CHAPTER 1

Introduction

1.1 Historical Background

The immune system, a complex network communication of cells and cell products within the body, normally directs the responses towards harmful pathogens while restraining these responses to self components. The ability of the immune system to discriminate between self and non-self antigens is the process known as self tolerance. When self-tolerance breaks down, the immune system will generate high level of the immune responses against self-antigens, subsequently lead to an inflammation and damage of self tissue and organ which is the characteristic of the disease called autoimmune diseases (ADs). Currently, more than 5% of the population worldwide are living with ADs and the prevalence of ADs seems to be increasing [1]. Though the etiology and pathogenic mechanisms of ADs are still unclear, it is generally accepted that the risk factors may be multifactorial involving the interaction of genetic, hormones and environmental factors. Several studies reported the significant associations of genetic variants with ADs. Human leukocyte antigen (HLA) and cytotoxic T lymphocyte antigen-4 (CTLA-4) are prominent genes associated with the increased incidence of ADs [2]. Previous studies also suggested that the environmental factors such as UV radiation, chemical, infectious agent, stress, drug and diet behavior were associated with ADs [1]. Also steroid hormones, including estrogen and androgen are known to influence antibody production and immune cell proliferation [3]. Additionally, infection leads to the changes of the environment inside the body, such as the alteration of the pro-inflammatory cytokines. This alteration has been shown to associate with the patho-gennesis of ADs. An imbalance of cytokine has been described in several autoimmune diseases. One theory of immune regulation involves homeostasis between cytokines produced by T-helper type 1 (Th1) and T-helper type 2

(Th2) cells. Some autoimmune diseases such as multiple sclerosis (MS) and rheumatoid arthritis (RA) [4], have shown the dominant productions of IFN- β, TNF-α and IL-12, the Th1-type cytokines. Others such as systemic lupus erythematosus (SLE) are mostly Th2-type cytokines (i.e. high IL-4 production) [4, 5], though higher Th-1 type cytokine production may be exhibited at some stages of the diseases.

Recently, it has been demonstrated that there is a new subtype of Th cells known as Th17 cells that play a significant role in ADs. Th17 cells were first described in mice in 2005, as IL-17 secreting cells [6]. These cells protect against fungal and extracellular bacterial infections which are not efficiently cleared by Th1 and Th2 responses. In addition, Th17 cells participate in inflammatory reactions and autoimmunity [7]. To date, accumulated evidence showed that Th17 cells strongly influence the induction of ADs [8, 9]. Numerous studies showed the elevated IL-17 levels in patients with systemic lupus erythematosus (SLE), a prototypic ADs [10-12]. In the presence of pro-inflammatory cytokines, particularly IL-6 and transforming growth factor β (TGF- β), the differentiation of naïve T cells to Th17 cells is promoted [8]. However, when pro-inflammatory cytokines are absence, TGF- β induces the differentiation of naïve T cells to regulatory T cells (Tregs) [13]. This balance between Th17 and Treg may play significant roles in the development of ADs.

The hallmark of HIV infected individuals is characterized by the depletion of CD4+ T cells and initial increase of HIV-specific cytotoxic T lymphocytes (CTL). Due to the insertion of viral DNA into host genome, HIV-specific immune responses fail to completely eliminate this virus and leads to chronic immune activation. In addition, HIV infection is associated with several B cell aberrations including polyclonal B cell activation and increase the production of autoantibodies [14].

Therefore, we hypothesize that the immune depletion and chronic activation found in HIV infection may cause the imbalance of Th17 and Treg productions that leads to the generation of autoantibodies. The aim of this study is to determine the level of antinuclear antibodies as well as the percentages of Th17 and Treg production in HIV infected patients. This knowledge will provide the insight mechanism of how autoantibodies may be produced in HIV infection.

1.2 Objective

1) To investigate the production of antinuclear antibodies in HIV-infected individuals.

2) To study the percentages of T regulatory and T helper 17 cells in HIV-infected individuals.

1.3 Literature review

1.3.1 Immune system

Immune system is a network of cells, cellular products, tissues, and organs that work together to defend host from the attacks by variety of pathogens. There are two main types of the immune defense mechanism; innate and adaptive immunity [15].

1) Innate immunity, also called natural or native immunity, is the first line of defense mechanism against any invasion of the body. Its presence exists since the time of birth and can actively function before the exposure to any pathogens. Therefore, this system can immediately block the entry of the microbes or rapidly eliminate them upon its first encounter. Nevertheless, this system is unable to recall any microbes it has previously confronted, hence unable to mount specific means of responses to effectively handle a particular antigen. The components of the innate immune response include epithelial barriers, innate immune cells (phagocytes, dendritic cells, natural killer or NK cells) and complement [15, 16].

