CHAPTER 9

A NEW EPIGENETIC CHALLENGE: Systemic Lupus Erythematosus

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Abstract: In recent years, compelling evidence has been gathered that supports a role for epigenetic alterations in the pathogenesis of systemic lupus erythematosus (SLE). Different blood cell populations of SLE patients are characterized by a global loss of DNA methylation. This process is associated with defects in ERK pathway signalling and consequent DNMT1 downregulation. Hypomethylation of gene promoters has been described, which permits transcriptional activation and therefore functional changes in the cells and also hypomethylation of the ribosomal RNA gene cluster. Among the identified targets undergoing demethylation are genes involved in autoreactivity (ITGAL), osmotic lysis and apoptosis (PRF1, MMP14 and LCN2), antigen presentation (CSF3R), inflammation (MMP14), B- T-cell interaction (CD70 and CD40LG) and cytokine pathways (CSF3R, IL-4, IL-6 and IFNGR2). DNA methylation inhibitors are also known to induce autoreactivity in vitro and cause a lupus-like disease in vivo. Further, altered patterns of histone modifications have been described in SLE. $CD4^+$ lymphocytes undergo global histone H3 and H4 deacetylation and consequent skewed gene expression. Although multiple lines of evidence highlight the contribution of epigenetic alterations to the pathogenesis of lupus in genetically predisposed individuals, many questions remain to be answered. Attaining a deeper understanding of these matters will create opportunities in the promising area of epigenetic treatments.

Epigenetic Contributions in Autoimmune Disease, edited by Esteban Ballestar.

Table 1. Symptoms described in SLE patients[1,6]

Affected Organ	Symptoms
Nonspecific	Fever, fatigue, weight loss
Circulatory system	Heart failure, pericarditis, endocarditis, myocarditis, coronary thrombosis
Cutaneous system	Rash, photosensitivity, alopecia, changes in pigmentation
Gastrointestinal system	Abdominal pain, peritonitis, pancreatitis, mesenteric vasculitis, nausea, dyspepsia
Haematological system	Leucopenia, lymphopenia, anaemia, thrombocytopenia
Musculoskeletal system	Arthralgia, myalgia, arthritis
Nervous system	Headache, mood, cognitive and movement disorders, psychosis, delirium, seizures
Pulmonary system	Pleuritis, dyspnea, serositis, pneumonitis, haemoptysis
Renal system	Glomerulonephritis, hypertension, haematuria, oedema, hyperlipidaemia.
Reproductive system	Miscarriage, pre-eclampsia, intrauterine growth restriction

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by the production of non-organ-specific autoantibodies against host components that generate inflammation and multisystem injury.[1,2] This disease can affect all sexes, ethnicities and ages although the highest prevalence is in women of African descent during their reproductive years.[1,3] The prevalence of SLE in Northern Europeans has been estimated at approximately 40 cases per 100,000 persons, in contrast to more than 200 per 100,000 persons among African-American populations.[4] Women are most commonly affected and the female to male ratio is 9:1. With respect to life expectancy, the 15-year survival rate is currently around 80% and the pattern of mortality is bimodal, with some dying earlier from consequences of the active autoimmune disease and others dying later from atherosclerotic cardiovascular disease.[5]

SLE is characterized by a broad range of clinical manifestations and unpredictable exacerbations and remissions. All systems and organs can be affected through autoantibody mediated inflammation. Cutaneous manifestations are the most common symptom of SLE, since 85% of patients develop various rashes, although there is a wide range of symptoms that do not include skin[1,6] (Table 1). The diagnosis of SLE is based on eleven criteria established by the American Rheumatism Association: malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neuropsychiatric alterations, haematological disorders, immunological alterations and the presence of antinuclear antibodies. At least four of these criteria are required to make the diagnosis with certainty.

The pathogenesis of SLE is complex and remains unclear. However, alterations of apoptotic processes and altered cytokine levels are two major mechanisms contributing to the loss of tolerance and consequent development of autoantibody production. Abnormalities in apoptosis are the source of autoantigens that induce inflammatory injury through autoantibody production. Moreover, this altered process may explain the fact that SLE autoantibodies mainly react with intracellular components. Defects in

