INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 26, no. 3, 585-596 (2013)

EDITORIAL

SYSTEMIC LUPUS ERYTHEMATOSUS: IMMUNOPATHOGENESIS AND NOVEL THERAPEUTIC TARGETS

E. BECCASTRINI[1], M.M. D'ELIOS[1,2], G. EMMI[2], E. SILVESTRI[1,2], D. SQUATRITO[1,2], D. PRISCO[1,2] and L. EMMI[1]

[1]SOD Medical Pathology, Center for Autoimmune Systemic Diseases, Behçet Center and Lupus Clinic, AOU Careggi, Florence, Italy; [2]Department of Experimental and Clinical Medicine, University of Florence, Italy

Received October 29, 2012 – Accepted August 30, 2013

Systemic lupus erythematosus (SLE) is the prototype of autoimmune diseases with multiorgan involvement. SLE presents many genetic and epigenetic associations and the pathogenesis is characterized by a complex network of alterations affecting both adaptative and innate immunity. The disclosure of novel mechanisms of SLE pathogenesis suggested new therapeutic targets, based on interference with the cytokine pathways or on depletion of the immune cells.

Systemic lupus erythematosus (SLE) is an autoimmune disease that can affect many organs, with a relapsing/remitting course (1). Although considered infrequent, SLE is relatively common in certain groups of the population. Diagnostic advances led to the identification of many cases that, otherwise, might not have been diagnosed. The incidence of SLE has been estimated to range from 1 to 10 cases per 100,000 persons per year, and the prevalence has been reported to range between 20 and 150 cases per 100,000 persons. The variability may result from differences in the methodology of the studies. Current evidence indicates that ethnic and geographic factors play a key role in the onset of the disease. Overall the incidence of SLE is higher in African American, Hispanic and Asian individuals than Caucasians. SLE is characterized by a 9:1 female to male ratio of incidence, with a higher peak during reproductive years (2).

The etiology of SLE still remains unknown. The pathogenesis of SLE, however, is multifactorial and involves the interplay of genetic and environmental factors. SLE is classically known as a dysregulation of the adaptive immune response. However, many studies demonstrated a key role of dendritic cells, phagocytes and their cytokines in SLE pathogenesis (3).

Genetic and epigenetic factors

Genetic alterations promote the development of systemic lupus erythematosus. Familial aggregation studies have shown that siblings of SLE patients have a greater relative risk for the disease compared with the population as a whole. Monozigotic twin pairs display a higher rate of concordance compared with dizygotic (34% *vs* 3%) (3).

Several genes have been associated to SLE susceptibility. Genetic mutations that cause the disease in a Mendelian fashion account only for a small percentage of cases. In the majority of

Key words: systemic lupus erythematosus, SLE pathogenesis, biologic therapies, belimumab, autoimmune diseases, autoantibodies

Mailing address: Lorenzo Emmi, MD
SOD Patologia Medica,
Center for Autoimmune Systemic Diseases,
Behçet Center and Lupus Clinic,
AOU Careggi, L.go Brambilla 3,
50134 Florence, Italy
Tel.: +39 055 7947134 Fax: +39 055 7947252
e-mail: lorenzoemmi@yahoo.it

0394-6320 (2013)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

SLE patients a combination of genes, instead of a single gene, predisposes to the disease and many different environmental factors are able to trigger immunopathological responses. Moreover, epigenetic factors have also been claimed to play a role in the pathogenesis of SLE (3).

The human leukocyte antigen (HLA) system on the short arm of chromosome 6 contains many crucial immunity-related genes, such as first class major histocompatibility complex (MHC class I) genes (HLA-A, B and C) and MHC class II genes (HLA-DR, DQ and DP). HLA gene mutations have been associated with many autoimmune diseases, including SLE (4).

Several mutations of genes located between MHC class I and II, known as class III, can induce deficiencies of components of the classical complement pathway and are associated with SLE. Homozygous C1q deficiency and genetic mutations inducing a reduction of C2 and C4 levels are associated with SLE (4). Recent studies proposed a role for the mannose-binding lectin (MBL) in the pathogenesis of SLE. MBL can opsonize mannose-rich microorganisms and activate macrophages and complement cascade, with similarities to the classical pathway. Several polimorphysms resulting in reduction of MBL levels have been described in association with SLE. Complement activity reduction can lead to a defective clearance of the apoptotic material that can induce an overexpression of autoantigens to the immune system, stimulating autoimmune mechanisms (5).

Several genome-wide association studies (GWAS), microarray technology based, have demonstrated the association between many new genetic *loci* and SLE. The majority of the genes involved are related to Toll-like receptor (TLR) or type I Interferon (IFN-I) pathways, lymphocyte development and signaling cascade (3).

Increased levels of serum IFN-I have been observed in SLE patients. Moreover, peripheral blood mononuclear cells from these patients display an IFN-I-inducible gene-expression signature which defines the most severe cases of SLE, often associated with renal involvement. Lupus-like syndromes can indeed be the consequence of treatment with recombinant IFN-α that is widely used for HCV infection. SLE patients can present an increased expression of Interferon regulatory factor 5 (IRF5), a transcription factor expressed by plasmocytoid dendritic cells (pDCs), in association with increased levels of IFN-α activity and, sometimes, with anti-RNA binding proteins or anti-double stranded DNA autoantibodies. The IFN-α transcription factors IRAK1, STAT4, the Janus kinase TYK2 and the genetic variants of tumor necrosis factor-α-induced protein 3 (TNFAIP3), involved in the regulation of the IFN-α cytokine function, are also associated with SLE. The observation that an increased IFN-α activity can be a heritable risk factor for lupus development, suggests a key role for the IFN-I system in SLE pathogenesis (3, 6).

