/100,000 patients; women (89% cases) tends to be highest from a total of 41,804 patients diagnosed with SLE, according to Columbia National records (Fernandez-Avila et al., 2019), as compared to the study by Bae et al., (2019), the annual prevalence of SLE slightly increased from 21.25/100,000 patients in 2005 to 35.45/100,000 patients in 2015 as recorded in National Health Insurance of South Korea (Bae et al., 2019).

Being a multiracial country, Malaysia consists of three largest ethnic groups in Peninsular Malaysia which are Malays (69.3%), Chinese (22.8%) and Indians (6.9%), A change in the SLE epidemiology over time could be an outcome of age and ethnic diversity between population (Rees et al., 2016).

2.2.2 Clinical features and manifestations of SLE

SLE presents a range of features and manifestations from indolent to fulminant, depending on which part of the body is affected (Bartels and Muller, 2019) (Table 2.1). Disease assessment and diagnosis can be challenging as lupus imitates several other diseases; thus, it needs clinical and serologic assessment. As depicted by Cojocaru et al., (2011), there are multiple manifestations in SLE namely constitutional, musculoskeletal, dermatological, renal, neuropsychiatric, pulmonary, gastrointestinal, cardiac, vascular, ocular, obstetric, endocrine and hematologic manifestations (Cojocaru et al., 2011).

SLE patients may show diverse systemic symptoms such as fever, headache, arthralgias, myalgias, malaise, food aversion and weight loss. Fever, nonspecific fatigue, weight changes and arthralgia are the prevailing signs for recurrent active

SLE or new SLE cases. Musculoskeletal manifestation is common as patients regularly seek medical attention for joint pains as osteonecrosis, myopathy, arthritis and arthralgia. A meta-analysis on clinical manifestations of childhood- and adult-onset SLE showed that sicca and Raynaud's phenomenon are more common in adults than adolescents and children SLE (Livingston et al., 2011). On the other hand, fever, haemolytic anaemia, ulcers or mucocutaneous involvement, malar rash, proteinuria, renal involvement, urinary cellular cast, lymphadenopathy and thrombocytopenia are more common in childhood-onset SLE (Bartels and Muller, 2019). The common clinical features of SLE are explained in Table 2.1.

Table 2.1 Common clinical features of SLE

Clinical features	**Clinical manifestations**
Unexplained fever	• Acting as an early sign of SLE with low-grade fever (36.9˚C to 38.3˚C) • Fever occurs intermittently
Headache	• Inflammation in the brain causes headaches, fatigue, vision problems, seizures or behaviour changes • May also be triggered by vasculitis (blood vessels swelling or inflammation)
Rash/ lesions	• Butterfly-shaped rash on the bridge of the nose and both cheeks • May appear abruptly or occur after sunlight exposure (sensitive to sunlight)
Hair loss	• Thinning hair triggered by skin and scalp inflammation • Patients might also have facial thinning, brows, eyelashes and other body hair loss due to hair fragility
Dry mouth and eyes	• Gritty and dry eyes and mouth due to malfunction of salivary glands • In some instances, women may also feel the dryness of the skin and vagina
Swollen and painful joints	• Inflammation may cause stiffness, pain and noticeable swelling of the joints, especially in the morning • Intermittent occurrence and pain may be progressively more evident

2.2.3 Laboratory characteristics of SLE

Early diagnosis and treatment are crucial in SLE. However, medical symptoms of SLE typically resemble other autoimmune diseases and thus serological measures are required to validate the diagnosis. These laboratory tests include ANA test, anti-dsDNA, CRP level and anti-ENA as summarised in Table 2.2.

