An Update on Pathogenesis of Systemic Lupus Erythematosus

Pedram Ahmadpoor, Nooshin Dalili, Mehrdad Rostami

Systemic lupus erythematosus is a group of diseases that seem to become separate entities by etiology in the near future. Its pathogenesis remains elusive; however, multifactorial interactions among genetic and environmental factors may be involved. Systemic lupus erythematosus is the perfect prototype of autoimmune disorder with multiple derangements, starting from innate immunity to adaptive immune system leading to loss of self-tolerance. An overview of the pathogenesis of systemic lupus erythematosus is the focus of this review.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with various presentations from mild to life-threatening multiple organ involvements. It is a group of disease that seems to become separated etiologically in near future. Its pathogenesis remains elusive; however, multifactorial interactions among genetic and environmental factors may be involved. Systemic lupus erythematosus is the perfect prototype of autoimmune disorder with multiple derangements, starting from innate immunity to adaptive immune system leading to loss of self-tolerance. Understanding the pathogenesis of SLE is the focus of interest not only to clinicians who are engaged directly in treating SLE patients, but also for those who are engaged in organ transplantation because of the lessons learnt from pathogenesis of SLE that can be applied for reaching the tolerance in organ transplantation.

The incidence of SLE is variable in different parts of the world and in different age groups, but generally, it is estimated to be about 5 per 100 000 people per year with a prevalence of 40 to 80 per 100 000. Systemic lupus erythematosus is about 10 times more frequent in women and is usually a disease of child-bearing ages, but in some parts of the world like Sweden, the number of newly diagnosed cases in women aged over 45 years is high.1

PATHOGENESIS

Role of Genes

Genetic factors confer a predisposition to the development of SLE. Although in rare cases, SLE may be associated with the deficiency of a single gene (for example, the complement components C1q and C4), the disease more commonly results from the combined effect of variants in a group of genes.2 Some genes have been associated with several autoimmune diseases and appear to increase the risk of SLE specifically, but concerning the effect size of each of these mutations that are not big enough, it seems that there are multiple checkpoints for controlling the process of self-tolerance and a threshold is needed for development of a clinical presentation. The abnormal immune responses that lead to emergence and persistence of pathogenic B and T cells has multiple components that include abnormal processing of self-antigens by antigen-presenting cells, hyperactivation of T and B cells, and failure of multiple regulatory networks to interrupt this process.3

Other Factors

The pathogenesis of lupus can be divided into 2 stages: (1) loss of tolerance to self-antigens and generation of auto-antibodies, and (2) pathogenic auto-antibodies and immune complexes that cause inflammation and disease.4 External factors, for example ultraviolet light, by inducing apoptosis,
release self-antigen (eg, nucleosomes, U1 RNP, and Ro/SS-A), which can in turn be processed by antigen-presenting cells and B cells in an aberrant milieu. These processed peptides stimulate T cells and bind to antigen receptors on B cells, thus driving the production of harmful antibodies, which can in turn combine with antigen to form harmful immune complexes. The target cells (glomeruli, endothelial cells, and platelets) then release more self-antigen to perpetuate the process. Meanwhile, multiple regulatory circuits/suppressor immune components that are supposed to alter this response to self so that it is harmless are not working effectively. Indeed SLE should be considered as a syndrome with different presentations, and these diverse clinical manifestations present a challenge to the clinician for targeting therapy based on the pathogenesis of disease in each subgroup of patients.

Role of Abnormal Apoptosis

It is becoming increasingly obvious that SLE and lupus nephritis develop from combinations of genetic variants that impair proper apoptotic cell death and rapid clearance of apoptotic cells to avoid the exposure of nuclear auto-antigens to the immune system. Certain nuclear and cytoplasmic auto-antigens become clustered in the surface blebs of apoptotic cells. Mechanisms of uptake of apoptotic cells are via tyro 3, axl, mer (TAM) receptors, early components of complements, CD36, and DNAase.

Under normal circumstances, apoptotic cells are engulfed by macrophages in the early phase of cell death without inducing inflammation. In SLE, however, the clearance of apoptotic cells by macrophages is impaired, which may allow apoptotic cells to serve as immunogens for the induction of autoreactive T and B cells and drive the production of autoantibodies. Macrophages recognize apoptotic cells through an array of surface receptors. Among them, the TAM receptors, play an especially important role in the clearance of apoptotic cells. Mice lacking TAM have impaired clearance of apoptotic cells and develop progressive lupus-like autoimmunity. The two ligands that bind to and activate TAM are growth arrest-specific 6 (Gas6) and protein S, which in turn bind to phosphatidylserine residues exposed on the surface of the apoptotic cell. Protein S, but not Gas6 levels, reflect disease activity in SLE. Low levels of protein S in SLE, could be contributing to the thrombotic propensity in certain SLE patients.

Free protein S decreased in subsets of SLE patients with a history of serositis and neurologic, hematologic, and immunologic disorders. It is especially important that the concentrations of free protein S correlate with C3 and C4.

Hence, protein S may play a particularly significant role in the removal of apoptotic cells because of its high plasma concentration, despite its apparent lower affinity for the receptor than Gas6. In the study of c-mer-mediated phagocytosis of apoptotic cells, protein S stimulated phagocytosis as well as or better than Gas6. Therefore, it is possible that insufficient levels of protein S may lead to inefficient clearance of apoptotic cells, resulting in exposure of cellular contents to immune cells and promoting an autoimmune response. A new role for glucocorticoids is that they can increase protein S-dependent uptake of apoptotic neutrophils by human macrophages.