Toll-like receptors (TLRs) are transmembrane proteins that are expressed in various cells such as lymphocytes, dendritic cells (DCs), macrophages and others. TLRs can recognize various pathogen-associated molecular patterns (PAMPs) from bacteria, viruses, fungi or protozoan parasites. Upon activation by pathogens, the TLR signaling pathway induces the production of proinflammatory cytokines and, subsequently, the activation of the adaptive immune response. In predisposed subjects, endogenous ligands, such as DNA or RNA, can activate TLRs, inducing an autoimmune response against self antigens. It is hypothesized that mutations resulting in the hyperactivation of this pathway may predispose to the development or perpetuation of the immune response in SLE. In particular, the majority of studies suggested the involvement of endosomal TLR-7 and TLR-9. Patients with active SLE showed an increased expression of TLR-9 on B-cell surfaces and monocytes in comparison with inactive SLE ones. Several factors involved in TLR activation and signalling, such as IRAK1, IRAK4 and TRAF6 seem to be involved in SLE pathogenesis. It is well known that IFN-I and TLR activities are tightly related. Pathogenic variants of the FC receptor for IgG (FcRγ2A) may also contribute to SLE pathogenesis impairing the clearance of immune complexes (ICs) and inducing the activation of TLR and IFN system. Environmental factors, such as viral infections, could contribute to the disease pathogenesis through the activation of TLRs and IFN-I system. Considered as a whole, these observations highlight the key role of innate immunity in SLE pathogenesis (7).

In addition to the above mentioned MHC genetic

abnormalities, several genetic mutations are involved in T and B-lymphocyte signaling and activation. These mutations can induce an overactivation of the adaptative immunity, through abnormalities of cellular receptors, co-stimulatory molecules and cytokine levels and activity. Many genes involved in downstream receptor lymphocyte signaling, such as BLK, PTPN22, RASGRP3, BANK1, LYN, ITPR3 and TNFSF4, can be associated with SLE and other autoimmune diseases. Some of the above-mentioned genetic alterations may be associated with susceptibility to target organ damage (3).

Epigenetic modifications have also been claimed to play a role in SLE pathogenesis. DNA methylation and histone modification are the major mechanisms of epigenetic gene regulation. These modifications induce an alteration in gene expression that does not involve changes in DNA sequence. SLE patients were found to have a reduced capacity of DNA methylation (8), leading to an overexpression of several molecules, such as CD11a, CD70, CD40LG and perforin. In SLE patients, due to gene hypomethylation, perforin is overexpressed, so that $CD4^+$ T lymphocytes kill the autologous monocytes. IL-4 and IL-6 seem also to be hypomethylated, and increased levels of these cytokines have been shown in a murine model treated with DNA methylation inhibitor. Drug-induced SLE has also been described in patients treated with DNA demethylating agents, such as hydralazine or procainamide, suggesting that epigenetic modifications could represent a crucial mechanism contributing to drug-induced lupus-like disease. As a whole, the hypomethylation mechanism increases apoptotic rate of the peripheral blood mononuclear cells. In SLE patients, such an increase is associated with the impaired clearance of apoptotic cells. Therefore, in SLE patients, the release of nucleic acids during apoptosis could play a key role in the activation of autoimmunity (9).

Expression patterns of the histone modification enzymes are also abnormal in SLE. In mouse models the expression of histone acetyltransferases and histone deacetylases is abnormal, inducing a reduction of the histone acetylation as well as alterations of histone methylation. Histone modifications are all strongly associated with SLE, particularly in active forms. Further studies, however, are required to clarify the exact mechanism of such an association. MicroRNAs (MiRNAs), noncoding molecules that regulate the expression of target genes in a post-transcriptional manner, could modulate DNA methylation and histone modifications, potentially linking the two mechanisms. Abnormal patterns of MiRNAs have been demonstrated in SLE patients (10, 11).

Hormonal and environmental factors

As is well known, hormonal and environmental factors can trigger the development of SLE in predisposed individuals. Recent studies revealed a link between genetic, environmental factors and immunological mechanisms involved in SLE pathogenesis.

Many theories have been postulated in order to explain why SLE has a female predominance. A key role has been attributed to estrogen hormone signaling by the estrogen receptor α. Previous studies reported that females with SLE present an increased level of estrogens and a reduced level of androgens, probably due to an abnormal metabolism of sex hormones. Aromatase, an enzyme that converts androgens into estrogens has been shown to present an increased activity in SLE patients. However, the correlation between estrogen hormone concentration and disease activity is still a matter of debate. Based on mouse models and evidence in humans, the X chromosome and sex-associated genetic alterations can play a key role in predisposing to SLE. Also in humans the XXY phenotype induces an increased risk of developing the disease. In addition, elevated levels of prolactin have been observed in SLE subjects of female gender. The increased risk of disease flare during pregnancy and puerperium may be based on these alterations. *In vitro* studies demonstrated an increased production of inflammatory cytokines, especially of IFN-α, in DCs exposed to estrogens. By contrast, progesterone could block TLR-7, inducing a reduction of the above-mentioned cytokine levels. Some immunological alterations have also been revealed in T and B lymphocytes and cytokine activity, probably due to NF-kB increased expression (12, 13).

Much evidence supports the role of infections as trigger factors in SLE induction and exacerbations through different immunological mechanisms. Complement deficiencies, mannose-binding lectin

pathway alteration, FCγRECs polymorphism and other genetic alterations predisposing to SLE can promote the aberrant activation of immune response against chronic infections, initiating autoimmunity. Cross-reactivity between self and non-self epitopes seems to be a pivotal mechanism in SLE development. Based on molecular mimicry mechanism, sequence similarities between pathogen-derived and self-antigens can induce the activation of the autoimmune response, breaking the immune tolerance. For example, the production of several autoantibodies by the immune system in SLE patients could be based on the cross-reactivity between EBV Nuclear Antigen-1 (EBNA) and self-antigens. In mice, chronic viral infections could promote the aberrant production of IFN-I and could up-regulate TLR expression, inducing innate immunity activation. Furthermore, bacterial and viral DNA are hypomethylated and can induce many immune alterations, as previously described, among which is the production of many cytokines leading to autoreactive cells survival (14, 15).