2) Adaptive immunity, also called specific or acquired immunity, is an immune response initiated by the recognition, subsequently activation of lymphocyte surface receptors to specific antigens that pass through epithelial barrier and are being delivered to lymphoid organs. Therefore, it responds slower but more specific than innate immunity. Adaptive immunity is divided into 2 types; humoral-mediated immunity (HMI) and cell-mediated immunity (CMI). The humoral immune response is mediated by antibody molecules which are the proteins secreted by B lymphocytes (plasma cells). This immune response is especially good at dealing with extracellular microbes. HMI begins with the activation of naïve T-helper cells, by the antigen processed and presented on the major histocompatibility complex (MHC) molecules on the antigen presenting cells (APCs). The activated T-helper cells then release certain cytokines to further activate B cells. These activated B cells are then proliferated and differentiated into antibody secreting plasma cells. Antibodies are secreted into the circulation to neutralize and eliminate microbes and toxins. On the other hand, CMI is mediated by T lymphocytes. This system can eliminate both extracellular and intracellular organisms. For extracellular microbes, naïve T-helper lymphocytes (CD4+ T cells) will be activated after recognizing the peptide antigen which are digested and

presented by antigen-presenting cells (APCs) displayed on MHC class II (MHC-II). These cells function by sending the signals to other types of immune cells including B cells and innate immune cells to destroy the infectious particles or organisms. For intracellular microbes, cytotoxic T lymphocytes (CD8+ T cells) will be activated after infected cells were digested and displayed on MHC class I molecules (MHC-I). The role of these cells is to kill or destroy the infected and tumor cells. In adaptive immunity, they also generate memory cells for secondary infection with a stronger, faster and more effective immune response than primary infection [15, 16]. As mentioned earlier, the immune system directs its responses mostly toward foreign pathogens while turning blind eyes to host components. It accomplishes such task via the mechanism called immunological tolerance.

1.3.2 Immunological tolerance

Immunological tolerance or self-tolerance is a lack of response to self-antigens that is induced by exposure of lymphocytes to these antigens. Mechanisms contribute to self-tolerance can be divided into two types based on where the state is originally induced. If the tolerance state is induced within the thymus and bone marrow (central lymphoid organs), the mechanism is called central tolerance. Another type, peripheral tolerance is induced in lymph nodes and other tissues (peripheral lymphoid tissues) [15].

1) Central tolerance

This mechanism of self-tolerance is induced when self-antigen encounter with developing lymphocytes in central lymphoid organs (thymus for T lymphocytes and bone marrow for B lymphocytes). In central T-lymphocyte tolerance, the induction mechanisms involve cell death or negative selection of thymocytes and generation of regulatory T-cells (Treg). Immature T-lymphocytes, those with receptors strongly recognize to self-antigen that present by MHC molecules, are cells preferentially received the signals to trigger apoptosis. This mechanism is called negative selection. However, apoptotic process is not launched in some CD4+ T lymphocytes that recognize self-antigens, but instead develop to regulatory T-lymphocytes to regulate responses of other self-reactive cells in peripheral tissues. For B-lymphocyte central tolerance, immature B cells that recognize self-antigens in the bone marrow may receive

the signals that trigger them to change their receptor specificity, the process called receptor editing. If editing fail, cells will receive death signals and undergo apoptosis [15].

2) Peripheral tolerance

Tolerance to self-antigen that are not presented in central lymphoid organs must be induced and maintained by peripheral mechanisms [15]. Peripheral tolerance is immunological tolerance developed after T and B cells mature and enter the peripheral lymphoid organs. T cell peripheral tolerance includes functional inactivation (anergy), cells death or suppression by regulatory T cells. Anergy mechanism occurs when T cells recognize antigens without adequate levels of co-stimulatory signals which are important for fully T cell activation. Autoantigen-recognized T-cells are able to recognize the antigens but do not receive any co-stimulatory signal typically provided by an innate immune response. T cells may interact with CD28-inhibitory receptor known as CTLA-4 (cytotoxic T lymphocyte-associated antigen 4), resulting in long-lasting anergy. Some autoreactive T-cells may be suppressed by regulatory T-cells (Treg) proteins; hence cell death is induced. On the contrary, when T cells recognize microbes, co-stimulatory signals arise and drive the expression of anti-apoptotic proteins which will overcome the effect of pro-apoptotic proteins. Self-antigen recognition may also enable the expression of death receptors and their ligands on lymphocytes, such as Fas and Fas ligand (FasL), which will also accelerate cell apoptosis. For B-cell peripheral tolerance, Mature B cells that recognize self-antigen may be rendered an anergic state by the reason of not receiving the helping signal from T cells [15].