Binding of phosphatidyl serine expressed in apoptotic blebs to TAM receptor by activating suppressor of chemokine signaling prevents activation of interferon or Toll-like receptor signaling. If apoptotic cells persist longer, the clearance will be enhanced by the phagocytes recognition via interaction with complement receptors. It is shown that phagocytes from lupus patients engulf far less during a 7-day period in vitro than phagocytes from healthy patients.

The important role of the complement system in the process of clearing apoptotic materials has also been documented. The defective clearance of apoptotic cells in SLE could be the result of quantitative or qualitative defects of the early complement proteins, such as complements C2, C4, or C1q. Patients with homozygous deficiencies in these complement components develop a severe lupus-like disease early in life. The C1q receptors on the surface of phagocytes constitute an extremely important mechanism for the clearance of apoptotic cells. It is demonstrated that C1q binds to surface blebs of apoptotic human keratinocytes and that C1q is essential for the proper clearance of apoptotic cells, which are considered as the primary source of self-antigens. Patients or mice with homozygous C1q deficiency develop autoantibodies and a lupus-
like syndrome apparently because of the inability to eliminate apoptotic cells effectively, which leads to an increase in the exposure of antigens to the immune system. Mice with a targeted deletion of C1q show glomerulonephritis with deposits of immune complexes and apoptotic cells in the glomeruli. This strong association invokes the role of complement in physiological waste disposal, particularly in the processing and clearance of dying cells and immune complexes (Figure 1).

Complement also plays a role in the activation of B and T lymphocytes, and complement deficiency can cause autoantibody production by impairing the normal mechanisms of tolerance. It has also been shown that C1q is a potent modulator of dendritic cells, resulting in an impaired capacity for cytokine production, downregulation of co-stimulatory molecules and a limited T-cell response. In addition, the binding of C1q results in complement activation, causing opsonization of apoptotic cells with C4b and C3b. This opsonization will further accelerate their uptake by phagocytes and contribute to additional anti-inflammatory responses. Anti-C1q antibodies can be found in a large proportion of patients, particularly those with kidney disease. This may result in a functional deficiency of the receptor protein. It is unlikely that anti-C1q antibodies represent the primary abnormality in most patients with SLE, but they would certainly provide a mechanism for persistence of the disease and flares.

Monocytes and macrophages constitutively express several receptors like CD14, CD36, and scavenger receptors, all involved in the recognition, binding, and internalization of apoptotic cells. It is believed that apoptotic cells are efficiently removed by reticuloendothelial cell system via surface receptors such as scavenger receptor A (CD36) and the phosphatidylserine receptor (CD68). CD36 is a transmembrane glycoprotein of the class B scavenger receptor family that has pro-atherogenic properties. Human and animal studies as well as cell culture experiments provide strong evidence that CD36 influences propensity to foam cell formation and development of atherosclerosis, and it is also involved in uptake of necrotic cells by macrophages. Increased CD36 expression may be implicated in the etiology of

Figure 1. Apoptotic cell can be recognized via phosphatidyl serine interactions with several molecules. Detection of phosphatidyl serine by tyro3-axl-imer receptor (TAM) via protein S/GAS 6 leads to activation of suppressor of chemokine signaling (SOCS). Activation of SOCS blocks interferon-α production and TLR expression and activity. Attachment of TIM-4 expressed on APCs to phosphatidyl serine prevents formation of antigen-specific T cells. Binding of CD36 expressed on dendritic cells reduces interleukin-12 production and induces anergic T-cell binding of C1q by releasing interleukin-10 without immunoglobulin G that leads to expression of CCR7 in dendritic cell without expression of CD80/86. These dendritic cells induce a tolerogenic response to antigen in naive T cells in lymph nodes. DNAse-1 degrades DNA and works as a secondary backup system to keep DNA out of reach of immune detection. Serum amyloid P binds to extracellular double stranded DNA. DNAse-1 deficiency or decreased activity and SAP deletion leads to autoimmunity. APC indicates antigen-presenting cell; DC, dendritic cell; NET, neutrophil extracellular trap; SAP, serum amyloid P; and PTS, phosphatidyl serine.
accelerated atherosclerosis in some autoimmune disease states.17

There are other molecules that involve in disposal of apoptotic bodies; those that digest autoantigens (deoxyribonuclease I [DNase I]/serum amyloid protein P [SAP]). DNase I facilitates chromatin breakdown during apoptosis and has been implicated in the pathophysiology of SLE and SAP and phospholipase A2 can bind to the apoptotic cell and facilitate the interaction with phagocytes. Serum amyloid protein P can bind to extracellular double-stranded DNA and chromatin. It is shown that reduced levels of renal DNase I coincides with deficient fragmentation of chromatin from dead cells, implying that the lack of this enzyme may have caused delayed clearance. The DNase I-mediated degradation of nuclear material derived from secondary necrotic cells that had not been cleared during the early phases of apoptosis is considered as a backup mechanism of the phagocytic system. DNase I might have a protective task in the removal of DNA from nucleoprotein complexes, preventing immune stimulation. Indeed, deletion of DNase I resulted in the occurrence of classical symptoms of SLE, including the production of antinuclear antibodies and the development of glomerulonephritis.

It is known that there is a subset of lupus patients with lower DNAase I activity.18 Similar findings have been reported in SAP-deficient mice. In mice with a targeted deletion of the SAP gene, autoimmune disease developed spontaneously, characterized by the presence of autoantibodies to DNA and chromatin and severe glomerulonephritis.

**Dendritic Cells**

At the interface of innate and adaptive immunity, dendritic cells (DCs) play a pivotal role in the regulation of immune responses. Dendritic cells are distributed throughout the body for optimal antigen capture, and as the most potent antigen presenting cells, they are well suited to activate naive T cells. On the other hand, immature DCs can promote epitope-specific peripheral tolerance by presenting antigens acquired from dying cells without co-stimulation to T cells. This results in anergy or deletion of self-reactive T cells and the development of regulatory T cells.