In subjects with genetic predisposition, the exposure of keratinocytes to ultraviolet (UV) light induces apoptosis and causes translocation of autoantigens to the cell surface of apoptotic blebs. These bleb-associated antigens may be presented to lymphocytes, inducing the activation of an autoimmune response. UV light can also promote the binding of autoantibodies to selected nuclear antigens located on blebs and seems to be capable of inducing a reduction of ICs clearance (15). Moreover, UV light increases the production of inflammatory cytokines, such as IL-1, IL-6 and TNF-α, by keratinocytes and lymphocytes. Such an inflammatory skin reaction will be further amplified through the activation of mast cells, fibroblast and endothelial cells, through the production of chemokines and adhesion molecules and the recruitment of different leukocytes from the blood flow. UVB exposure seems to be capable of promoting pDC recruitment in lesional cutaneous lupus skin. As a consequence, increased levels of IFN-α have been demonstrated in skin specimens from SLE patients. Apoptotic mechanisms and IFN-α production by pDCs can play a crucial role in the development of SLE (16).

Several drugs, such as procainamide, hydralazine and quinidine have been reported as trigger factors for SLE development. In the majority of cases, however, drug-induced autoimmunity consists only of circulating autoantibodies. Drug-induced lupus-like forms can appear with skin or joint inflammatory involvement, non-specific symptoms, such as arthralgia, fever and asthenia, but very rarely affect kidney or brain. Drug-induced SLE tends to resolve spontaneously after drug discontinuation. To date, the exact pathogenic mechanism remains unclear. As previously stated, however, the reduction of DNA methylation activity could play a pivotal role (6, 10).

Apoptosis and tolerance break-down

In individuals with a genetic predisposition, enviromental factors such as infections or UV could induce an increase of the apoptotic rate. Although in mice an aberrant apoptosis results in autoimmunity with lupus-like features, in human SLE only little evidence supports the presence of an increased apoptotic rate but, on the other hand, genetic alterations have been associated with a reduction of the apoptotic material clearance, leading, however, to the same result. In presence of an aberrant load of apoptotic material, phagocytes themselves undergo apoptosis, attempting to remove that material. The role of phagocytes in the onset of autoimmunity is confirmed by studies showing that in the absence of macrophages, apoptotic cells undergo a gradual elimination without an increase of DNA levels in the blood (7, 17).

As a consequence of the apoptotic mechanism alteration, SLE patients can present increased levels of circulating self-DNA that can offer antigenic targets promoting an autoimmune response. The immune system, however, is constantly in contact with self-antigens and tolerance mechanisms prevent the onset of an autoimmune response. An imbalance of immune tolerance has been demonstrated. Increased levels of B lymphocyte stimulator (BAFF/BLyS) and A proliferation-inducing ligand (APRIL), promoting lymphocyte longevity and survival, were observed in SLE. Memory B lymphocyte survival is independent of BLyS, mature B cells survival is promoted both by BLyS and APRIL and plasma cell survival is essentially stimulated by BLyS. Such a cytokine, however, seems to play a role also in T cells, DCs and other cell activity. SLE activity correlates with leukocyte expression of BLyS mRNA. To date,

BLyS emerged as a crucial therapeutic target in SLE treatment. In addition, several studies focused on regulatory T cells (Treg) as potential protagonists of the break-down of immune tolerance, revealing both numerical and functional alterations associated with SLE. Further studies, however, will be required for a better comprehension of the role of Treg in SLE pathogenesis (18).

Several observations, however, highlighted the role of other crucial mechanisms. Apoptotic material accumulation in the tissues results in secondary necrosis and promotes inflammatory response. Due to apoptosis, cells undergo nuclear fragmentation and membrane blebbing. As a consequence, modified or cryptic autoantigens are expressed on the cell surface. In addition, in SLE patients high mobility group box protein 1 (HMGB1) can bind and stabilize the nucleosome structure, acting as proinflammatory mediator, when released from dying cells. Finally, the antigens are presented to auto-reactive lymphocytes in an immunogenic contest by antigen-presenting cells (APC), such as dendritic cells, promoting an autoimmune response (19).

Dendritic cells and innate immunity activation

DCs can recognize damage-associated molecular pattern (DAMPs), such as endogenous ligands, via pathogen recognition receptors (PRRs) and, in particular, via TLRs. When apoptotic material is not promptly removed, autoantigens can be recognized by DCs, inducing a maturation of those cells. Immature DCs normally present self-antigens without co-stimulatory signals, inducing a tolerogenic effect. After maturation, however, DCs can present self-antigen to T lymphocytes with co-stimulatory signals, promoting an autoimmune response. Thereby, activated T cells can provide the necessary help for B lymphocyte activation (20).