1.3.3 Breakdown of tolerance and autoimmunity

Normally, the function of the immune system is to protect host against harmful pathogens by specifically recognizes and eliminates foreign agents. The immune system can distinguish between self and non-self antigens via the mechanism called immunological tolerance. Therefore, during maturation, immune cells that react against self tissues are eliminated. However, if these mechanisms fail or the tolerance mechanism is impaired, autoimmunity will occur. Autoimmunity is defined as an immune response against self antigens. It is a natural phenomenon that can present in all

normal individuals. If autoimmunity is poorly regulated, the immune system may attack the individual's own cells and tissues that can cause the autoimmune diseases.Self-tolerance may be broken both at the T- and B-cell levels. Studies have found that self-reactive antibodies usually are the product of T-helper cell dependent activated B cells. In normal individuals, self-reactive antibodies can be found at low concentration in serum and most of them are IgM isotypes with low avidity for the antigen. But in autoimmune disease patients, self-reactive antibodies are produced at high concentration and are mainly IgG isotypes with high avidity to self-antigens. The mechanism to switch from harmless natural autoantibodies to the production of dangerous IgG autoantibodies is still a question. However, there are several hypotheses proposed to explain the breakdown of tolerance [17].

1.3.4 Breakdown of tolerance hypotheses [17]

1) Failure to delete autoreactive lymphocytes

Some individuals fail to remove autoreactive T- and B-cell clones during ontogenesis because of genetics or inherited impairment.

2) Molecular mimicry

The breakdown of tolerance occurs when immune response against a microbial antigen containing epitope similar or identical to a self-antigen is generated. This immune response may then cross react with self-antigen [18].

3) Abnormal presentation of self-antigens

3.1) Aberrant expression of major MHC class-II molecules: MHC class-II molecules may be expressed on cells that do not usually express them by the induction of inflammatory cytokine such as IFN γ that may lead to the presentation of unknown self-antigens to autoreactive CD4+T cells.

3.2) Binding of exogenous antigens to self-antigens (piggy-back): exogenous antigen may act as an autoantigen carrier. When autoantigen binds to exogenous antigen, it can also be presented to and stimulate T helper cells along with the exogenous antigen.

3.3) Overproduction of autoantigen: some low affinity autoreactive clones are not deleted during ontogenesis and remain inactive (immunological ignorance). However, during inflammation, an excess production of autoantigens can be released and can cause the breakdown of immunological ignorance.

3.4) Disclosure of cryptic T-cell epitopes: Some proteins or antigens act as immunodominant, whereas some others may be cryptic that they do not reach the threshold for T-cell activation. Therefore, autoreactive T cells recognizing these types of antigens will escape the negative selection process. However, some events such as inflammation or infection may trigger the presentation of these cryptic antigens to T cells that will give rise to the loss of self-tolerance.

3.5) Release of sequestered self antigens: Antigens within the immunological privileged sites such as brain, eyes, testis and uterus are usually not exposed to self-reactive lymphocytes. But some unfortunate circumstances may let them accidently run into one another and cause the stimulation of these self-reactive lymphocytes.

1.3.5 Autoimmune diseases

Autoimmune diseases (ADs) are characterized as diseases with high level of immune responses produced against specific self antigens leading to inflammation and tissue damage. There are nearly 150 different autoimmune diseases reported to date which are often categorized into 2 main groups based on the number of tissues or organs affected by each disease. If the disease involves or affects only one organ, it is called organ- or tissue-specific autoimmune disease. Some representatives of this group of ADs are Graves' disease which is the disease with hyperactivity of the thyroid gland due to the continuous stimulation of thyroid stimulating hormone receptor (THSR) by an autoantibody recognizing this receptor. Another noticeable example is myasthenia gravis of which the blocking of acetylcholine receptor (AChR) by an autoantibody causes the weakness of the skeletal muscles in patients. Another group of ADs is known as systemic autoimmune disease due to the profusion of affected organs. The principal disease of systemic diseases is systemic lupus erythematosus (SLE) in which the deposition of the complex between autoantibodies and nuclear antigens causes inflammation almost all parts of the body [19]. ADs affect approximately 5% of the

population worldwide [1]. They are the third most common disease in industrialized countries following cardiovascular disease and cancer. Many ADs start at young age and continue throughout life that can cause morbidity and mortality. The etiology and pathogenic mechanisms of ADs is not clearly known but it is believed that the causes of ADs are multifactorial.