Table 2. Main autoantibodies described in SLE patients

Autoantibody	Prevalence	Autoantigen	Antigen Location	Tissue Target
Anti-dsDNA	≈50-80%	Ds genetic material	Nuclear	Kidney and skin
Antinucleosomes	≈50-90%	Histones	Nuclear	Kidney and skin
Anti-Ro (SS-A)	≈25-40%	52 KDa or 60KDa proteins	Nuclear	Kidney, skin, foetal heart, lung
Anti-Sm	≈10-30%	Spliceosomal snRNP	Nuclear	Foetal heart
Anti-La (SS-B)	≈10-20%	48 KDa transcription terminator protein	Nuclear	Kidney
Anti-ribosomal-P	≈15%	60S ribosomal subunit phosphoproteins (P0,P1, P2)	Nuclear	Kidney, brain, liver
Anti-nRNP	≈23-40%	Spliceosomal snRNP	Nuclear	Muscles, circulatory system
Anti-Ku	≈20-40%	P70/p80 DNA reparation proteins	Nuclear	Joins, heart, lung
Anti-NMDA receptor	≈33-50%	NMDA Receptor	Membrane	Brain
Antiphospholipids	≈20-30%	Phospholipids	Membrane and extracellular	Circulatory system
Anti-α actinin	≈20%	α-actinin	Cytoplasm	Kidney
Anti-C1q	≈40-50%	C1q complement component	Extracellular	Kidney

Ds (double-stranded), Sm (Smith), nRNP (nuclear riboprotein), snRNP (small nuclear riboprotein), NMDA (N.-methyl-D-aspartate.[2,6,9,127-132]

apoptotic clearance and alterations of complement components related to phagocytosis have also been described.[7] Both alterations could lead to aberrant antigen uptake by antigen-presenting cells (APCs) and consequent presentation to B and T cells. On the other hand, a significant decrease of tingible body macrophages of the germinal centres (GCs) has been described in a subgroup of SLE patients. These phagocytes rarely contain apoptotic material. Mistakes in the elimination of apoptotic B cells induced after somatic mutation, or other cells undergoing apoptosis such as monocytes and macrophages, could be a source of autoantigen release. This apoptotic debris could then be presented by germinal node follicular dendritic cells (FDCs), providing survival and stimulatory signals for autoreactive B cells.[8] Autoantibody production against nuclear antigens is a hallmark of systemic lupus erythematosus. These antibodies are responsible for inflammatory injury through immune complex formation and are also useful for SLE diagnosis, prognosis and patient management. Moreover, these antibodies can bind to

autoantigens or crossreact with other components blocking or increasing the functions of their targets. More than 50 autoantibody specificities have been described, most of which are to nuclear autoantigens and some are correlated with effects on tissues, disease manifestations and clinical stage (Table 2). For example, the anti-double-stranded DNA (anti-dsDNA) antibody is the most specific autoantibody in SLE since it is detected in 50-80% of lupus patients at some point during the course of the disorder, but in fewer than 0.5% of healthy people. However, the anti-dsDNA antibody is not very sensitive due in part to its transience. The presence of this autoantibody tends to be associated with clinical activity.[9] Indeed, in 80% of the SLE population, its first peak in serum presages the onset of clinical manifestations within five years.[10] Autoantibodies are also present in healthy people, where they have a nonpathological role.[11] The main difference between pathogenic SLE autoantibodies and those of healthy people is the high affinity of the pathogenic forms, which is due to the strong stimulation of B cells by $CD4^+$ lymphocytes that induces an antibody switch from IgM to IgG and a change in the molecular sequence of the secreted antibody.[2] Cytokines are also involved in SLE. Indeed, patients who suffer from lupus are characterized by the "interferon signature", due to overexpression of Type I interferons. Several cytokines and cytokine regulators are altered in SLE for genetic or epigenetic reasons. For example, a polymorphism of interferon regulatory factor 5 (IRF5) is an important risk factor for SLE development and a decrease of interleukin 2 production has been reported in T cells from SLE patients.[12,13] In contrast, increased serum interleukin 10 levels are associated with SLE activity.[14]

Currently, experts do not fully understand the aetiology of SLE. A combination of genetic risk and hormones is necessary but not sufficient to explain its development. The most widely accepted model of the disease highlights the importance of environmental events or factors in the onset of the pathology when the genetic context is predisposing. Genetic susceptibility is an important source of risk for developing SLE, as family aggregation and concordant twin research shows. One such study reported a concordance rate for SLE of over 25% in monozygotic twins compared with 2% in dizygotic siblings.[15] SLE is a multifactorial disease with complex genetics. Linkage and association studies have identified multiple loci that confer risk for lupus development[2,16,17] (Table 3). Some genes included in the disease susceptibility regions code for important immune system proteins, especially those of the cytokine signal transduction pathways, apoptosis and complement systems. Alterations of these genes can lead to a loss of tolerance and increased apoptosis of lymphocytes and monocytes. Hormonal and sexual genetic factors are also implicated in SLE aetiology, since ~85% of lupus patients are women, most of them of childbearing age. However there is also a greater prevalence of SLE in men with Klinefelter's syndrome suggesting that having 2 X chromosomes is also important for disease development.[18] Oral contraception increases the risk of SLE development and the number and severity of flares.[19] Conversely, menopause induces the opposite effects.[20] Hormonal analysis of women affected by SLE indicates an increase in prolactin levels and estradiol hydroxylation and a decrease in androgen levels in some patients.[21-24] The role of these hormones has been confirmed in studies using SLE mouse models, where prolactin and estrogens exacerbate the symptomatology in contrast to a suppressive effect of androgens.[25-27] Chimerism, the presence of cells from one individual in another person, is another potential aetiological factor in autoimmune disorders. Chimerism has been detected in a high percentage of women with SLE. Moreover, injection of chimeric cells into healthy mice induces a lupus-like disorder, indicating a potential role for this process in SLE aetiology.[28] Other evidence also suggests that viruses, such as Epstein-Barr virus