While myeloid DCs (mDCs) are able to recognize phagocyte and present uncomplexed

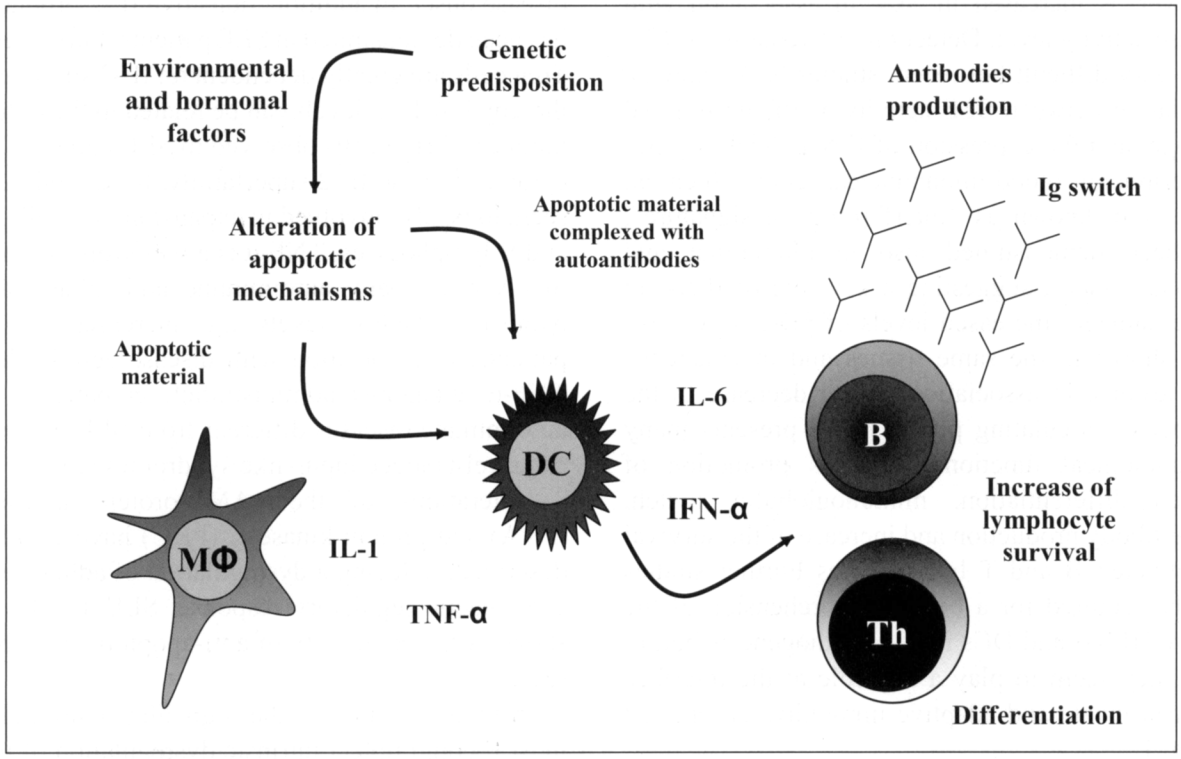

Fig. 1. *Role of innate immunity in SLE pathogenesis. The alteration of apoptotic mechanisms induces the maturation of dendritic cells (DC). As a consequence, DCs present autoantigens to T helper lymphocytes (Th) in association with co-stimulatory signals, promoting an autoimmune response. Moreover, DCs produce IFN-α, inducing many immunological effects, such as lymphocyte activation and differentiation, antibody production and immunoglobulin switch. Many other cytokines and cells, such as macrophages (MΦ), are involved in innate immunity in SLE.*

apoptotic material, plasmocytoid DCs (pDCs) usually recognize apoptotic material complexed with antibodies. Both in response to self-nucleic acids antigens and to self-nucleic acids containing immune complexes, DCs produce IFN-α. Although any cell can virtually produce IFN-I, the major producers of this cytokine are pDCs. Many different ICs can activate pDCs, but RNA containing ICs triggering endosomal TLR seems to be the best IFN-α inducer. IFN-I production, however, can occur without TLR activation. Based on the observations reported above, at the onset of SLE pathogenesis, environmental factors associated with alteration of the apoptotic mechanisms could induce a maturation of mDCs, presenting self-antigens to T and B lymphocytes in an immunogenic way. pDCs, however, could play a key role in producing high concentrations of IFN-I, thereby perpetuating the autoimmune response initiated by mDCs (7).

Normally, IFN-I production is triggered by viral infection with a strict regulation. IFN-I activity, however, is increased in SLE in association with disease activity level. Detection of circulating IFN is often very difficult but recent studies have shown a correlation between SLE activity, particularly renal damage, and the expression of IFN-inducible genes in peripheral blood mononuclear cells. Such an expression, known as "Interferon-gene signature", has been demonstrated also in glomerular and synovial tissues, suggesting a key role of IFN-I in tissue damage. Increased levels of pDCs have also been shown in the same tissues and in cutaneous SLE lesions, in association with a decrease in the number of circulating pDCs. IFN-I presents many immunological functions, such as promotion of B cell differentiation, immunoglobulin switch, autoantibody production and increase of the survival of activated B and T lymphocytes Further studies will be required for a better comprehension of the roles of IFN-I and DCs in SLE pathogenesis but, to date, they seem to play a key role at the interface between innate and adaptive immunity, as reported in Fig. 1.

Adaptive immunity

T cells undergo activation when mature DCs present self-antigen in MHC-restricted conditions. In SLE the engagement of the T-cell receptor (TCR) leads to an early and increased signaling, inducing an aberrant intracytoplasmatic calcium flux and cytosolic protein tyrosine phosphorylation. CD3ζ chain is a crucial component of the TCR complex, playing an important role in coupling antigen recognition to intracellular signal-transduction pathway. In SLE patients, CD3ζ chain is replaced by FcRγ common chain, promoting the hyperactivation of the T cells downstream of the TCR. Functional alterations of co-stimulatory factors can also contribute to the alteration of the TCR signaling. Among these, the increased activity of Nuclear factor of activated T cells (NFAT) could play a fundamental role, increasing the expression of the costimulatory molecule CD40L (21).

In SLE patients, T lymphocytes show increased lipid raft clustering, promoting the hyperactivation of T cells. Lipid rafts are high-cholesterol membrane zones that undergo polarization on the cell surface upon activation. In a murine model of SLE, administration of clustering enhancers promotes the disease onset. In addition, defective IL-2 production has been demonstrated in SLE patients. Furthermore, Treg cells are greatly dependent on IL-2 stimulation, thereby low IL-2 levels can be related to the reduced number of Treg cells observed in SLE patient. Other cytokines of the IL-2 superfamily, IL-15 and IL-21, seem to be also involved in expansion of T cells, NK and B lymphocytes. TNF-α is a pleiotropic cytokine involved in many autoimmune and inflammatory pathways. TNF-α levels are increased in SLE patients, in association with disease activity level. TNF-α antagonist administration in patients with autoimmune diseases different from SLE, however, can rarely induce lupus-like syndromes (21).

Alterations of the cAMP-protein kinase A (PKA) and protein kinase C (PKC) have also been described, inducing a dysregulation of adhesive and chemotactic regulatory response. SLE T cells also show increased activity of anti-apoptotic gene bcl-2 (21).

Although many of the signaling abnormalities in SLE could reflect intrinsic dysregulation of the T lymphocyte activity, several of such abnormalities can reflect the increased stimulation by the other cells of the immune system, such as DCs. Many other alterations of lymphocyte activity collaborate to induce the hyperactivation of Th cells (21).