Table 2.2 Laboratory tests for screening and diagnosis of SLE

Laboratory tests	Explanations
Antinuclear antibody (ANA)	ANA test is widely used to measure autoantibodies that target the nucleus of the cells or 'control' area. ANA is a main serological test to indicate the presence of autoimmune diseases involving multiple organs systemically. ANAs are found in over 95% of SLE patients in elevated titres (Isenberg et al., 2019). Nevertheless, due to its low positive predictive value, a positive ANA test on its own is inadequate to diagnose SLE or other autoimmune diseases. Therefore, a corresponding serological analysis such as anti-dsDNA and CRP levels are required. Testing by immunofluorescence on HEp-2 cells, ANA at a titre of ≥1:80 on HEp-2 cells or an equivalent positive test at least once.
Anti-double stranded DNA (anti-dsDNA)	Anti-dsDNA autoantibodies mistakenly target and attack the body's genetic material, causing inflammation and tissue damage. Anti-dsDNA autoantibodies in an immunoassay with demonstrated > 60 (IU/ml) was considered positive, while intermediate results (50-60 IU/ml) were re-tested and re-evaluated.
C-reactive protein (CRP)	CRP is formed in the liver and a proxy of systemic inflammation. CRP is an acute reactant phase which indicates that its concentrations rise in response to inflammation. Normal CRP levels are below 3.0 mg/l, while a high-sensitivity CRP test can detect levels below 10.0 mg/l. An inflammatory condition will be signified when CRP levels are over 10.0 mg/l.
Anti-extractable nuclear antigen (anti-ENA)	An anti-ENA screen is usually prescribed following a positive result in an antinuclear antibody (ANA) test. Anti-ENA screen determines the levels of seven different autoantibodies including anti-Sm, anti-RNP, anti-SSA, anti-SSB, anti-Jo-1, anti-Scl 70 and anti-CENP-B.

2.2.4 Clinical evaluation of SLE disease activity

SLE diagnosis criteria developed by American College of Rheumatology (ACR) state that if an individual encountered at least four out of 11 symptoms with no other explanation behind them, physicians can use as a reference to decide whether a patient with symptoms have lupus. Some of the symptoms are discoid and malar rash, mouth or nose sores, arthritis, kidney problem (e.g. blood or protein present in urine), pleuritis or pericarditis, neurologic problem (e.g. psychosis, strokes, or seizures), and abnormal blood tests (such as anaemia, abnormality of specific antibodies; e.g. anti-dsDNA, or positive ANA result) (Rheumatology, 2019). When patients meet the ACR criteria as listed in Table 2.3, a set of blood tests will be conducted to validate the diagnosis where if ANA is positive, more specific tests including anti-dsDNA and ENA antibodies are required to confirm the diagnosis.

Table 2.3 ACR criteria for the classification of SLE

Criterion	Definition
Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
Discoid rash	Erythematosus raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by the physician
Non-erosive Arthritis	Involving 2 or more peripheral joints, characterized by tenderness, swelling or effusion
Pleuritis or Pericarditis	Pleuritis -- a convincing history of pleuritic pain or rubbing heard by a physician or evidence of pleural effusion **Or** Pericarditis -- documented by electrocardiogram or rub or evidence of pericardial effusion
Renal disorder	Persistent proteinuria > 0.5 grams per day or > than 3+ if quantitation not performed **Or** Cellular casts – may be a red cell, haemoglobin, granular, tubular, or mixed
Neurologic disorder	Seizures – in the absence of offending drugs or known metabolic derangements; e.g., uraemia, ketoacidosis, or electrolyte imbalance **Or** Psychosis – in the absence of offending drugs or known metabolic derangements, e.g., uraemia, ketoacidosis, or electrolyte imbalance
Hematologic disorder	Haemolytic anaemia--with reticulocytosis **Or** Leukopenia --< 4,000/mm³ on ≥ 2 occasions **Or** Lymphopenia --< 1,500/mm³ on ≥ 2 occasions **Or** Thrombocytopenia --< 100,000/mm³ in the absence of offending drugs
Immunologic disorder	Anti-DNA: antibody to native DNA in abnormal titre **Or** Anti-Sm: the presence of antibody to Sm nuclear antigen **Or** A positive finding of antiphospholipid antibodies on: 1. An abnormal serum level of IgG OR IgM anti-cardiolipin antibodies, 2. A positive test result for lupus anticoagulant using a standard method, or 3. A false-positive test result for at least 6 months confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test
Positive Antinuclear Antibody	An abnormal titre of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs

To date, 2019 SLE European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria has been developed for SLE (Aringer et al., 2019). Compared to previous criteria, SLE ACR 1997 (Hochberg, 1997) and Systemic Lupus International Collaborating Clinics (SLICC) 2012 (Petri et al., 2012), EULAR/ACR criteria have been developed to find a better compromise of specificity and sensitivity.