Table 3. Candidate risk loci in SLE development[2,16,17]

Name	Location	Function
ATG5	6q21	Apoptosis. Ubiquitination
BANK1	4q22-q24	B cell-specific scaffold. Immune adaptive system regulation.
BLK/ FAM167A	8p23-p22	Kinase. Immune adaptive system regulation/Unknown function.
C1q	1p36	Complement system member. Immune innate system regulation.
C2	6p11-21	Complement system member. Immune innate system regulation.
C4A	6p21.3	Complement system member. Immune innate system regulation.
C4B	6p11-21	Complement system member. Immune innate system regulation.
Chrom 8p21.1	8p21.1	Unknown function.
Chrom 5q33.3	5q33.3	Unknown function.
Chrom 1q25.1	1q25.1	Unknown function.
CRP	1q21-23	C-reactive protein. Clearing apoptotic debris. Immune innate system regulation.
FCGR2A	1q23	Receptor. Immune innate system regulation.
FCGR2B	1q22	Receptor. Immune innate system regulation.
FCGR3A	1q23	Receptor. Immune innate system regulation.
FCGR3B	1q23	Receptor. Immune innate system regulation.
HLA	6p11-21	Human leukocyte antigen. Immune adaptive system regulation.
ICA1	7p22	Unknown function.
IRAK1	Xq28	Kinase. IL1R pathway.
IRF5	7q32	TF. Interferon pathway. Apoptosis. Immune adaptive system regulation.
ITGAM	16p11.2	Adherence and phagocytosis. Immune innate system regulation
IKZF1	7p13-p11.1	TF. Lymphoid differentiation.
LYN	8q13	Kinase. Innate and adaptive immune system regulation.
MBL2	10q11-21	Mannose-binding lectin. Complement. Immune innate system regulation.
MECP2	Xq28	Methyl CpG binding protein
NMNAT2	1q25	Nicotinamide mononucleotide adenyltransferase
PARP	1q41-42	Apoptosis regulation.
PDCD1	2q37.3	B- and T-cell differentiation and apoptosis. Adaptive immune system regulation
PHRF1	11p15.5	Transcription.
PTPN22	1p13	Phosphatase. TCR pathway. Adaptive immune system regulation.
PXK	3p14.3	Kinase. Inflammatory response.
SCUBE1	22q13	Inflammatory response.

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Table 3. Continued

Name	Location	Function
STAT4	2q32	TF. Cytokine response (IL12). $CD4^+$ differentiation.
TLR5	1q41-42	Antigen receptor. Innate immune system regulation
TNFAIP3	6q23	TNF pathway. Apoptosis. Inflammation. Innate immune system regulation.
TNFSF4	1q25	Cytokine. T-cell-APC interaction. Adaptive immune system regulation.
TREX1	3q21	Exonuclease. Repair system. Granzyme A-mediated apoptosis
TYK2	19p13.2	Kinase. Cytokines and interferon pathway.
UBE2L3	22q11.21	Ubiquitination
XKR6	8p23.1	Unknown function
ZNF432	16q12	Transcription factor. Adaptive immune system regulation

Chrom (Chromosome), TCR (T-cell receptor), TF (transcriptional factor).

(EBV), or viral retro-elements, including human endogenous retrovirus (HERVs), are possible factors in SLE development through molecular mimicry or mutational mechanisms. In the particular case of EBV, increases in the percentage of infected B cells, in the viral load and in the viral gene expression have been described in SLE patients in comparison with healthy people. Such differences also occur between quiescent and active SLE in people.[29,30] In mice, immunization with the EBV nuclear antigen 1 (EBNA-1) induces production of Smith (Sm)-antibodies and anti-double-stranded DNA-antibodies.[31] The molecular mimicry of EBV may also be important because the Sm autoantigen is similar to EBNA-1 protein and both can induce lupus-like autoantibodies following direct immunization. Moreover, anti-Ro antibody crossreacts with EBNA-1 antigen.[32,33] Likewise, retrotransposable elements, mainly human endogenous retrovirus (HERVs), are implicated in SLE aetiology.[34] Molecular mimicry between retroviral proteins and autoantigens has been identified in SLE patients. For example the C-HERVs p30 *Gag* protein shares a homologous region and therefore crossreacts with human U1snRNP protein. Moreover, these repetitive sequences have the ability to deregulate immune genes in cis or trans, provoking loss of autotolerance. The MRL/lpr mouse model is a clear example in which the integration of a transposable element in the Fas gene alters the apoptosis process and induces SLE development by producing a nonfunctional Fas protein.[35] Finally, the environment plays a key role in SLE development. More than 100 drugs have been reported to cause a lupus-like disease and this disorder disappears after withdrawl of the compound. The drugs most commonly causing a lupus-like disease are hydalazine, quinidine, procainamide, phenytoin, isoniazid and d-penicillamine.[1] Exposure to sunlight, silica, mercury or pesticides are other common factors that unleash SLE development.[36] The majority of these drugs or exposures induce, directly or indirectly, changes in DNA methylation and histone modifications, highlighting the importance of epigenetics in SLE.