Recently, Savino MT et al. have highlighted, both in mice and humans, a possible role in SLE pathogenesis of Rai, an Shc adapter family member. Indeed Rai(-/-) mice develop a lupus-like phenotype associated to the spontaneous activation of self-reactive lymphocytes. In particular, it has been demonstrated that Rai(-/-) mice present Th1 and Th17 cell infiltrates in the kidneys, suggesting that Rai(-/-) might contribute to the development of lupus nephritis. Moreover, T cells derived from SLE patients were found to have a defect in Rai expression, suggesting a possible role for this adapter protein in SLE pathogenesis (22).

T-helper cells are classically distinct in Th1 and Th2, according to cytokine receptors and effector functions. Th1 cells are usually associated with autoimmunity. IL-12 stimulates the differentiation of naïve CD4+ T cells into Th1, which produce IFN-γ. In murine models of SLE, T cells have increased IFN-γ levels. IL-4, IL-5 and IL-13 are Th2 cytokines involved in many T and B cell functions such as proliferation, activation and isotype switching and have been demonstrated to be also increased in SLE patients. Recent studies, however, have also revealed a crucial role for Th17 in SLE. Th17 cells, involved in pathogenesis of several autoimmune diseases such as rheumatoid arthritis or inflammatory bowel diseases, are a subset of T-helper lymphocytes producing the cytokines of the IL-17 family. Current evidence revealed that IL-17 could also play a role in SLE pathogenesis. SLE patients present increased circulating levels of IL-17, in association with disease activity. Th17 cells have been found in glomerular tissue from patients with active lupus nephritis. Increased levels of IL-6 and IL-21, correlated to Th-17 response, have been also demonstrated in SLE. IL-17 is able to induce the production of inflammatory cytokines and autoantibodies, supporting the inflammatory response. In murine models, IL-6 along with TGF-β induces the production of IL-17A and IL-17F, while IL-23, produced by APC, seems to be the major inducer of Th17 expansion and maintenance (23).

In addition to the helper activity abnormalities, an impaired cytotoxic response has also been reported. A reduced cytotoxic T-cell activity against EBV-infected cells has been demonstrated. Such an alteration can be involved in the trigger activity of EBV in SLE pathogenesis. In addition, lupus-like disease can develop in cytotoxic function-deficient mice (21).

B cells are involved in SLE pathogenesis and are responsible for the production of an array of autoantibodies against soluble and cellular constituents, such as nuclear antigens. Similar to T cells, B lymphocytes have been reported to present a generalized hyperactivation. This hyperactivation seems to result from an increased stimulation and an impaired regulation. Increased phosphorylation of several signaling molecules and an abnormal calcium flux in B cells have been reported in SLE patients. High levels of co-stimulatory molecules, as B7 and increased levels of immature B-cells, memory B cells, plasmablasts and plasma cells have been also demonstrated. A key point is the increase of BLyS and APRIL levels, promoting the survival of B cells and the tolerance check-point avoidance. Many alterations of the cytokine levels and activity have been reported in SLE, in association with the alteration of B-cell function. Serum IL-6 levels are elevated in SLE, with a correlation with disease activity. Increased levels of IL-10 seem to be associated with Treg function but also with B-cell activation. The administration of IL-10-antagonists in murine models and in SLE patients seems to reduce joint and skin disease activity (24, 25).

Relevant elements of the complex pathogenetic mechanism of T and B cell hyperactivation are reported in Fig. 2. The deregulation of the adaptive immunity results in a wide range of effects. Autoantibody production, as is well known, represents one of the most important mechanisms contributing to tissue damage.

Targeting immunological pathways: therapeutic perspectives

In recent years, many biologic agents have been developed for SLE treatment. As summarized in Fig. 3, the main targets are represented by neutralization of autoreactive B cells, induction of tolerance, inhibition of costimulatory signals and inhibition of cytokine pathway. Due to their role in SLE pathogenesis, B cells could represent a major therapeutic target. In addition to autoantibody secretion, B lymphocytes can present antigens to T cells and can therefore play a composite role in the development of immune