Under physiological conditions, the presence of apoptotic cells is interpreted by the immune system as an anti-inflammatory signal. Thus, when DCs pick up apoptotic cell fragments, auto-antigens are presented in a manner that leads to the inactivation of possible autoreactive T cells. Whereas ingested necrotic cells are able to induce DC maturation, apoptotic cells fail to activate DCs under normal circumstances. When an antigen is perceived by DCs as being harmful, T cells recognizing the antigen will be activated. In order to activate T cells, DCs undergo a process called maturation, migrate to secondary lymphoid organs, and present antigens in an immunogenic context to T cells.

During the process of maturation, DCs are transformed from predominantly antigen-capturing cells towards antigen-presenting cells. Dendritic cells thereby increase the expression of antigen-loaded major histocompatibility complex molecules, upregulate the expression of co-stimulatory molecules such as CD86 and CD40, and secrete pro-inflammatory cytokines and chemokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α. Dendritic cells also produce high amounts of C1q and are known to induce tolerogenic responses. Dendritic cell activation leads to maturation associated with a reduced capacity to produce C1q and the induction of immunogenic responses.19,20 Hence, a correct interplay between apoptotic cells and DCs is essential for the maintenance of peripheral tolerance. In normal conditions, DCs capture a limited amount of apoptotic cell-derived debris and present it to T cells in a noninflammatory fashion, such a process induces T cell tolerance. Currently, at least 2 main types of DCs are known: myeloid DCs, and plasmacytoid DCs. An important characteristic of myeloid DCs in the context of SLE is their ability to take up apoptotic and necrotic cell material and present them to T cells. In contrast to myeloid DCs, plasmacytoid DCs are unable to ingest uncomplexed apoptotic and necrotic material. However, when apoptotic material attached to auto-antibodies, plasmacytoid DCs ingest these complexes. Plasmacytoid DCs can produce high amounts of type I interferon, mainly interferon-α, therefore, referred to as the natural interferon-producing cells. Interestingly, high levels of interferon-α are found in SLE patients, suggesting the involvement of plasmacytoid DCs in the pathogenesis of SLE.21

Due to aberrant apoptosis, or insufficient clearance of apoptotic cells, combined with an
SLE-susceptible genetic background, high (local or systemic) concentrations of apoptotic nucleosomes and blebs are present in SLE patients. These apoptotic nucleosomes and blebs can then be ingested by myeloid DCs. Nucleosomes that are modified during apoptosis or endogenous danger ligands incorporated in blebs are responsible for the maturation of myeloid DCs, which subsequently secrete pro-inflammatory cytokines such as IL-6, and present apoptosis-induced, modified auto-antigens in an immunogenic way to T cells.

Plasmacytoid DCs encountering these immune complexes produce high concentrations of interferon-α, which adds to the maturation of myeloid DCs and supports isotype switching and autoantibody production by autoreactive B cells. In this way, plasmacytoid DCs can amplify the autoimmune response initiated by myeloid DCs. Moreover, the immune complexes can bind to basement membranes in different organs, causing inflammation, which leads to the various disease manifestations, including lupus nephritis, and tissue destruction. In turn, the tissue destruction can result in a local increase in apoptosis, yet another positive feedback loop that contributes to a progressive course of the disease in time.

Recently, 2 novel markers of plasmacytoid DCs are identified, BDCA2 and BDCA4. BDCA2 is selectively expressed on human plasmacytoid DCs acting as a hallmark of human plasmacytoid DCs. It is shown that BDCA2 expression on plasmacytoid DCs decreased along maturation and TLR7 or TLR9 agonists could further significantly downregulate expression of BDCA2. Functionally, BDCA2 ligation significantly inhibited upregulation of CD40, CD86, and CCR7 expression and interferon-α, interferon-β, and IL-6 production.

Moreover, BDCA2 ligation suppresses plasmacytoid DCs to mediate T helper 1 response, including T cell proliferation, interferon-γ production, and CD4+CCR5+T helper 1 development, confirming that BDCA2 is a negative regulator of TLR9-dependent activation of human plasmacytoid DCs. BDCA2 expression on plasmacytoid DCs from SLE patients decreased significantly, but interferon-α production of these patients increased markedly as compared to that from healthy donors. Therefore, these results suggest that downregulation of BDCA2 expression on plasmacytoid DCs may reflect the activation of plasmacytoid DCs accumulated in SLE patients, and may be one marker for indication of disease activity of SLE patients. When the apoptotic-DC ratio is increased and perhaps when apoptotic cells are opsonized by autoantibodies, DCs may become activated and induce a productive immune response against apoptotic cell-borne autoantibodies.

**Toll-like Receptors**

Toll-like receptors (TLRs) constitute a family of pattern recognition molecules that function to discriminate “self” from microbial “nonself.” Toll-like receptors are localized to either the cell surface or endosomes of several cell types, such as antigen-presenting cells (APCs) such as DCs and B cells.

It has become apparent that TLRs can participate in cell activation by self-molecules such as immune complexes containing DNA or RNA. Indeed TLRs have an important role in the pathogenesis of lupus with recruitment of adapter proteins, activation of protein kinases and transcription factors, and expression of inflammatory cytokines (TNF, IL-1, and IL-12), chemokines (monocyte chemoattractant protein-1, IL-8, and regulated upon activation normal T cell expressed and presumably secreted [RANTES]), endothelial adhesion molecules, co-stimulatory molecules (CD80 and CD86), and antiviral cytokines.