There is no permanent cure for SLE yet. Current treatments ameliorate symptoms by reducing inflammation and autoimmune activity. The use of anti-inflammatory drugs, such as nonsteroidal anti-inflammatory agents or corticosteroids, in combination with immunosuppressive medications such as mycophenolate mofetil or cyclophophamide, remains the most common treatment for SLE. In recent years, new strategies based on

antibodies to immune cells, immunoadsorption and plasmapheresis, among others, have permitted some improvements in the treatment, although great efforts are still needed to find a cure for SLE. As discussed below, epigenetic therapy is an area that promises to help fulfil that objective.[1]

THE ROLE OF EPIGENETICS IN SLE

Epigenetics is one of the most rapidly expanding fields in biomedicine. Since Waddington defined epigenetics in 1939, great steps have been made in cell biology and disease pathogenesis, although much of the terrain remains unexplored.[37] Epigenetics, the study of reversible and potentially heritable changes in gene expression that do not depend on changes in DNA sequence, includes marks such as DNA methylation and histone modifications that define the cell transcriptome and ultimately the cell phenotype. Epigenetic regulation is essential for the normal development and function of the immune system and disruption of this regulatory mechanism can destroy the fine balance between a correctly functioning defence system and autoimmunity.[38]

Autoimmune diseases such as SLE arise when the immune system recognizes self-components of the body as damaged materials and reacts against them. Several lines of evidence indicate that environmental factors, including diet and lifestyle, can modulate the onset of SLE in a genetically predisposed person in part through epigenetic changes. For example, several drugs and ultraviolet light trigger a lupus-like disease in genetically predisposed people and twin studies reveal incomplete concordance (25-57%) between monozygotic siblings and a lower percentage among dizygotic ones (2-9%) indicating a requirement for exogenous triggers from the environment.[39,40]

In the following sections we review the most relevant publications in the field to give an overview of the implications of epigenetics in SLE pathogenesis and to summarize objectives for the near future.

CHANGES IN DNA METHYLATION OCCUR IN SLE

DNA Methylation: A Fundamental Epigenetic Mechanism

DNA methylation is the most extensively studied of the epigenetic mechanisms. In non-embryonic mammals, DNA methylation consists of the postsynthetic addition of a methyl group to the fifth carbon of the cytosine (C) pyrimidine ring located within a CpG dinucleotide.[41,42] CpG dinucleotides are statistically underrepresented in the genome due to spontaneous deamination of methylcytosines (mCs) to form thymidine during evolution.[43] In contrast, CpGs cluster in regions known as CpG islands that frequently coincide with gene regulatory sequences. With the exception of imprinted genes, X-chromosome genes and certain tissue-specific genes, CpG islands of healthy genomes are generally unmethylated and consequently permit transcription of the affected gene.[44] However, the majority of CpGs are located within intronic and intergenic DNA regions, particularly within the repetitive sequences and in normal cells these CpGs are methylated, thereby ensuring genomic stability and parasitic sequence silencing.[45,46] DNA methylation profiles are established during development by the de novo DNA methyltransferases (DNMTs) 3A and 3B and are maintained during mitosis by the