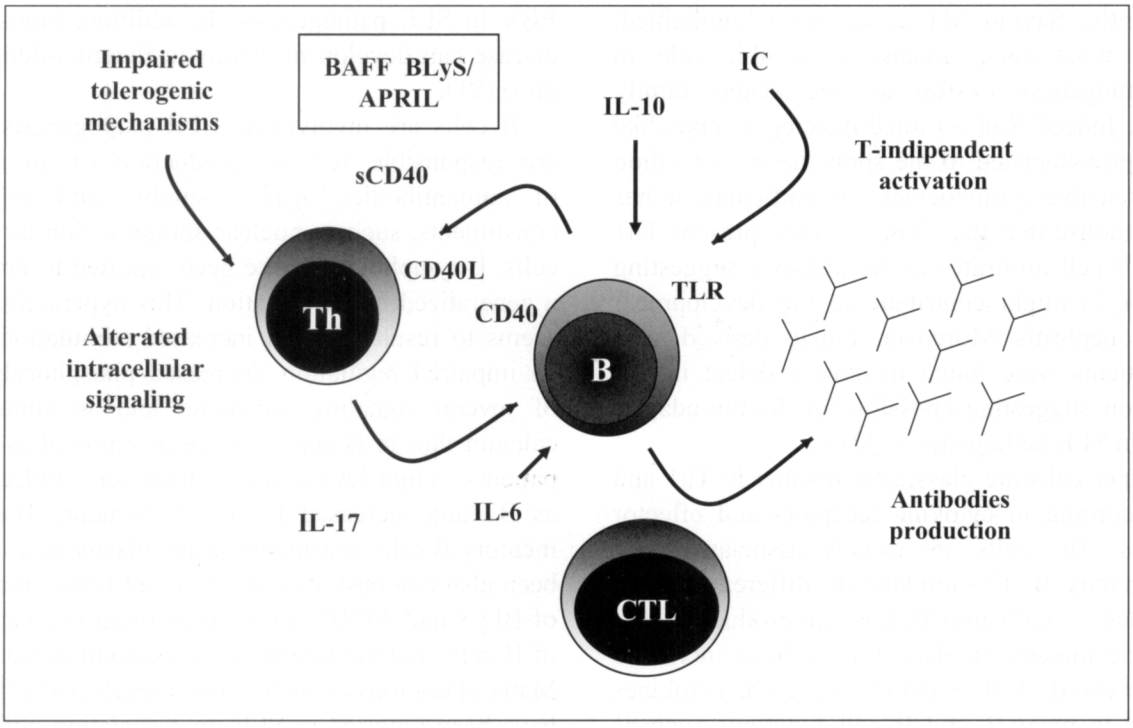

Fig. 2. *Role of adaptive immunity in SLE pathogenesis. The breakdown of central and peripheral tolerance leads to expansion and differentiation of autoreactive T cells that activate B lymphocytes. B cells may also be activated by the link between immunocomplexes of self DNA and Toll like receptors (TLR) in a T lymphocyte independent manner.*

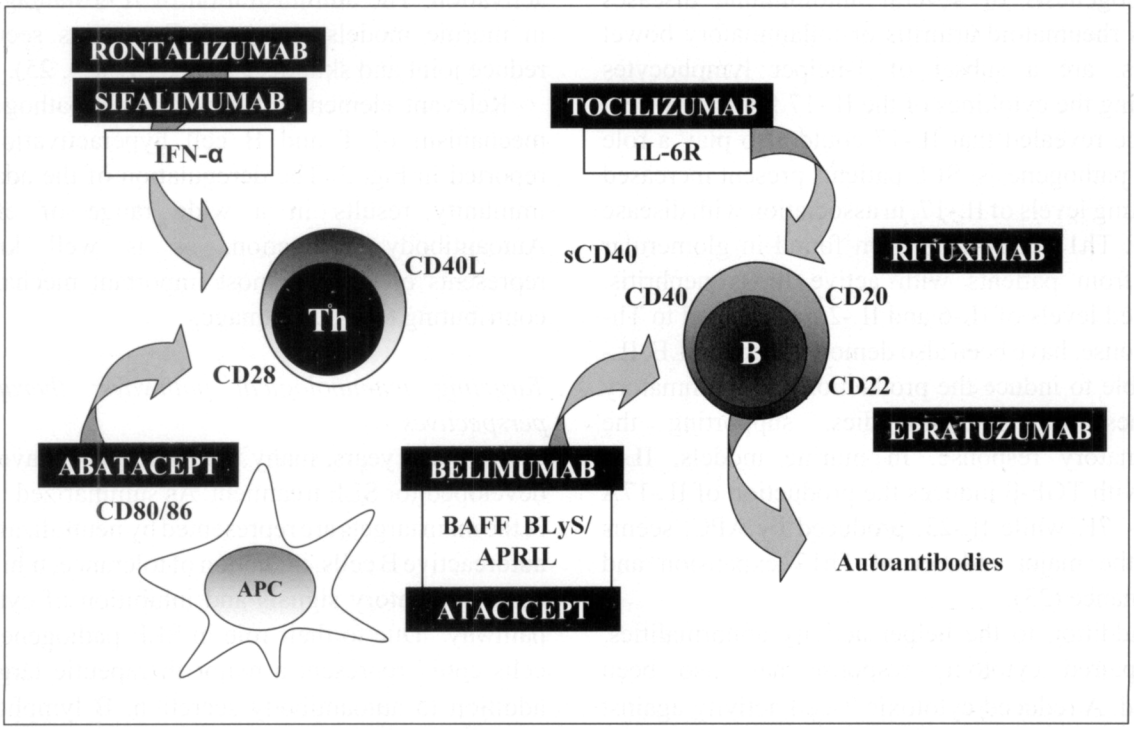

Fig. 3. *Targeting SLE pathogenesis with biological agents.*

response, as mentioned above.

Current and ongoing therapeutic approaches targeting B-cell compartment include B cell depletion, blocking of the costimulatory molecules and inhibition of specific cytokines (26).

Rituximab, a chimeric monoclonal antibody (mAb) against B lymphocyte specific antigen CD20, inducing B cell depletion, was promising in active and refractory SLE. In murine models, Rituximab targets B cells from the pre-B stage in the bone marrow to the mature lymphocyte and memory B cells. But, unlike mice, in humans Rituximab targets B cells from peripheral blood and does not target plasma cells that do not express CD20 on their surface, so that the normal antibody levels are maintained. However, the effect of Rituximab on the tissue B cells remains not completely understood. Clinical experience and case series have suggested a clinical improvement and a consistent reduction of the activity disease index (SLEDAI, BILAG). Recently, however, two double blind placebo-controlled trials (EXPLORER and LUNAR) have failed to meet their end-points in SLE. Despite the normalization of anti-dsDNA and complement levels, clinical outcomes did not appear to differ between Rituximab and the control arm. Further studies would be useful to explain the gap between the failure of the clinical randomized controlled trials and the effectiveness of Rituximab in SLE. The study end-point design may have contributed to the negative results of these trials. Alternative response index can reveal beneficial effects of Rituximab, particularly in lupus nephritis, as suggested by clinical experience (26, 27).

The mAb against CD22, Epratuzumab, modulating the BCR-signaling of the B cells, induces a reduction of the B cell count and a slight reduction of immunoglobulin levels. A phase-IIb trial demonstrated a significant response rate in SLE patients, measured with a combined clinical index. The safety and efficacy of Epratuzumab must be confirmed by the phase-III trial, currently ongoing (26).

The blockage of costimulatory signals can interfere with B-T-cell signaling and, as a consequence, inhibit B cell proliferation and activation. Clinical trials are currently ongoing, targeting CD28 and CTLA4. Abatacept, a fusion protein between the extracellular domain of CTLA-4 and the FC of IgG1, binds B7-1 and B7-2 receptors on B cells and the other APC and inhibits the activation of T cells. Results were promising in mouse models of SLE and in other autoimmune diseases. Abatacept was evaluated in SLE patients in a a randomized, placebo-controlled trial. Despite a reduction in disease flares, particularly in patients with articular involvement, no differences were observed between Abatacept and the control group overall. As mentioned for Rituximab, different response indexes may reveal an usefullness of Abatacept in SLE (28, 29).