1) Factors involved in autoimmune diseases

1.1) Genetics

Genetic has been proven to be one of the factors influencing the development of ADs. When one individual in a family has an autoimmune condition, the risk of the other members of having ADs is increasing. The prevalence studies of ADs concordance rate in the twins showed a 15-50% and 2-5% in monozygotic and dizygotic twins, respectively [20]. Genetic is accounted for approximately 30% or one-third of all the risks contribute to the developing of ADs. But the remaining 70% risks are from others factors, especially the environment [3].Most ADs result from the combination effects of several genes. Human leukocyte antigen haplotype genes, is prominently associated in ADs development. HLA molecules have significant roles in the regulation of the immune responses since they assist in the processing and presentation of antigens to specific T cells. In addition, there are non-MHC genes that are also associated with ADs. For instance, cytotoxic T lymphocyte antigen-4 (CTLA-4), which is the component of costimulatory pathway, function as lymphocyte proliferation inhibitor. Therefore, blocking or deleting CTLA-4 in experimental animal have enhanced ADs [20].

1.2) Hormones

Most ADs have higher prevalence in women than men. Approximately 80% of individuals with autoimmune diseases are women [3]. Many studies suggested that sex hormones may drive the development of ADs by directly interact with the immune cells via their receptors on the surface or inside the cells. Estrogens and androgens are known to influence antibody production and immune cell proliferation. They can amplify or inhibit the immune response [3]. However, how sex hormones regulate the immune response and the bias between men and women is not clearly known.

1.3) Environmental factors

It has been long proposed that the development of any autoimmune disease depends on a combination of genetic and environmental factors, and it contributed to approximately 70% as the causes of ADs [3]. Environmental factors such as UV radiation, chemicals, infectious agents, stress, drugs, diet and behavior have all been the prime suspects. For example, the studies found that drugs such as procainamide and hydrolyzine can induce a lupus-like syndrome. Exposure to various metals in autoimmune disease has been explored. Mice develop a lupus-like condition when dosed with mercury, silver, or gold suggested that these metals can induce lymphocyte proliferation and autoimmunity. Infectious agents are the environmental most often triggers ADs. For example, group A beta-hemolytic streptococcus can induce the development of rheumatic heart disease [3]. Lifestyle factors may also be associated with AD development and progression. Several studies suggested that smoking has been associated with an increased risk of rheumatoid arthritis and SLE [21]. However, there is no report of any factor mentioned above that is solely responsible for the generation of ADs. Rather, the development of ADs is considered to be the unfortunate perfect interplay of all elements.

1.3.6 Diagnosis of autoimmune diseases

The diagnosis of ADs requires a combination of symptoms, physical examination and blood tests. Blood tests include autoantibody and inflammation tests. However, diagnoses of most ADs are complicated because there are no specific signs and symptoms, as well as the lack of standardized diagnostic criteria and biomarkers of early disease diagnoses. Therefore, definitive diagnosis requires repeated evaluation and monitoring over time. Many professional societies have developed classification criteria for several autoimmune diseases. For example, the American College of Rheumatology has developed classification criteria for rheumatoid arthritis, lupus, and several other rheumatologic diseases [20].

1) Autoantibodies detection

An autoimmune disease occurs when the immune system begins to attack its own host tissues. A hallmark is the production of high-affinity autoantibodies. Therefore, diagnosis of autoimmune diseases depends on the identification of disease-

associated clinical symptoms and detection of autoantibodies. Different techniques have been developed to test for autoantibodies including immunodiffusion, immunoblotting techniques, immunofluorescence, enzyme immunoassays and recently flow cytometry for multiplex bead-based assays [22].

1.1) Antinuclear antibody (ANA) assay

Antinuclear antibody (ANA) assay is the most commonly performed autoantibody-detection test in the autoimmune laboratories. ANA are autoantibodies that react with components located in the cell nucleus and, to the lesser extent, cytoplasm. ADs especially SLE, a prototypic autoimmune disease are characterized by the production of antibodies to nuclear molecules. Therefore, detection of ANA has been used as one of the criteria for lupus or autoimmune disease diagnosis. A positive ANA test means autoantibodies are present and more than 95% of individuals with lupus are ANA positive [23]. However, the study found that up to 20% or more of healthy people can be positive in ANA test [24]. There are many subtypes of ANA such as anti-Ro/SSA (Sjögren's Syndrome A) antibodies, anti-La/SSB (Sjögren's Syndrome B) antibodies, anti-Sm (Smith) antibodies, anti-snRNP (small ribo-nucleoprotein) antibodies, anti-PM/Scl-70 (polymyositis/scleroderma) antibodies, anti-dsDNA antibodies, anti-histone antibodies, antibodies to nuclear pore complexes, anti-centromere antibodies and anti-sp100 antibodies.The most widely used method for ANA test is indirect immunofluorescence assay (IFA) on cultured human epidermal carcinoma cells (HEp-2 cells) and determining the pattern by fluorescence microscope (Figure 1.1) [25]. The resultant ANA staining pattern is correlated with the type of autoantibody synthesized against particular antigen. For example, the nuclear coarse speckled pattern is strongly associated with anti-Smith (anti-Sm), as well as the antibodies against the intracellular small ribonucleoprotein particles primarily Ro/SSA, La/SSB, and snRNP. The nuclear homogeneous pattern is correlated with antibodies to native DNA, nucleosomes, and histones. The centromeric pattern is associated with the chromatin containing the histone H3 variant proteins generally known as centromere proteins (CENP-A, CENP-B, and CENP-C). The peripheral nuclear is found correlated with lamins A, B, and C, and glycoprotein 210. Lastly, the nucleolar pattern is associated with PM/Scl (exosome), nucleolin, fibrillarin, RNA polymerase I, and human upstream binding factor [26-28]. Moreover, different antigens are associated with