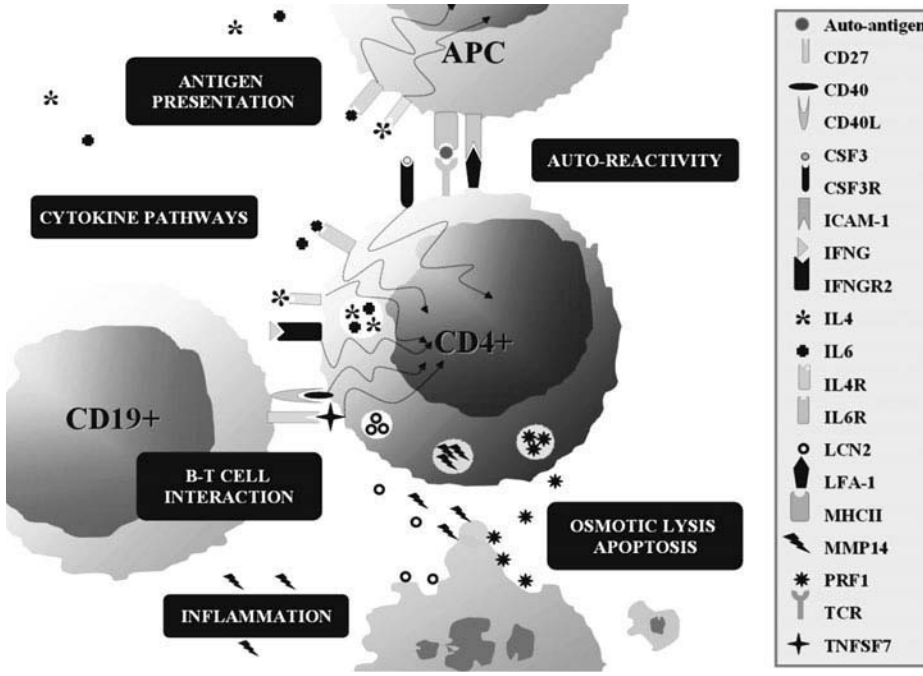

Figure 1. Altered immunological processes in SLE due to gene promoter demethylation. SLE is characterized by DNA methylation decrease in several gene promoters and this epigenetic deregulation induces autoreactivity (LFA-1), osmotic lysis and apoptosis (PRF1, MMP14 and LCN2), impaired antigen presentation (CSF3R) and inflammation (MMP14) as well as deregulated B-T-cell interaction (CD70 and CD40LG) and cytokine signalling (CSF3R, CD70, CD40LG IL-4, IL-6 and I FNGR2).

maintenance methyltransferase DNMT1.[47] DNA demethylation is also an epigenetic mechanism although its importance remains controversial. Unlike passive demethylation, the mechanism for active demethylation is still unclear, although it may be catalyzed by an enzymatic complex made up of a deaminase (AID), a glycosylase (MBD4) and gadd45α.[48]

Impaired DNA Methylation in SLE

The importance of DNA methylation in autoimmunity and especially in SLE, was established in the 1990s and has since been consolidated by many other observations. The first evidence of the involvement of DNA methylation in autoimmunity was the induction of self-reactivity in $CD4^+$ T cells by 5-azacytidine (5-aza C). Human or mouse $CD4^+$ T cells treated with 5-aza C or other DNA methylation inhibitors can be activated by autologous macrophages alone, responding to self-major histocompatibility complex (MHC) II molecules without the usual requirement for specific antigen.[49-53] Moreover, the adoptive transfer of $CD4^+$ T cells treated with 5-aza C, procainamide or hydralazine into syngeneic mice induces a lupus-like disease. Notably, several medications, such as procainamide, hydralazine and 5-aza C and ultraviolet light, all inhibit DNA methylation, induce or aggravate SLE and trigger $CD4^+$ T-cell autoreactivity in mice and humans.[52,54] Interestingly, mutations in the epigenetic machinery can cause other immune problems

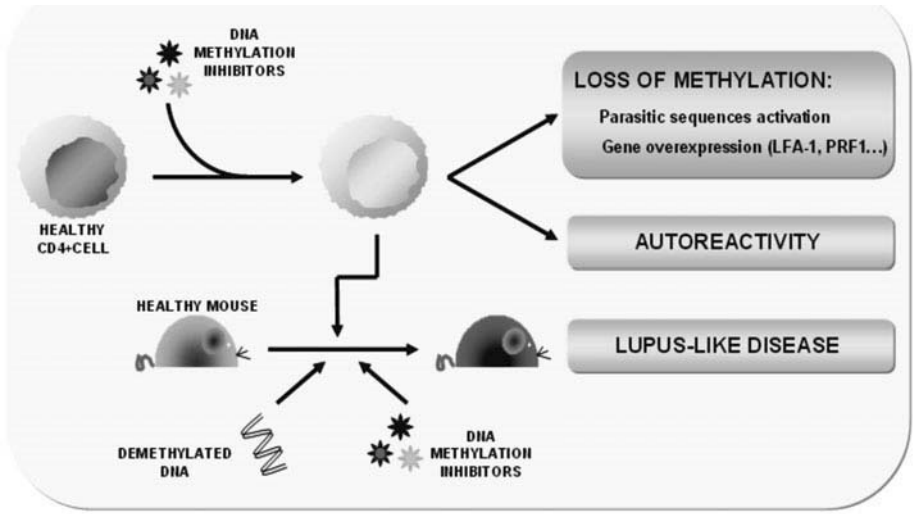

Figure 2. Relationship between DNA methylation and healthy biological systems. Treatment of human or mouse healthy $CD4^+$ T cells with DNA methylation inhibitors induces loss of global methylation, activation of parasitic sequences and gene overexpression. All of these epigenetic changes provoke $CD4^+$ T-cell autoreactivity and consequent self-tolerance break. Adoptive transference of these treated cells into healthy mice as well as administration of DNA methylation inhibitors or injection of hypomethylated DNA in this animal induces lupus-like disease.