As mentioned above, the BAFF/BLyS pathway plays a key role in the survival and selection of autoreactive B cells. Belimumab, a fully human IgG1λ monoclonal antibody against BLyS, decreases B cells and autoantibody level, inducing a significant clinical improvement. A phase-II trial did not meet its co-primary endpoints. A new index of clinical response (SLE responder index or SRI), however, allowed a close post hoc analysis of the trial data, demonstrating a significant response in Belimumab-treated SLE-patients at 52 weeks. Therefore, two randomized, double-blind, placebo-controlled phase-III trials (BLISS-52 and BLISS-76) were performed. The first-one, largely conducted in Asia, South America and Eastern Europe, demonstrated a good response rate at 52 weeks of treatment. SRI rate of responders was 51% at 1 mg/kg Belimumab dose and 58% at 10 mg/kg, in comparison with 44% in the placebo group. BLISS-76, however, involving US, Western Europe and Canada, demonstrated a 41% SRI rate of responders at 1 mg/kg Belimumab dose and 43% at 10 mg/kg, in comparison with 34% in the placebo group. In brief, phase-III Belimumab trial demonstrated the efficacy of Belimumab, particularly at 52 weeks. It has been reported that therapy with Belimumab improves SLE disease activity, particularly of cutaneous and articular involvement; moreover, it induces the reduction of autoantibodies and normalizes low complement levels. Several questions, such as the usefulness of a long-term treatment and the effectiveness of Belimumab in any SLE subset, still remain unanswered. Recent evidence, however, confirmed the safety of Belimumab over 4-year therapy. Belimumab was approved by the US Food and Drug Administration (FDA) and also recently by the European Medicines Agency (EMEA) and at the

moment is the only approved biologic treatment for SLE (30, 31).

In addition to Belimumab, other agents target the BLyS/BAFF pathway. Atacicept, a fusion protein between TACI and the Fc portion of IgG, binds both BLyS and APRIL. Despite the favorable safety profile demonstrated in preclinical and phase-I studies, a clinical phase II/III trial in lupus nephritis patients was interrupted early because of severe reduction of B cell numbers and serum immunoglobulin levels, inducing the comparison of severe infections. A new trial, however, is actually ongoing to confirm the safety and efficacy profile of Atacicept (31, 32).

Based on the alteration of cytokine level and function in SLE, many agents interfering with cytokines activity are currently under examination.

Anti-TNF-α-agents are used to treat many autoimmune diseases, such as rheumatoid arthritis, with benefits. Despite the involvement of TNF-α in SLE pathogenesis, monoclonal antibodies against TNF-α blockers are not generally used in SLE therapy, except in uncommon conditions and for a short term. This is because of the paradoxical effect of the anti-TNF-α agents in inducing autoimmunity. As well reported in literature, TNF blockage can induce autoantibody production and, rarely, drug-induced lupus-like syndrome (33).

Increased levels of IL-1 have been observed in SLE patients. Anakinra, an IL-1 receptor antagonist, interferes with the biological activity of IL-1 and is used to treat patients affected by rheumatoid arthritis and several other diseases with articular manifestations. Some evidence suggested the usefulness of Anakinra in SLE patients with lupus arthritis not responding to common treatments (26).

Tocilizumab, a humanized IgG1 monoclonal antibody against IL-6 receptor, inhibits the IL-6 pathway that, as mentioned above, is involved in the development of inflammation and in B-cell activation. Based on pathogenetic mechanisms of SLE, Tocilizumab could be considered as a future therapeutic alternative (34).

Elevated serum levels of IFN-α have been demonstrated in SLE. IFN-α pathway is involved in SLE pathogenesis in a different manner, such as DC activation, T- and B-cell proliferation and activation, TLR signaling. As mentioned above, peripheral blood mononuclear cells from SLE patients present an increased expression of the IFN-I-inducible genes, referred to as "IFN-gene signature". The evaluation of IFN-gene over-expression in peripheral blood of SLE patients can be useful to identify the subgroup of patients in SLE population that presents high possibilities of response to anti-IFN treatment. Sifalimumab and Rontalizumab, monoclonal antibodies against IFN-α, are currently being evaluated in phase II clinical trials (35).

CONCLUSIONS

Recent advances in our understanding of SLE pathogenesis have offered new targets for treatment, but several fundamental questions remain unsolved. As for pathogenesis and therapy, SLE remains elusive because of the wide spectrum of manifestations, suggesting a complex network of different factors, explaining the difficulty of SLE biological treatment trials in meeting the end-points. We speculate that various SLE subsets of patients, characterized by different pathogenetic and clinical profiles, could request a tailored therapeutic approach based on a better understanding of the disease immunopathogenesis.

REFERENCES

1. Rahman A, Isemberg DA. Systemic lupus erythematosus. N Engl J Med 2008; 58:929-39.
2. Pons-Estel GJ, Alarcón GS, Sconfield L, Reinlib L, Cooper GS. Understanding the epidemiology and progression of systemic lupus erythematosus. Semin Arthritis Rheum 2010; 39(4):257-68.
3. Flesher DL, Sun X, Behrens TW, Graham RR, Criswell LA. Recent advances in the genetics of systemic lupus erythematosus. Expert Rev Clin Immunol 2010; 6:461-79.
4. Crow MK. Collaboration, genetic associations and lupus erythematosus. N Eng J Med 2008; 358:956-61.
5. Monticielo OA, Mucenic T, Machado Xavier R, Tavares Brenol JC, Bogo Chies JA. The role of mannose-binding lectin in systemic lupus erythematosus. Clin Rheumatol 2008; 27:413-19.
6. Rönnblom L, Alm GV, Eloranta ML. The type I interferon system in the development of lupus.