Eleven TLRs have been identified in the human genome. Cell surface TLRs (TLR-1, TLR-2, TLR-4, TLR-5, and TLR-6) are designed for the detection of extracellular pathogens, whereas the intracellular TLRs (TLR-3, TLR-7, TLR-8, and TLR-9) are against intracellular pathogen-derived products and, among these, TLR-7 and TLR-9 are of significant interest because they may contribute to the immunological response in SLE to well-known self-antigens such as single-stranded RNA and DNA, respectively. The TLR-4 and TLR-9 have been suggested to instigate T helper type 1 responses, while TLR-2 and TLR-5 are related to T helper 2-related immunity.

Nucleic acids complexed with immunoglobulin G autoantibody bind to FcγRIIa (CD32) on DCs and then are transported into an endosomal compartment where the DNA interacts with TLR-9 and the RNA with TLR-7. Plasmacytoid DCs constitutively express TLR-7 and TLR-9, and they are a major source of the innate cytokine, interferon-α. Plasmacytoid DCs secrete large...
amounts of interferon-α when exposed to immune complexes. A single plasmacytoid DC synthesizes around 1 billion interferon-α molecules in a 12-hour period—200 to 1000 times as many as other cell types.

Genomic studies indicate that around 95% of children and 70% of adults with SLE have a type I interferon signature, of which interferon-α is a hallmark. The TLR signaling in B cells stimulates B cell proliferation, differentiation, and immunoglobulin class switching, all in a T cell-independent manner. The proportion of TLR-9-expressing B cells is expanded in SLE patients with active disease and correlates with the presence of anti-double stranded DNA antibody, suggesting a role for TLR-9 hyper-responsiveness to anti-DNA immune complex in SLE.

In fact, patients with active SLE have increased expression of TLR-9 in peripheral blood memory and plasma B lymphocytes, suggesting that endogenous nucleic acids released during apoptosis may stimulate B lymphocytes via TLR-9 and contribute to SLE pathogenesis. Upregulated expression of TLR-7 and TLR-9 mRNA, together with interferon-α mRNA in peripheral blood mononuclear cells, may also contribute to the pathogenesis of human lupus.

Since a critical level of TLR signaling may convert the potential for autoreactivity to overt autoimmunity and end-organ damage, pharmacologic modulation of TLR-directed pathways may offer a new therapeutic approach for the treatment of SLE. Hydroxychloroquin by blocking TLR-9 and impaired endosomal acidification reduce TLR-induced inflammation. However, the ideal anti TLR agent should block at least both TLR7 and TLR 9. Blockage of just one of these TLR may shift the antibody formation to the other, ie, if TLR9 is blocked, the anti-double-stranded DNA may decrease but the TLR-7 mediated anti RNA levels may rebound and vice versa.

**Importance of Interferon-α**

Interferons are the first cytokine family that was discovered more than 40 years ago. It was reported that interferon was detectable in supranormal concentrations in the serum of patients with active SLE. The observation that intracellular vesicular inclusions in renal endothelial cells of patients with SLE and lupus mice are inducible by interferon suggested that the interferon produced in lupus might have functional consequences for cell structure or function.

But what is the source of interferon-α in SLE? Plasmacytoid DCs of SLE patients were found to produce increased amounts of interferon-α. Interferon-α is known to have diverse effects on immune function, and many of interferon-α activities, including maturation of DCs and induction of pro-inflammatory chemokines, are consistent with observations of altered immune function in patients with SLE (Figure 2).

Further support for the role of interferon-α in pathogenesis of SLE comes from induction of SLE flares and drug-induced lupus in cases of pharmacologic administration of interferon-α for hepatitis or other diseases. Immune complexes are important stimuli for both interferon-α and TNF. In the case of interferon-α, immune complexes containing nucleic acids can access intracellular TLRs after binding to Fc receptors and induce interferon-α.

Consistent with a concomitant role for both interferon-α and TNF in lupus, those patients with gene expression profiles showing activation of both pathways had a higher prevalence of kidney disease than those with either pathway activated. It appears that in well-controlled lupus and in health, both interferon-α and TNF will control each other. On the other hand, when TNF is diminished below a certain threshold, such as by TNF blockers in SLE patients, interferon-α production may be increased, rising the ability to create autoantibodies.

Recently, a group of type I interferon-inducible genes that are significantly upregulated in peripheral blood cells from SLE patients have been identified, expression of which found to be closely associated with increased disease activity, specific autoantibody profiles, and significant organ damage in SLE patients. There are 7 interferon-inducible chemokines (RANTES; monocyte chemoattractant protein-1; chemokine (C-C motif) ligand 19; CXCLs 9, 10 and 11; and IL-8), and of these 7 chemokines, monocyte chemoattractant protein-1, RANTES, and CCL19 are members of the CC family, and recruit monocytes, macrophages, T cells and DCs. In contrast, chemokine (C-X-C motif) ligands 9, 10, and 11 and IL-8 are from the chemokine (C-X-C motif) ligand family, the first 3 of which are chemoattractants of activated T cells, whereas
IL-8 is chemotactic for neutrophils. All of these chemokines have been reported to have consensus sequences for interferon responsive elements.\(^3\)

Antimalarial agents, such as quinine, have long been used in the treatment of SLE, first in the context of cutaneous lupus, and then, as hydroxychloroquine (HCQ) in SLE. In a randomized double-blind placebo-controlled study, SLE patients treated with HCQ had fewer disease flares and disease exacerbations compared to those receiving a placebo. The principal mechanism of action of agents such as HCQ relates to their ability to increase the intracytoplasmic pH and to prevent acidification and maturation of endosomes. Interferon-\(\alpha\) in SLE patients can be produced by plasmacytoid DCs in response to continuous stimulation by circulating immune complexes that are internalized by CD32 (FcgRIIA), with subsequent detection of DNA and RNA by endosomal TLR-9 and TLR-7 in plasmacytoid DCs. Hydroxychloroquine would block TLR-9/7 stimulation and thus play a beneficial role in the treatment of SLE. Hydroxychloroquine can inhibit the production of interferon-\(\alpha\) in plasmacytoid DCs in vitro, either after induction by DNA-containing immune complexes or by stimulation with TLR-9 agonists.\(^3\) Plasmacytoid DC production of interferon-\(\alpha\) by TLR-9 or TLR-7 stimulation was markedly reduced in SLE patients treated with HCQ.