as well. One example is the ICF (immunodeficiency, centromeric instability and facial abnormalities) syndrome, a disorder produced by a DNMT3B mutation and characterized by B cell immunodeficiency. Another is the mouse strain lacking a functional Gadd45α gene which develops lupus-like disease.[55] Interestingly, SLE is characterized by an increased apoptotic rate of peripheral monocytes, macrophages and lymphocytes coupled with impaired clearance of the resultant cellular debris, which provides a major source of autoantigens.[56,57] In addition, hypomethylated DNA, such as apoptotic or microbial DNA, is more antigenic than normal or necrotic DNA, which are characterized by a higher degree of methylation.[58,59] Thus, BALB/c mice immunized with apoptotic DNA develop a lupus-like disease, unlike mice immunized with necrotic or normal DNA. In addition, demethylation of necrotic or normal genetic material results in the induction of the pathogenic state.[60] These results suggest that circulating apoptotic DNA may mimic microbial DNA, potentially inducing autoimmunity.[61,62] Finally, a significant positive correlation between T-cell DNA hypomethylation, aging and increased probability of SLE development has been established.[63,64] Taking all these findings together, the importance of DNA methylation alterations in SLE pathogenesis is beyond doubt (Fig. 2).

A Global Decrease of DNA Methylation Characterizes SLE Individuals

The relationship between DNA hypomethylation and SLE pathogenesis was first proposed in the 1980s, but its direct involvement was not demonstrated until 1990 when Richardson and colleagues demonstrated impaired DNA methylation in SLE T cells.[65] These findings have been corroborated by multiple studies over recent years

and similar results have been obtained from the analysis of subacute cutaneous lupus erythematosus (SCLE) patients.[66-68] These reports have also characterized the specific cell populations that undergo epigenetic alterations. In particular, a significant decrease in DNA methylation has been identified in $CD4^+$ T cells but no differences have been detected in other peripheral blood populations.[68] Moreover, the methylation level of peripheral blood T cells from patients with active SLE is lower than that from patients with the inactive disease, emphasizing a direct relationship between lupus symptoms and DNA methylation.[65] The loss of global methylation can induce activation of endogenous retroviruses and dormant transposons, erase imprinting signals and deregulate gene expression, breaking immune-tolerance as the final consequence.[69] Regarding parasitic DNA activation, a controversial role for HERV in SLE aetiology due to molecular mimicry has been proposed. Indeed, the levels of transcription and translation of HERV clone 4-1 as well as of the production of antibodies against this retrovirus are significantly higher in SLE patients than in normal individuals.[70-72] In addition, the peptide p15E derived from the HERV clone 4-1 is able to induce the same immune abnormalities associated with SLE.[73] As mentioned above, DNA methylation is maintained by DNMT1, an enzyme regulated by the ras-MAPK pathway.[74] Similar to DNA methylation, $CD4^+$ T cells of SLE patients also have lower DNMT1 activity levels and the decrease is associated with disease activity.[67,74,75] Recent studies have identified impaired protein kinase C (PKC) delta phosphorylation as being responsible for the ras-MAPK pathway alteration and subsequent decrease in DNMT1.[76] According to other reports, treating $CD4^+$ T cells with hydralazine, which inhibits ERK pathway signalling by preventing PKC delta phosphorylation, also induces autoreactivity in vitro and lupus-like disease in vivo.[52,54] Similarly, the PKC delta knockout mouse model develops SLE[77](Fig. 3). Expression analysis of other epigenetic effector molecules, such as the methyl-CpG binding domain proteins (MBDs), have been performed in SLE patients although no compelling evidence has emerged due to the contradictory results.[66,68,78] Interestingly, several animal models have been used to study SLE because of the many clinical features they share with human lupus, and impaired DNA methylation has been reported in some of these. One example is the MRL/lpr mouse, in which insertion of an endogenous retrovirus into the Fas gene causes defective elimination of self-reactive T cells due to impaired apoptosis and lupus-like autoimmunity.[79] T cells in the lymphatic nodules and thymus of the MRL/lpr mouse are globally hypomethylated compared to the MRL+/+strain.[80] Moreover, changes in DNA methylation levels have been detected in different lymphatic tissues with aging in this mouse strain, correlating with SLE progression. Specifically, significant differences have not been detected in peripheral blood in contrast to the methylation loss detected in axillary lymph nodes and thymus and an increase in the spleen.[80] As in humans, DNMT1 expression is significantly lower in $CD4^+$ T cells from 16-week-old MRL/lpr mice with active disease compared to younger mice in which autoimmunity has not yet been detected.[81] In contrast to SLE patients and other animal models, administration of 5-aza C to MRL/lpr mice has a protective effect, prolonging survival and reducing the splenomegaly, lymphadenopathy and autoantibody titers, although this may be due to DNA synthesis inhibition by 5-aza C, similar to other drugs used to treat human lupus, such as azathioprine or mycophenylate mofetil[82,83] (Fig. 4).