Semin Immunol 2011; 23:113-21.
7. Kontaki E, Boumpas DT. Innate immunity in systemic lupus erythematosus: sensing endogenous nucleic acids. J Autoimmun 2010; 35:206-11.
8. Richardson B, Scheinbart L, Strahler J, Gross L, Hanash S, Johnson M. Evidence for impaired T cell DNA methylation in systemic lupus erythematosus and rheumatoid arthritis. Arthritis Rheum 1990; 33(11):1665-73.
9. Zhang Y, Zhao M, Sawalha AH, Richardson B, Lu Q. Impaired DNA methylation and its mechanisms in CD4(+)T cells of systemic lupus erythematosus. J Autoimmun 2013; 41:92-99.
10. Zhao S, Long H, Qiaanjin L. Epigenetic perspectives in systemic lupus erythematosus: pathogenesis, biomarkers and therapeutic potentials. Clinic Rev Allerg Immunol 2010; 39:3-9.
11. Stagakis E, Bertsias G, Verginis P, Nakou M, Hatziapostolou M, Kritikos H, Iliopoulos D, Boumpas DT. Identification of novel microRNA signatures linked to human lupus disease activity and pathogenesis: mir-21 regulates aberrant T-cells response trough the regulation of PDCD4 expression. Ann Rheum Dis 2011; 70:1496-506.
12. Weckerle CE, Niewold TB. The unexplained female predominance of systemic lupus erythematosus: clues from genetic and cytokine studies. Clinic Rev Allerg Immunol 2011; 40:42-49.
13. Shoenfeld Y, Tincani A, Gershwin ME. Sex gender and autoimmunity. Autoimmun Rev 2012; 38(2-3):J71-3.
14. Doria A, Canova M, Tonon M, et al. Infections as trigger and complications of systemic lupus erythematosus. Autoimmun Rev 2008; 8:24-28.
15. Zandman-Goddard G, Solomon M, Rosman Z, Peeva E, Shoenfeld Y. Environment and lupus-related diseases. Lupus 2012; 21(3):241-50.
16. Walling HW, Sontheimer RD. Cutaneous lupus erythematosus: issues in diagnosis and treatment. Am J Clin Dermatol 2009; 10:365-81.
17. Pisetsky DS, Ullal AJ. The blood nucleome in the pathogenesis of SLE. Autoimmun Rev 2010; 10:35-37.
18. Gerli R, Nocentini G, Alunno A, Bocci EB, Bianchini R, Bistoni O, Riccardi C. Identification of regulatory T cells in systemic lupus erythematosus. Autoimmun Rev 2009; 8:426-30.
19. Ma CY, Jiao YL, Zhang J, Yang QR, Zhang ZF, Shen YJ, Chen ZJ, Zhao YR. Elevated plasma level of HMGB1 is associated with disease activity and combined alterations with IFN-alpha and TNF-alpha in systemic lupus erythematosus. Rheumatol Int 2012; 32:395-402.
20. Fransen JH, van der Vlag J, Ruben J, Adema GJ, Berden JH, Hilbrands LB. The role of dendritic cells in the pathogenesis of systemic lupus erythematosus. Arthritis Res Ther 2010; 12:207.
21. Peng SL. Altered T and B lymphocyte signaling pathways in lupus. Autoimmun Rev 2009; 8:179-83.
22. Savino MT, Ulivieri C, Emmi G, et al. The Shc family protein adaptor, Rai, acts as a negative regulator of Th17 and Th1 cell development. J Leukoc Biol 2013; 93(4):549-59.
23. Apostolidis SA, Lieberman LA, Kis-Toth K, Crispín JC, Tsokos GC. The dysregulation of cytokine networks in systemic lupus erythematosus. J Interferon Cytokine Res 2011; 31:769-79.
24. Jacob N, Stohl W. Cytokine disturbances in systemic lupus erythematosus. Arthritis Res Ther 2011; 13:228.
25. Chu VT, Enghard P, Schürer S, Steinhauser G, Rudolph B, Riemekasten G, Berek C. Systemic activation of the immune system induces aberrant BAFF and APRIL expression in B cells in patients with systemic lupus erythematosus. Arthritis Rheum 2009; 60:2083-93.
26. Yildirim-Toruner C, Diamond B. Current and novel therapeutics in the treatment of systemic lupus erythematosus. J Allergy Clin Immunol 2011; 127:303-12.
27. Rovin BH, Furie R, Latinis K, et al.; LUNAR Investigator Group. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. Arthritis Rheum 2012; 64:1215-26.
28. Mok MY. The immunological basis of B-cell therapy in systemic lupus erythematosus. Int J Rheum Dis 2010; 13:3-11.
29. Mok CC. Abatacept for systemic lupus erythematosus: the outlook. Expert Opin Biol Ther 2012; 12(12):1559-61.
30. Stohl W, Scholz JL, Cancro MP. Targeting BLyS

in rheumatic disease: the sometimes-bumpy road from bench to bedside. Curr Opin Rheumatol 2011; 23:305-10.

31. Merrill JT, Ginzler EM, Wallace DJ, et al. on Behalf of the LBSL02/99 Study Group. Long-term safety profile of Belimumab plus standard therapy in patients with SLE. Arthritis Rheum 2012; 64(10):3364-73.
32. Ginzler EM, Wax S, Rajeswaran A, Copt S, Hillson J, Ramos E, Singer NG. Atacicept in combination with MMF and corticosteroids in lupus nephritis: results of a prematurely terminated trial. Arthritis Res Ther 2012; 7;14(1):R33.
33. Aringer M, Smolen JS. TNF inhibition in SLE: where do we stand? Lupus 2009; 18:5-8.
34. Dall'era M, Chakravarty EF. Treatment of mild, moderate, and severe lupus erythematosus: focus on new therapies. Curr Rheumatol Rep 2011; 13(4):308-16.
35. Lichtman AI, Helfgott SM, Kriegel MA. Emerging therapies for systemic lupus erythematosus – Focus on targeting interferon-alpha. Clin Immunol 2012; 143:210-21.