In lupus, regulatory T cells which are a part of mechanism for immune regulation and suppression of autoimmunity are often found in lower numbers than in controls. Those regulatory T cells that are present in lupus are inefficient in suppressing inflammation and T-cell proliferation. Regulatory T cells development is suppressed by treatment of DCs with interferon-\(\alpha\). In lupus patients, regulatory T cells activity is diminished, due at least in part to the action of interferon-\(\alpha\) indicating that increased interferon-\(\alpha\) levels in lupus patients is contributing to the development of autoimmunity through suppression of regulatory T cells cells.\(^3\)

Interferon-\(\alpha\) can prevent apoptosis and enhance proliferation of primary B cells, even in the absence of mitogenic stimuli. Isolated B cells are inhibited from developing into antibody producing plasma cells by blocking interferon-\(\alpha\). However, this inhibition is reversed if the B cells are allowed to come into contact with monocytes, in which case interferon-\(\alpha\) actually stimulates B-cell development and antibody production.\(^3\)

**Follicular T-Helper Cells**

It has been known for a while that T-helper cells are involved in the regulation of B-cell responses. The characterization of a precise subset of these
specialized cells, called follicular T-helper cells, was first reported in 2000. Follicular T-helper cells, a special CD4+ T-cell subset localized in the B-cell follicle, were first reported in tonsils where immune cells are constantly exposed to foreign antigens, resulting in the expansion of immune cells and the formation of germinal centers.

Follicular T-helper cells are characterized by the expression of CXCR5, which is a receptor for CXCL13 and allows them to localize to the follicular regions of lymphoid organs to form stable contacts with antigen-primed B cells. Chemokine receptor 5 (CXCR5) is involved in follicular T-helper cell homing to the B cell follicles. During germinal center formation, follicular T-helper cells with strong expression of CXCR5 are attracted to the gradient expression of CXCR5 cognate (C-X-C motif) chemokine ligand 13 (CXCL13) in germinal centers, allowing follicular T-helper cells to migrate and form stable contacts with antigen-primed B cells in the B cell follicles. Inducible T-cell co-stimulator (ICOS or CD278) is a co-stimulatory molecule that belongs to the CD28 superfamily. It interacts with its ligand expressed on antigen-presenting cells or B cells. Inducible T-cell co-stimulator plays an important role in the regulation of follicular T-helper cell development, T-cell-dependent antibody response, and germinal center reactions. Inducible T-cell co-stimulator supported follicular T-helper cell formation and maintenance via the production of IL-21 and c-Maf expression.

The ICOS/ICOS ligand interaction plays a crucial role in the development and maturation of follicular T-helper cells in vivo. Mutation (M199S) in Roquin that is associated with increased expression of ICOS increases the risk of autoimmunity. In addition, it is demonstrated that IL-21, a type I cytokine, is produced by follicular T-helper cells and functions as an autocrine growth factor for follicular T-helper cells. Programmed cell death protein-1 (PD-1 or CD279) is generally considered a potent inhibitory receptor expressed by T cells, and associated with T-cell tolerance and CD8 cytotoxic T-cell exhaustion during chronic virus infection and cancer, thus playing a negative role in immune response. Compared to other T-cell subsets, follicular T-helper cells express the highest levels of PD-1. Through the interaction between PD-1 on follicular T-helper cells and its ligand on germinal center-B cells, follicular T-helper cells deliver survival signals to germinal center-B cells. Dendritic cells from SLE patients fail to express PD-1 ligand (B7H) to tolerize lymphocytes by PD-1. There is also PD-L2 (B7DC), but overall this is less widely express.

Follicular T-helper cells also interact with B cells through the expression of surface molecules such as the CD40 ligand, a member of the tumor necrosis factor family. CD40 ligand is well known for its role in immunoglobulin isotype switching and B-cell survival via its interaction with CD40 on B cells. Patients with CD40 ligand deficiencies or mutations have a severe lack of memory B cells and an absence of germinal centers.

Follicular T-helper cells are characterized by their homing capacity in CXCL13-rich areas, such as B-cell follicles, through CXCR5 expression and their ability to support immunoglobulin production. Follicular T-helper cells belong to a subset of T-helper cells that differ from the other T-helper cell subsets. Indeed, T-helper 1 cells express STAT4, STAT1, and T-box transcription factor/Tbet, T-helper 2 cells express GATA3, T-helper 17 cells express retinoid-related orphan receptor and regulatory T cells express FOXP3.