2019 EULAR/ACR is designed to enhance the sensitivity and specificity of SLE classifications, which have been suggested for usage in SLE research studies and trials rather than for diagnostic purposes. It is advised that the involvement of positive ANA at a titer 1:80 or higher by immunofluorescence (IFA) as an entry criterion for classification of SLE (Rodrigues Fonseca et al., 2019) and refining the SLICC 2012, requiring the present of at least 1 immunologic criterion (Aringer et al., 2019).

Measurement of SLE disease development or activity remains challenging due to the dynamic multi-system of lupus complexity. However, it is essential for the assessment of treatment outcomes as well as response to novel therapies in clinical trials. There are two main protocols used to measure SLE disease activity: (1) Global Score System for comprehensive activity measurement as well as individual organ assessment such as Systemic Lupus Activity Measure (SLAM), European Consensus Lupus Activity Measurements (ECLAM) and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI); (2) British Isles Lupus Assessment Group Index (BILAG) is the System Assessment Scale to assess particular disease activity in specific organs (Fernando and Isenberg, 2005).

SLEDAI is a global index for the measurement of lupus disease occurrence in the intervening 10 days. SLEDAI score comprises of 24 weighted medical and experimental factors in nine organ systems. The descriptor scores vary from 1 to 8 and

the total possible score for all 24 descriptors is 105. Currently, the 30-day continuation of the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) is proportionate to the initial 10-day version (Mikdashi and Nived, 2015; Touma et al., 2010). SLEDAI-2K descriptors are listed as present or absent, each has a weighted ranking and the total score of SLEDAI-2K is the total of all 24 descriptor scores, allowing the detection of persistent active disease in mucous membrane ulcers, proteinuria, alopecia and rash. The total score of SLEDAI-2K ranging within 0 and 105. The decision-making to treat feasibility of therapy initiation in more than 50 per cent cases are affected by a deemed medically significant, a score of 6 and meaningful change and progress is best defined as a SLEDAI-2K reduction by 4.

2.3 Cytokines

The intermediary intracellular protein signals of the immune system are generally known as cytokines; consisting of interleukins (produced by one leukocyte to modulate the activities of another leukocyte), chemokines (for chemotaxis of cells), interferons (involved in antiviral reactions), lymphokines, monokines and tumour necrosis factor (TNF) (Kindt et al., 2007). Cytokines are formed by a wide range of cells including immune cells such as T and B cells, macrophages, mast cells, granulocytes, stromal cells, endothelial cells and fibroblasts (Lackie and O'Callaghan, 2010). Cytokines play vital roles in the differentiation, maturation and activation of numerous immune cells, and to mount a potent immune response (Zhang and An, 2007). At such, overproduction of pro-inflammatory cytokines may cause or exacerbate autoimmune diseases including SLE (Oppenheim, 2020).

2.3.1 Types of inflammatory cytokines

Pro-inflammatory cytokines such as IL-1, IL-6, IL-12, TNF-α and IFN-γ are produced by immune cells such as T helper (Th) cells and macrophages. Pro-inflammatory cytokines could render the infection worse by inducing fatigue, tissue damage and in certain instances, causing shock and death (Dinarello, 2000). Meanwhile, IL-4, IL-10, IL-13, IFN-α and transforming growth factor-beta (TGFβ) are anti-inflammatory cytokines essential for the suppression and resolution of diseases (Chen et al., 2019; Murray et al., 2014; Cavaillon, 2001).