specific autoimmune diseases. For example, anti-double-stranded native DNA (anti-dsDNA) or anti-Sm antibodies is associated with SLE [29, 30], anti-Ro/SSA or anti-La/SSB antibodies with Sjögren's syndrome, anti-Jo-1 antibodies with dermato-polymyositis [31], anti-centromere or anti-topoisomerase I (anti-Scl70) antibodies with cutaneous systemic sclerosis [32], and anti uridinerich 1 ribonucleoprotein (U1RNP) antibodies with mixed connective tissue disease (MCTD) [33, 34] (Table 1.1) [25]. Therefore, different ANA patterns may be associated with different autoimmune diseases.

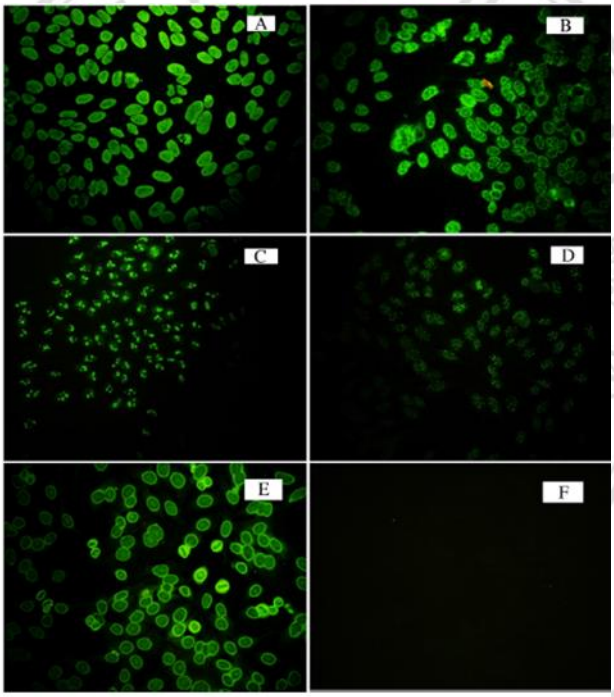

Figure 1.1 Illustrations of ANA staining patterns. (A) Homogeneous, diffuse staining of interphase nuclei. (B) Speckled, granular nuclear staining of interphase cell nuclei. (C) Nucleolar, coarse speckled staining within the nucleus, less than six in number per cell. (D) Centromere, uniform fine sand-like particles. (E) Peripheral nuclear, staining primarily around the outer region of the nucleus. (F) Negative staining pattern [25].

Table 1.1 Common ANA patterns associated with specific antigens and diseases [25]

ANA pattern	Related antigen	Related diseases
Speckled pattern	ENA, RNP, Sm, SSA/Ro, SSB/La, Scl-70, Jo-1	Sjögren's syndrome, SLE, Mixed connective tissue disease (MCTD), Systemic sclerosis(SS), Polymyositis (PM)
Homogeneous	Native DNA, nucleosome and histone	SLE, Drug induced SLE
Centromeric	CENP-A, CENP-B, CENP-C	Cutaneous systemic sclerosis, limited SS
Nucleolar	PM/Scl, nucleolin, fibrillarin, RNA polymerase I, U3-RNP, To RNP	MCTD, SS, PM
Peripheral	RNP, Sm, SSA/Ro	SLE, SS

1.3.7 Cytokines and cells involve in autoimmune diseases

Autoimmune diseases occur when the immune system attacks and destroys healthy tissue by mistake. These auto-aggressive responses result from the complex interactions among different immune cell types including T lymphocytes, B lymphocytes and antigen presenting cells that communicate to each other principally through the production of cytokines. Therefore, cytokines, particularly inflammatory cytokines and chemokines appear to be involved in ADs pathogenesis. An imbalance of cytokines has been described in several autoimmune diseases. One theory of immune regulation involves homeostasis between T-helper 1 (Th1) and T-helper 2 (Th2) activity. Some autoimmune diseases such as multiple sclerosis (MS) and Rheumatoid arthritis [4] are caused by Th1 dominant immune responses. Others, such as systemic lupus erythematosus (SLE) , are Th2 dominant [4,5]. Tang and colleague reported markedly elevated levels of the Th1 cytokine, IL-12, in MS [35]. Gerli and collaborators isolated T-helper cells from the blood and synovial fluid of four patients with active RA and they found that all had prominent production of Th1 clones in the

blood and a slight prevalence of naive, undifferentiated T-cell clones in the joints [36]. Chang and collaborators found no differences in IFN-gamma (Th1-type cytokine) or IL-4 (Th2-type cytokine) in untreated SLE patients [37] but Akahoshi and colleague found that SLE patients with serositis or CNS involvement had higher IL-4 than those who did not [38]. Moreover, it has been demonstrated that there is a new subtype of Th cells known as Th17 cells that play a significant role in Ads [6].