Follicular T-helper cell differentiation program is controlled by Bcl6. Follicular T-helper cells upregulate Bcl6, which in turn blocks T-helper 1, T-helper 2, and T-helper 17 cell differentiation by repressing their selective transcription factors. On the other hand, mutation (M199S) in Roquin that is associated with increased expression of ICOS increases the risk of autoimmunity overexpression of Bcl6 in activated T-helper cells induces expression of IL-6 receptor and IL-21 receptor, which are both required for follicular T-helper cell generation. Bcl6 also represses the expression of miR-17-92, a micro RNA that downregulates CXCR5 expression, which is essential for follicular T-helper cell function. Inducible T-cell co-stimulator is also implicated in the regulation of follicular T-helper cell differentiation. Once engaged with its ligand, expressed on antigen-presenting cells including B cells, ICOS induces the production of helper cytokines such as IL-2, IL-4, and especially IL-10 and IL-21. Inducible T-cell co-stimulator deficiency is associated with a reduction of germinal center formation and fewer follicular T-helper cells, suggesting that ICOS signaling is essential in follicular T-helper cell generation.
CXCR5, follicular T-helper cells express markers such as CD25, CD69, CD95, CD57 (in humans only), OX40 (CD134), and CD40L (CD154), and induce overexpression of activation-induced cytidine deaminase in B cells. Follicular T-helper cells also produce cytokines such as IL-10 and IL-21 that promote B-cell survival and antibody production.37

Mutations of CD40L or ICOS genes in humans are associated with follicular T-helper cell deficiency and affect B-cell differentiation and humoral responses, indicating the important role of the co-stimulatory molecules in regulating B-cell responses. Follicular T-helper cells express some molecules, which play a key role in the stability of the antigen-dependent T cell-B cell interaction, such as the signaling lymphocytic activation molecule-associated protein, which is required to form stable contacts between B and T cells and supports follicular T-helper cell generation and germinal center formation.

Expansion of the circulating follicular T-helper cell subset in lupus patients correlated with increased serum titers of auto-antibodies as well as with the incidence of glomerulonephritis, thromboembolic disease, and thrombocytopenia.38 CD57, B and T lymphocyte attenuator, members of the signaling lymphocytic activation molecule-associated protein family (SAP), CD84, and natural killer T-B antigen also contribute to germinal center formation and immune homeostasis.

Follicular T-helper cells also exhibit a unique expression profile of cytokines (such as IL-21, IL-4, and IL-10) and other soluble factors (such as CXCL13). Interleukin-21 is a signature cytokine for follicular T-helper cells and plays a critical role in promoting B-cell somatic hypermutation, immunoglobulin isotype switching, and plasma cell generation. Through the IL-21 receptor, IL-21 also induces follicular T-helper cell differentiation in an autocrine fashion. Furthermore, IL- 21 synergizes with IL-6 and B-cell activating factor to regulate follicular T-helper cell and B-cell differentiation.42

Physiologically attracted to the T-cell zone chemokine gradient of C-C motif chemokine ligand 19 (CCL19) and CCL21, peripheral naive CD4+ T cells expressing the counterparts (CCR7 and P-selectin glycoprotein ligand-1) of these chemokines migrate to the T-cell zone from the circulation. Upon encountering the antigens provided by DCs, naive CD4+ T cells expressing CXCR-5 and transcription factor Bcl-6 while losing expression of CCR-7 and P-selectin glycoprotein ligand-1, are attracted by the chemokines (such as CXCL-13) secreted by B cells, migrate to the B-cell follicle border, and become follicular T-helper cells.43

The data from autoimmune mice strongly suggest that excessive follicular T-helper cells are responsible for autoimmunity and the dysregulation of the germinal center response. In addition to the number of follicular T-helper cells, the quality of help from follicular T-helper cells also controls the extent of the immune response. Evidence has shown that excessive production of the follicular T-helper cell signature cytokine IL-21 leads to autoimmunity. Blocking IL-21 signaling in BXSB mice and lupus-prone MRL mice led to diminished pathogenic autoantibody production and other lupus-like features, indicating that IL-21 is required for autoreactive B-cell differentiation and that blocking IL-21 could be a potential therapy to treat SLE.44 Recent studies showed that the accumulation of circulating follicular T-helper cells strongly correlated with high levels of plasmablasts, anti-double stranded DNA, and antinuclear antibodies, as well as the severity of disease activity in patients with SLE.

**Neutrophils**

As part of the innate immune system, neutrophils are a crucial component in the first line of defense against invading microorganisms. Elimination of microbes occurs through a number of processes that include phagocytosis, generation of reactive oxygen species via the respiratory burst, and the release of microbicidal substances from cytoplasmic granules. In addition, a process characterized by the formation of neutrophil extracellular traps (NETs), is also involved in antimicrobial activity. Neutrophil-derived alarmins include various antimicrobial peptides such as α-defensins, the cathelicidin human cationic antimicrobial protein 18 and lactoferrin. Cathelicidin peptides such as LL-37, which is produced by proteolytic cleavage of the C-terminal antimicrobial domain of human cationic antimicrobial protein 18 and lactoferrin. Cathelicidin peptides such as LL-37, which is produced by proteolytic cleavage of the C-terminal antimicrobial domain of human cationic antimicrobial protein 18, are chemotactic to various leukocytes. Attachment of LL-37 promotes activation of pDC by increasing their expression of co-stimulatory molecules and production of type I interferons.45 LL37/cathelicidin can also complex with RNA to activate DCs through TLR7 and TLR8.46
Many abnormalities in various neutrophil functions have been reported in SLE. Serum from patients with SLE induces increased neutrophil aggregation, compared with serum from healthy donors, also serum from lupus patients interferes with phagocytosis and lysosomal enzyme release by normal neutrophils in vitro. An impaired phagocytic capacity of SLE-derived neutrophils is well established, and aberrant clearance of apoptotic material by phagocytes, including neutrophils, has been proposed to play a part in the pathogenesis of SLE. In addition, decreased responsiveness to cytokines, including IL-8, has been described in SLE-derived neutrophils. Moreover premature telomere shortening, which is suggestive of enhanced senescence is described in these patients. Delayed clearance of neutrophils extracellular trap is called NETosis.