2.3.2 Interleukins

2.3.2(a) Interleukin-17 (IL-17 or IL-17A)

IL-17 is a potent pro-inflammatory cytokine that mediates protective immunity (Zenobia and Hajishengallis, 2015) and acts as a host defence against microbial pathogens (Chen and Kolls, 2017). The family members of IL-17 consist of IL-17A (also known as IL-17), IL-17B, IL-17C, IL-17D, IL-17E and IL-17F (Sakkas and Bogdanos, 2017). IL-17 is mainly produced by $CD4^+$ T helper 17 (Th17) cells (Jin and Dong, 2013; Lee et al., 2015) in response to their stimulation by IL-23 produced by macrophages and dendritic cells (DCs) (Iwakura and Ishigame, 2006; Suzuki et al., 2014). IL-17 is also produced by $CD8^+$ T cells, natural Th17 cells, innate lymphoid cells (ILCs), and natural killer T (NKT) cells as listed in Table 2.4 (Coquet et al., 2008; Gaffen et al., 2014; Korn et al., 2009; Liang et al., 2015; Marks et al., 2009). The activation and maturation of Th17 cells rely on adequate T cell receptor (TCR) expression, costimulatory molecules and cytokines such as IL-6 and IL-21, which play vital roles in the differentiation of naïve $CD4^+$ T cells (Lee, 2018).

Table 2.4 Sources, production sites and mode of IL-17 production

Sources	Production sites	Mode of IL-17 production	Reference
$CD4^+$ T cells	Thymus/peripheral lymphoid tissues	Upon activation and expansion, $CD4^+$ T cells develop into $CD4^+$ Th17 cells with the production of IL-6 by DCs that induce IL-17 production	(Korn et al., 2009)
$CD8^+$ T cells	Thymus/peripheral lymphoid tissues	$CD8^+$ T cells develop into Tc17 cells, inducing IL-17 production. In Tc17 cells maturation, IL-23 is required for their expansion and maintenance	(Liang et al., 2015)
	Skin		
Natural Th17 (nTh17) cells	Skin and mucosa	Both transcription factors, RORγt5 and RORα6 are expressed by Th17 cells, to produce IL-17 and also express the production of IL-23R	(Marks et al., 2009)
	Thymus	Similar to adaptive Th17, nTh17 cells also develop in the thymus and induce IL-17 production	(Gaffen et al., 2014)
Innate lymphoid cells (ILCs)	Gut and skin	Produce IL-17 in response to inflammatory cytokines and stress	
NKT	Thymus and liver	NKT cell subsets are categorized based on CD4 and NK1.1 expression, and tissue of origin. Activated $CD4^-NK1.1^-$ NKT cells produce high levels of IL-17	(Coquet et al., 2008)

IL-17 is critical for the protection against extracellular bacteria, protozoa and fungal infections at mucosal and epithelial barriers (Matsuzaki and Umemura, 2018). IL-17 signals through a heterodimeric receptor complex, IL-17RA and IL-17RC, where IL-17RA is found ubiquitously but can only signal in the presence of IL-17RC (Astry et al., 2015).

IL-17R, a type I surface receptor, consists of IL-17RA (mainly known as IL-17R), IL-17RB, IL-17RC, IL-17RD and IL-17RE (Xu and Cao, 2010). IL-17R interacts with IL-17 and activates the signalling cascades leading to the induction of chemokine production and release to recruit immune cells to the site of inflammation. However, this phenomenon also contributes to the pathogenesis of autoimmune diseases such as rheumatoid arthritis (RA) and spondylarthritis (SpA) where IL-17 directly aggravates the inflammation site by stimulating immune cells to produce pro-inflammatory cytokines, chemokines and other inflammatory mediators including nitric oxide (NO), prostaglandins and matrix metalloproteinases (MMPs) (Lubberts, 2015). Aberrant production of IL-17 has also been implicated in SLE, RA, inflammatory bowel disease (IBD) and psoriasis (Abdel Galil et al., 2015; Jin and Dong, 2013).