1.3.8 Th17 cells

Th17 cells were first described in mice in 2005, as IL-17 secreting cells [6]. Th17 cells secret IL-17 and other cytokines such as IL-21 and IL-22 [39]. There are six members of cytokines in IL-17 cytokine family. These members include IL-17A (also called IL-17), IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25) and IL-17F [40]. IL-17 members that have significant roles in the inflammatory process are IL-17A and IL-17F [41]. IL-17 cytokine family signal through the recognition, hence stimulation of their counter receptors namely IL-17RA, IL-17RB, IL-17RC, IL-17RD and IL-17RE. IL-17RA is recognized by both IL-17A and IL-17F although IL-17A binds to this receptor with higher affinity [42]. The binding of IL-17 to their cognate receptors brings about the mobilization of monocytes and neutrophils through the local productions of chemokines (mainly IL-8, chemoattractant protein-1 and growth-related oncogene protein-α) and some inflammatory mediators including interleukin-6 (IL-6), prostaglandin E2 (PGE2) or granulocyte/monocyte-colony stimulating factor (G/M-CSF). The recruitment augments host responses against fungal and extracellular bacterial infections which are not efficiently cleared by Th1 and Th2 immunity [43]. Nevertheless, skewing of IL-17 production have implicated in diseases such as infectious diseases, transplantation reaction, allergy, tumor, as well as autoimmune conditions [7]. Several evidences demonstrated that IL-17 production has been found at high level in SLE patients [44,45]. Palpable evidence also suggests rheumatoid arthritis [46], multiple sclerosis [47], and inflammatory bowel diseases [48] are all connected to the dominant production of IL-17. Therefore, Th17, an IL-17 producing cells, have been listed as one of the major players among pathogenic Th cell populations underlying the development of many autoimmune diseases [49].

In mice, Th17 cells are differentiated from naïve T-helper cells in the presence of an anti-inflammatory cytokine, TGF-β, combines with IL-6, a pro-inflammatory cytokine. TGF-β by itself, has been known to up-regulate the transcription factor involves in the generation of regulatory T cells (Treg) called forkhead box protein P3 (Foxp3). Surprisingly when IL-6 is present, the induction of Foxp3 by TGF-β is inhibited. Rather, the expression of the retinoic acid-related orphan receptor-gamma t (RORγt), a transcription factor necessary in the conversion of naïve T cells to Th17, is induced [50,51]. In the absence of IL-6, the differentiation of Th17 cells can also be induced by TGF-β plus IL-21, a cytokine produced by Th17, but IL-6 is more potent [52,53]. Role of IL-21 is believed to be more important to the amplification of Th17 cells rather than their differentiation. Another cytokine involving in the expansion and maintaining of Th17 cells is IL-23, an IL-12-related cytokine family produced by dendritic cells (DCs) and macrophages (MQs) upon an activation by microbial pathogen, and is typically driving the differentiation of Th1 cells [54,55]. However, the differentiation of Th17 cells in human has shown to be utilizing TGF-β and IL-21 which induces the transcription factor retinoic acid-related orphan receptor-c (RORc). While IL-1β plus IL-6 are used to enhance the amplification and IL-23 to maintain Th17 cell populations [56,57] (Figure 1.2) [6]. Numerous studies showed the elevated IL-17 levels in patients with SLE, a prototypic ADs [10-12].