NETosis is triggered by various stimuli and is characterized by active release of chromatin fibers containing antimicrobial peptides, which can trap and kill microorganisms. NETosis is achieved through translocation of neutrophil elastase into the nucleus from primary granules, where it partially degrades specific histones. Neutrophil elastase, together with reactive oxygen species, activates the autophagy machinery that promotes chromatin decondensation and release of DNA from the cell. Impaired NET breakdown has been identified in a subset of patients with SLE and occurs owing to increased abundance of DNase I inhibitors, and production of anti-NET antibodies that prevent access of DNase I NETs. In addition, increased NET formation has been documented in patients with SLE, and might contribute to development of autoimmunity. The LL-37 auto-antibodies with SLE, and might contribute to development of NET formation. In some patients with SLE, the degradation of NETs is impaired owing to DNase I inhibitors or antibodies to NETs. It has been observed that the antimicrobial peptide LL-37—a key mediator of plasmacytoid DC activation—was expressed at high levels in the blood of patients with SLE, suggesting that LL-37 is involved in immunogenicity of self-nucleic acids in immune complexes. It is confirmed that LL-37 and human neutrophil peptide (HNP) are essential for the immunogenicity of DNA-containing immune complexes. Autoantibodies in immune complexes interacted with the Fcγ surface receptor II (FcγRII) on plasmacytoid DCs and triggered receptor-mediated endocytosis of self DNA. Self DNA–antimicrobial peptide complexes, LL-37 and HNP, which would seem to be pivotal components of NETs, activate plasmacytoid DCs to produce interferon-α. Both anti–LL-37 and anti-HNP antibodies activate neutrophils to release NETs; exposure of neutrophils to interferon-α in vitro is followed by expression of the LL-37 and HNP peptides. DNase I deficiency or presence of autoantibody to DNase I in SLE patients lead to longer persistence of NET. Thus, in addition to defective clearance of apoptotic/ necrotic cell clearance, impaired NET clearance supposed to be a source of autoantigen and explain how infection may trigger a flare. In about half of lupus patients net degradation is slow and most of them develop anti NET antibody, it means that there is significant association between Lupus and NET degradation. Release of neutrophil
extracellular traps (NETs) by neutrophils and the activation of plasmacytoid DCs direct the chronic interferon-α production observed in SLE. Type I interferons prime the neutrophils for NETosis, with translocation of LL-37 to the surface. NETosis begins by the binding of surface LL-37 to anti–LL-37 autoantibodies. NETs released from dying neutrophils are taken up by plasmacytoid DCs as a NET-associated LL-37–DNA immune complex, together with anti–LL-37 or anti-DNA autoantibodies. NET-associated self DNA engages TLR-9 in endosomes, leading to interferon release and additional neutrophil priming. Moreover, neutrophil-derived antimicrobial peptides such as LL-37 are used as B-cell auto-antigens in combination with DNA. As a result, abundant NET creation may also prompt autoreactive B-cell activation, possibly through the capacity of NETs to engage B-cell receptors and TLR9 in B cells, leading to the release of anti–LL-37 and anti-DNA autoantibodies.

Role of B Cells

As SLE is characterized by the generation of large amounts of autoantibodies directed against chromatin and a variety of other self-antigens, the loss of B cell tolerance clearly plays a key role in the disease. Recent evidence shows that the breakdown of B-cell tolerance likely occurs very early in SLE and may precede or trigger other immune abnormalities. This can be claimed by demonstrating that SLE patients express antinuclear antibodies several years before the onset of clinical disease.

B-cell tolerance is established at multiple checkpoints throughout B cell development, both in the bone marrow and the periphery, and is enforced largely by negative selection (deletion, editing, or anergy) although positive selection and sequestration into the B1 and marginal zone compartments has also been described. Recent work has demonstrated the role of the breakdown of peripheral tolerance mechanisms in SLE in both mice and humans. It is shown that an important tolerance checkpoint operates in healthy subjects to censor autoreactive B cells in the mature naive compartment, thereby preventing the expansion of these cells into the memory compartment, a checkpoint recently shown to be faulty in SLE. Key checkpoints to censor autoreactive B-cell clones occur at the immature B cell stage in the bone marrow and the transition between new emigrant and mature B cells in the periphery, but with up to 20% of peripheral naive B cells still reactive with nuclear antigens. Moreover, in a small number of SLE patients, peripheral checkpoints were found to be defective, with a further increase in autoreactivity within the mature naive compartment.

Numerous studies in human SLE have documented significant abnormalities in B-cell homeostasis that may be reflective of the loss of B-cell tolerance and aberrant B-cell activation. Such abnormalities include naïve B-cell lymphopenia, increased transitional B cells, and an expansion of peripheral blood plasmablasts. Of note, the frequency and absolute number of plasmablasts in patients with SLE correlates with overall disease activity and autoantibody titers.

B cells may theoretically participate in the immune dysregulation of SLE at multiple levels by (1) serving as the precursors of antibody-secreting cells, (2) taking up and presenting autoantigens to T cells, (3) helping to regulate and organize inflammatory responses through cytokine and chemokine secretion (such as IL-10, IL-6, interferon-γ, and lymphotoxin-α), and (4) regulating other immune cells. B cells play a key role in the recruitment of CXCR5+ follicular T helper cells to the germinal center. Follicular T helper cells provide critical assistance for follicular and germinal center B cells, inducing activation, differentiation, and antibody production. The influence of B cells on follicular T helper cells via ICOS ligand and OX40L co-stimulation may be important in SLE, as excessive activity of follicular T helper cells induces hyperactive germinal center, breakdown of B cell tolerance, auto-antibody production, and a lupus-like phenotype. Another central function of B cells is cytokine secretion. B cells have been shown to produce IL-4, IL-6, IL-10, interferon-γ, transforming growth factor-β, and lymphotoxin-α. Lymphotoxin-α is important for the formation of tertiary lymphoid tissue. This tissue consists of organized collections of lymphocytes in nonlymphoid peripheral organs, where such immune aggregates are not normally found. In lupus, tertiary lymphoid tissue has been demonstrated in the kidney.