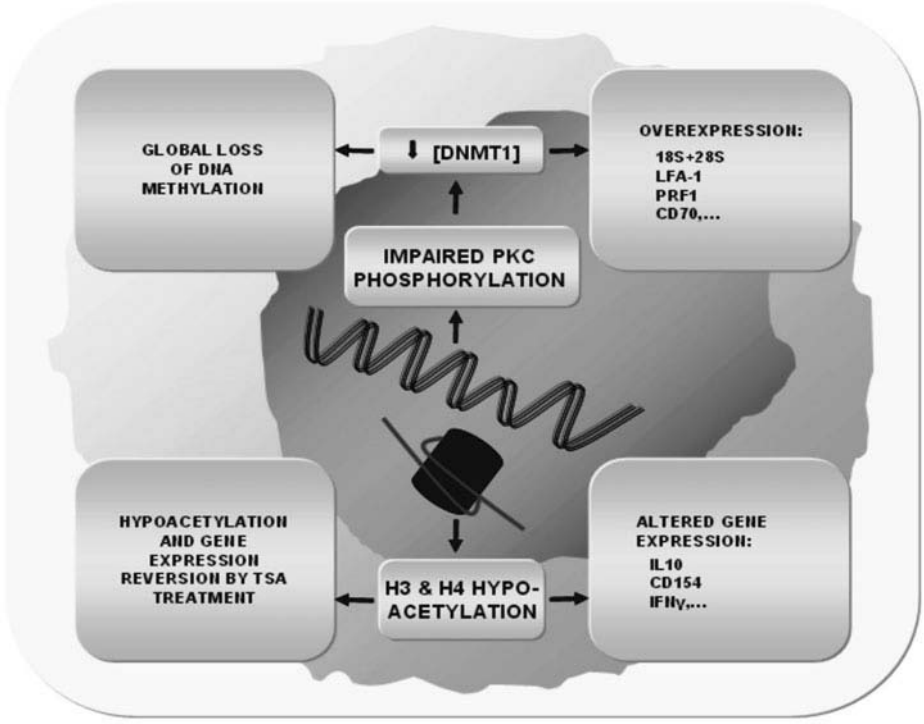

Figure 3. Epigenetic alterations of $CD4^+$ T cells of SLE patients. Human SLE $CD4^+$ T cells are characterized by global DNA hypomethylation and histone 3 and 4 loss of acetylation. Decreased DNA methylation results from the low level of expression of DNMT1 due to altered PKC phosphorylation and provokes gene and repetitive sequence overexpression. Global hypoacetylation induces skewed gene transcription and this altered expression profile can be reverted by HDAC inhibitors such as TSA DNMT1 (DNA methyltransferase 1), PKC (protein kinase C), H (histone), TSA (trichostatin A).

What Are the Specific DNA Sequences Undergoing Methylation Changes in SLE

As noted above, DNA methylation is an epigenetic modification that regulates gene expression and provides genomic stability in close collaboration with histone modifications. Basically, gene promoter methylation inhibits gene expression whereas the methylation of repetitive sequences allows silencing of parasitic elements and represses chromosomal recombination.[84] As promoter regions occupy a negligible genomic area compared with repetitive sequences, global methylation changes are mainly caused by the alteration of repetitive region methylation profiles. With respect to the repetitive sequences, recent data have identified the ribosomal RNA gene cluster as a region that is susceptible to the development of hypomethylation in SLE. The ribosomal gene is a repetitive sequence of about two megabases located in the short arms of acrocentric chromosomes.[85] The 18S and 28S regions undergo a loss of methylation and are overexpressed in SLE patients relative to healthy siblings.[67] These alterations can induce an increase in ribosomal particles that may provoke the synthesis of autoantibodies against them. Other repetitive sequences, such as D4Z4,