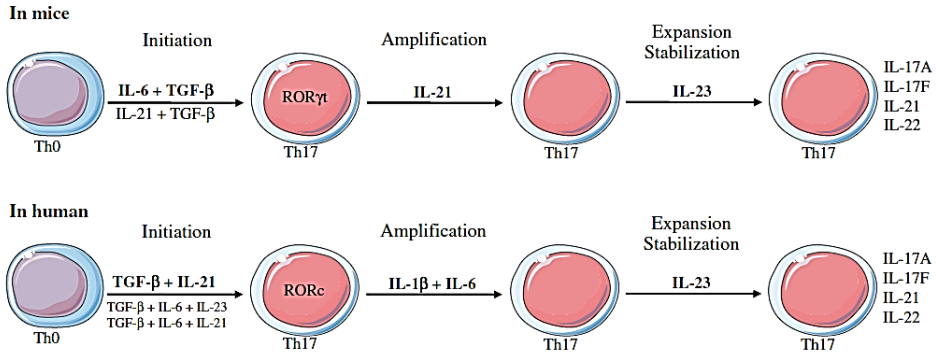

Figure 1.2 Differentiation of Th17 cells in mice and in human. In mice, TGF-β and IL-6 initiate the differentiation and activate RORγt. IL-21 acts on Th17 cell amplification, and IL-23 serves to expand and maintain Th17 cell population. In human, the differentiation is initiated by TGF-β and IL-21 which induce the transcription factor RORc. IL-1β plus- IL-6 are important to enhance the amplification of Th17 cells and IL-23 to maintain the Th17 cell population [6].

1.3.9 Regulatory T cells

Regulatory T cells are a subset of CD4+ lymphocytes that were originally termed "suppressor cells" [58]. These cells play important roles in the maintenance of self-tolerance, inhibition of autoimmunity and modulation of immune responses against infections and tumor cells. The expression of CD25 (alpha chain of IL-2 receptor or IL-2Rα), CTLA-4, and FoxP3 are often used as marker to identify Treg [59-61]. Moreover, the lack of CD127 (known as IL-7 receptor α chain, IL-7Rα) is also used to distinguish Treg cells [62,63]. However, markers to accurately identify human Treg cells have yet to be found [64]. Treg cells require the specific cytokine TGF-β and the transcription factor FoxP3 for their differentiation and also secrete inhibitory cytokines TGF-β and IL-10 to suppress the effector cells [65]. Moreover, Treg cells can suppress immune responses via cell-contact dependent by expressing CTLA-4 which is important to inhibit immune activation [66]. Treg cells can be divided into two subgroups based on maturation site; natural Treg cells (nTreg) and inducible Treg cells (iTreg) [6]. Natural Treg cells develop during normal T-cell maturation in the thymus and already expressed FoxP3 when enter the peripheral tissues. On the contrary, inducible Treg cells are induced in the peripheral lymphoid organs after an exposure of naïve T cells to the antigens [67,68].

1.3.10 Reciprocal roles between Th17 and Treg

As mentioned previously, differentiation of Th17 comes as the price of suspending the generation of Treg. The presence of pro-inflammatory cytokines such as IL-6 blocks the TGF-β from mediating the generation of Treg and drives the differentiation of Th17[6]. However, when pro-inflammatory cytokines are absence, TGF-β induces the differentiation of Treg (Figure 1.3) [6]. In addition, the accompanying of IL-6 earns effector T cells the resistance to Treg-mediated suppression [69]. Accordingly, the balance between Th17 and Treg may be important to the regulation of AD development. While Th17 cells promote autoimmunity, Treg cells serve to maintain self-tolerance and control the expansion and activation of auto-reactive effector CD4+ T cells. However, the studies regarding reciprocal roles of Treg and Th17 in ADs are still scarce.

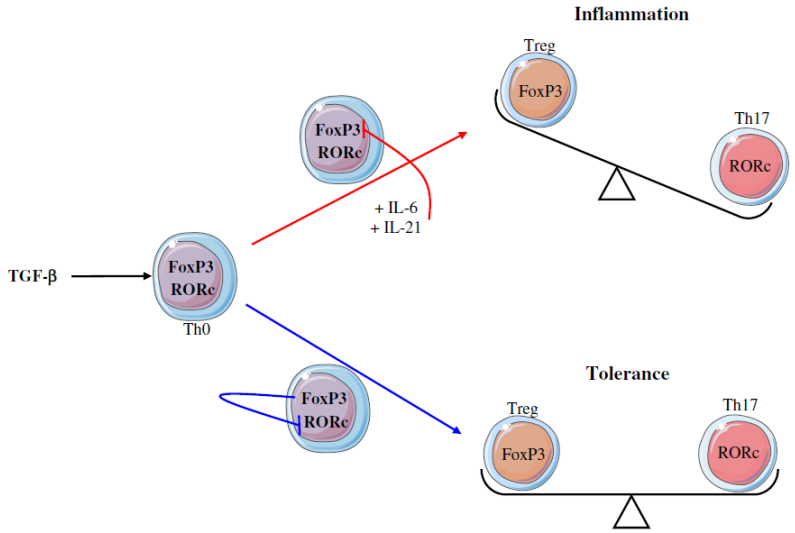

Figure 1.3 Balance between Th17 and Treg cells. TGF-β is able to induce expression of FoxP3 and RORc. In presence of pro-inflammatory cytokines, FoxP3 expression is reduced and RORc expression is up-regulated. In absence of inflammation, TGF-β promotes Treg differentiation [6].