Patients with SLE also exhibit high levels of B-cell activating factor, a protein that contributes
to the survival of these autoreactive cells. There is also overexpression of CD40 ligand on B cells, which results in excessive T cell co-stimulation, another mechanism for the survival of autoreactive B cells. B-cell activating factor, also known as B-lymphocyte stimulator, and its homologue APRIL (a proliferation-inducing ligand), are members of the TNF-superfamily critical in the development of B cells. They are both widely expressed by neutrophils, macrophages, monocytes, and DCs, as well as B and T cells. B-cell activating factor binds to 3 receptors of B-cell activating factor-R, transmembrane activator and calcium modulator ligand interactor, and B-cell maturation antigen. Where B-cell activating factor-R only binds B-cell activating factor, APRIL can bind to both transmembrane activator and calcium modulator ligand interactor and B-cell maturation antigen. These receptors are expressed at different times of B cell development. B-cell activating factor-R is expressed by all B cells with the exception of plasma cells in the bone marrow. Binding of B-cell activating factor to its receptor promotes B cell survival and development, allowing for progression from T1 stage into follicular and marginal zone cells. Binding of B-cell activating factor and APRIL to transmembrane activator and calcium modulator ligand interactor and B-cell maturation antigen allows further survival and immunoglobulin class switching and somatic hypermutation, although B-cell activating factor is not necessary for this to occur.55

B-cell activating factor levels have been found to be elevated in patients with SLE compared to normal controls. Whereas serum protein levels may be variable in patients, serum B-cell activating factor mRNA levels correlate better with disease activity. Belimumab was designed as a recombinant fully humanized antibody, with a high affinity to soluble BAFF. Binding of CD28 on T cells to its ligand CD80/86 on APCs and activated B cells provides the major co-stimulatory activation signal to T cells. Endogenous CTLA-4 is an inhibitory molecule on previously activated T cells that binds CD80/86 with higher affinity than CD28. Abatacept, a fusion protein of the extracellular portion of CTLA-4 with the Fc portion of immunoglobulin G, blocks the interaction of CD28 with CD80/86.56

Different B cell subsets may contribute differentially to disease flare. After antigen activation, B cells can become short-lived plasma cells, long-lived plasma cells, or memory cells. The former usually develop after T-independent activation, while the latter two are typically T-cell dependent. Short lived plasma cells live weeks to months, reside in the tissue where they are generated, and, in vitro, are unable to secrete antibody in the presence of anti-proliferative drugs. Long-lived plasma cells, on the other hand, home to the bone marrow where they live and secrete antibody for many years. These plasma cells have been shown to secrete antibody even in the presence of anti-proliferative drugs. This is of interest because different autoantibodies in lupus show different patterns of expression. Anti-RNP antibodies, for example, show a stable pattern of expression over a patient’s lifetime, and their levels are not typically affected by immune suppression. This pattern is suggestive of antibody secretion by long-lived plasma cells.57

Hence, B cells still have many mysteries yet to reveal with respect to how they mediate SLE and how we can successfully overcome these aberrant cells.

**SUMMARY**

As discussed in this review, multiple genetic, environmental, and hormonal factors instigate a number of cellular and cytokine abnormalities in SLE. These abnormalities lead to increased production of auto-antibodies, which either directly, or after forming complexes with auto-antigens and activating complement, deposit in tissues and ignite an inflammatory response. It is imperative to identify the specific molecular defects encountered in human lupus. This is the only way to design and use any novel and rational treatment, since currently treatment of lupus is largely empiric and more or less unsatisfactory.

Mouse models have also enhanced our understanding of the respective roles of various cells and molecules in the innate immune system and the interplay between the innate and adaptive immune systems in lupus pathogenesis. The challenge ahead is to better define the cellular and molecular players orchestrating lupus and to translate our improved understanding of lupus pathogenesis into better rationalized therapeutics targeting selected cells or molecules (or both) that facilitate lupus. There is increasing evidence that the
presence and accumulation of apoptotic cells can result in autoimmunity. Whether this accumulation in SLE patients is due to increased production of apoptotic cells, results from decreased phagocytic capacity, or from the combination of both has to be proven. Nevertheless, it has been shown that tolerance can be broken due to increased amounts of apoptotic cells. Alternatively, or in conjunction, posttranslational modifications occurring during the process of apoptosis of cellular antigens can bypass tolerance. Breaking tolerance induces the production of autoantibodies that will bind to their antigens whenever exposed on the cell membrane.

Understanding the processes underlying these inflammatory lesions will allow us in the near future to intervene therapeutically much more specific in autoimmune mediated disorders so reducing morbidity and mortality of patients suffering from these diseases.

CONFLICT OF INTEREST
None declared.

REFERENCES


37. King C, Tangye SG, Mackay CR. T follicular helper (TFH) cells and context-dependent requirements for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4+ T cell (Tfh) differentiation. PLoS One. 2011;6:e17739.


Correspondence to:
Pedram Ahmadpoor, MD
Department of Internal Medicine, Shahid Labbafinejad Medical Center, 9th Bousian, Pasdaran, Tehran, Iran
Tel: +98 21 2258 0333
Fax: +98 21 2258 0333
E-mail: pedram.ahmadpoor@gmail.com

Received January 2014