2.3.2(b) Interleukin-23 (IL-23)

IL-23 (p19/p40) is an important cytokine in the development, expansion and proliferation of Th17 cells (Du et al., 2014) where it is produced by inflammatory myeloid DCs (mDCs), monocyte-derived DCs (Mo-DCs), intestinal macrophages, eosinophils and epithelial cells as listed in Table 2.5 (Garg et al., 2015; Guerra et al., 2017; Krause et al., 2015; Macho-Fernandez et al., 2015; Shi et al., 2015). IL-23 is involved in the development and maintenance of autoimmune inflammation (Tang et al., 2012; Teng et al., 2015). IL-23 belongs to IL-12 cytokine family which includes

IL-12, IL-23, IL-27 and IL-35; IL-23 induces memory T cells to produce interferon-γ (IFN-γ) and potently enhances the expansion of Th17 cells for the production of IL-17 (Sherlock et al., 2015; Tang et al., 2012).

Table 2.5 Sources, production sites and mode of IL-23 production

Sources	**Production sites**	**Mode of IL-23 production**	**Reference**
Inflammatory myeloid dendritic cells (imDCs)	Bone marrow	gp120-treated with mDCs induced production of IL-23, which then upregulated the suppressor of cytokine signalling 1 (SOCS1) protein in T cells	(Garg et al., 2015)
Monocyte-derived DCs (Mo-DCs)	Bone marrow	Treatment with PGE2 has been demonstrated to act in a cAMP-dependent manner to elevate IL-23 production in human Mo-DCs	(Shi et al., 2015)
Intestinal macrophages	Intestine	As IL-10 is an anti-inflammatory cytokine which limits mucosal immune responses, the addition of IL-10 reduces IL-23 production by intestinal macrophages in mice	(Krause et al., 2015)
Eosinophils	Lung	Confocal microscopy on cells obtained by bronchoalveolar lavage 8- and 54-hours post-infection with *Aspergillus fumigatus* were performed to confirm that eosinophils produced IL-23p19 and IL-17A in mice	(Guerra et al., 2017)
Epithelial cells	Gut (Intestinal epithelial cells)	Lymphotoxin beta receptor (LTβR) signalling in intestinal epithelial cells promotes self-repair after mucosal damage (wound healing) and essential for epithelial IL-23 production	(Macho-Fernandez et al., 2015)

As a heterodimer, IL-23 is composed of a p19 and p40 subunit, the latter being shared with IL-12 (p35/p40) (Arnold et al., 2016; Astry et al., 2015; Tang et al., 2012). p19 expression is produced by antigen-presenting cells (APCs), T cells and endothelial cells, while p40 is particularly limited to APCs *e.g.* DCs, monocytes and macrophages (Tang et al., 2012). IL-23 forms a disulphide-linked complex with p19 and p40 secreted by activated macrophages and DCs in peripheral tissues *e.g.* lung, skin and intestinal mucosa where the synthesis of both p40 and p19 subunits are within the same cell that produces IL-23 (Tang et al., 2012). However, both IL-23 and IL-12 have distinct functions and their production in response to pathogens are also differently regulated where IL-23 stimulates Th17 to produce IL-17, while IL-12 promotes Th1 response (Lyakh et al., 2008).

The IL-23 receptor, IL-23R, is found on activated memory T cells, NKT cells, macrophages, and DCs (AlFadhli, 2013; Korn et al., 2009). Naïve T cells do not express IL-23R, while the receptor is expressed on activated Th17 cells (Astry et al., 2015). Binding of IL-23 with its receptor complex activates STAT3 signalling in Th17 cells that induce Th17 differentiation to gain effector functions including expression of pro-inflammatory cytokines IL-17 and IFN-γ (Bedoya et al., 2013; Lee et al., 2017). IL-23 is involved in the onset of several autoimmune inflammatory diseases such as psoriasis, colitis, gastritis, and arthritis (Fotiadou et al., 2018; Tang et al., 2012), and high serum levels of IL-23 have been demonstrated in patients with SLE (Du et al., 2014).

2.3.3 IL-23/IL-17 axis in autoimmunity

The initial steps of naïve $CD4^+$ T cells differentiation into IL-17 producing cells does not require IL-23, however, IL-23 plays an important role in stabilizing the phenotypic features of the Th17 lineage. IL-23 is important in the expansion and