1.3.11 HIV and autoimmunity

There is one form or another of abnormalities found in all major lymphocyte populations as result of HIV infection. Due to the insertion of viral DNA into host genome, HIV-specific immune responses fail to completely eliminate this virus and leads to chronic immune activation. Progressive immunodeficiency and CD4+ T cell lose are the hallmark of HIV infection that leads to the development of opportunistic infections and ultimately death in the majority of patients [70]. In addition, Rawson and colleague reported that the release of protein fragments from dying CD4+ T cells in HIV-infected patient may promote the formation of autoreactive CD8+ T cells [71, 72]. Moreover, HIV infection is associated with several B cell defects including polyclonal B cell activation, poor antibody response to specific antigens and increase the production of autoantibodies [14]. Autoantibody production in HIV-infected patients have been reported in many studies such as anti-cardiolipin, anti-beta-2-glycoprotein (β2-GPI), anti-DNA, anti-small nuclear ribonucleoproteins (snRNP), anti-thyroglobulin, anti-thyroid peroxidase, anti-myosin, and anti-erythropoietin antibodies (Table 1.2) [73]. Massabki and colleague studied the frequency and specificity of autoantibodies in HIV-infected subjects and their association with rheumatic manifestations, immunodeficiency, and prognosis compared to non-HIV-infected subjects. They found that HIV-infected patients presented higher frequency of autoantibodies and the most frequent autoantibodies were antibodies to cardiolipin and denatured DNA. The presence of autoantibodies was associated with lower $CD4^+$ cell counts and with higher mortality within 2 years [74]. Argüello and colleague found that patients with severe immunosuppression were positive for at least one out of seven autoantibodies (antinuclear factor, anti-DNA, anti-smooth muscle, anti-mitochondrial, anti-neutrophil cytoplasm, anti-cardiolipin, Rheumatoid factor) tested and displayed hypergammaglobulinaemia in the absence of clinical autoimmune disease [75]. Moreover, there are the wide range frequencies of ADs in HIV patients. The reported of ADs in HIV/AIDS include systemic lupus erythematosus, anti-phospholipid syndrome, vasculitis, primary biliary cirrhosis, polymyosits, Graves' disease, and idiopathic thrombo-cytopenic purpura [73]. Laura and colleague reported autoimmune diseases in 52 cases HIV infected patients, they found that there are 11 patients with vasculitis, 8 patients with immune cytopenias, 8 patients with rheumatic diseases, 7 patients with lupus, 7 patients

with sarcoidosis, 6 patients with thyroid diseases, 5 patients with hepatic diseases, and 4 patients with antiphospholipid syndrome (4 patients presented 2 ADs) [76]. Zandman-Goddard and Shoenfeld suggested that autoimmune disease can be found in stage I (acute HIV infection) and stage IV (immune restoration after anti-retroviral therapy) HIV-infected patient, but not found in stage II (declining CD4 count without manifestation) and stage III (immunosuppression with a low CD4 count; AIDS) patient [73]. There are several hypotheses about the development of ADs in HIV infected patient, including direct role of viral particles, immune complex mediated diseases, dysregulation of the B/T lymphocyte interaction [77], molecular mimicry [78, 79], and polyclonal B lymphocyte activation that might favor the synthesis of autoantibodies (ANA positive present in up to 23% of HIV-infected patients [80] However, the association of immune dysfunction in HIV infected patient and the development of autoimmune diseases is still unclear. In this study, we interested in the role of Th17 and Treg balance in ADs development. We hypothesize that the immune depletion and chronic activation found in HIV infection may cause the imbalance of Th17 and Treg productions that lead to the generation of autoantibodies since there are several studies reported that Th17 and Treg balance are correlated with disease progression in HIV infected patient. For example, in 2009, Favre and colleague found that in high Th17 and Treg balance (2.0 Th17/Treg ratio) is associated with limited immune activation and no disease progression in SIV infection monkeys (81). In 2010, Kanwar and colleague also reported that progressive HIV disease is associated with depletion of Th17 cells and loss of Th17/Treg balance [82]. However, the association between Th17/Treg balance and autoantibody production remain to be investigated.

Table 1.2 Autoantibodies in HIV [73]

Autoantibody	Disease	Frequency
Anti-α myosin	Left ventricular dysfunction	43%
Anti-EPO	Anemia	23.5%
Anti-TPO	Grave's disease	5 patients
Anti-TSHR	Grave's disease	5 patients
Anti-cardiolipin	APS	1 patients
Anti-PS	APS	56%
Anti-PI	APS	34%
Anti-PC	APS	43%
Anti-β2 GPI	APS	5-27%
Anti-prothrombin	NG	NG
Anti- DNA	NG	NG
ANTI-snRNP	NG	68.1%

*APS, antiphospholipid syndrome; EPO, erythropoietin; NG, not given