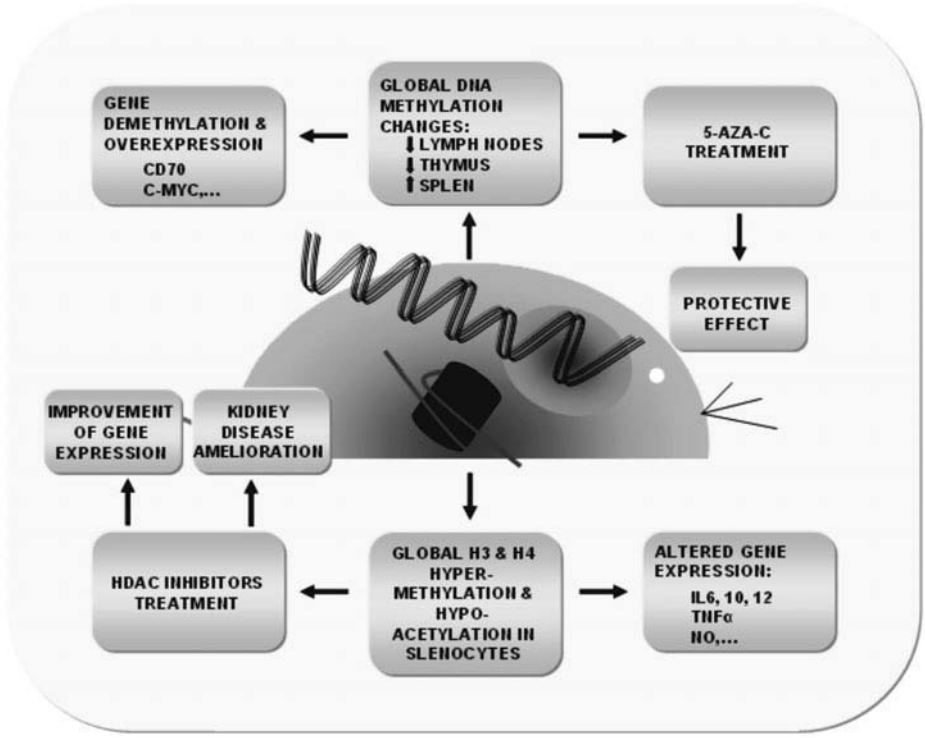

Figure 4. Epigenetic alterations of MRL/lpr SLE mouse model. The MRL/lpr model is characterized by global changes in DNA methylation and histone modifications compared with its mouse control. Lymph nodes and thymus DNA have low levels of methylation compared with spleen genomic material, which experiences methylation gain. These alterations correlate with altered gene expression. Unlike in humans, administration of DNMT inhibitors, such as 5-aza cytidine, has a protective effect. MRL/lpr splenocytes are characterized by global histone 3 and 4 hypermethylation and hypoacetylation and consequent altered gene expression. Administration of HDAC inhibitors improves kidney disease and gene expression profiles. 5-aza-C (5-aza cytidine), HDAC (histone deacetylases), H (histone).

NBL2, Alu, Satellite 2 or LINE-1, have been analysed, but no significant differences associated with SLE have been detected[67] (Fig. 3).

Even though repetitive sequence hypomethylation is the main factor responsible for the decrease in global DNA methylation, changes in gene promoters can also occur. Indeed, there is strong evidence of gene deregulation due to impaired DNA methylation; and T cells are particularly susceptible to gene promoter demethylation in lupus. One example of a gene characterized by loss of promoter methylation in SLE is CD11a. This gene (also called ITGAL) encodes one of the two proteins that comprise lymphocyte function-associated antigen-1 (LFA-1), an adhesion molecule of the integrin family involved in T-cell activation and signalling.[86,87] LFA-1 helps immunological synapse formation, conferring stability to the MHCII-TCR complex and costimulating T cells. Increases in the LFA-1 protein, such as occurs with CD11a overexpression, can break tolerance, possibly by overstablizing the lower affinity interaction of the T-cell

antigen/MHC receptor with MHC molecules alone.[50] An upstream region of the ITGAL promoter enriched in Alu repeats is characterized by a loss of methylation that induces gene overexpression in $CD4^+$ and $CD8^+$ T cells of SLE patients.[51,88] Indeed, a direct correlation between disease activity and degree of CD11a promoter demethylation has been reported. Treatment with DNA demethylating drugs such as 5-aza C, procainamide or hydralazine induces a similar loss of methylation and stronger gene expression as well as conferring autoreactivity in vitro and lupus-like disease in vivo.[51] Moreover, the stable transfection of healthy T cells with an ITGAL expression construct induces the same effects.[53,62,88] Interestingly, this gene is damaged in a wide range of leukaemias and lymphomas.[89,90] Another example is the perforin (PRF1) gene,[91] a sequence that encodes a protein that integrates into target cell membranes, where it forms lethal pores.[92,93] PRF1 overexpression is positively correlated with disease activity in $CD4^+$ T cells of SLE and SCLE patients as result of promoter hypomethylation.[91,94] The stronger expression may partly be responsible for the promiscuous T-cell-mediated killing of monocytes and macrophages that typifies SLE pathogenesis.[91] Similarly, $CD4^+$ cells of healthy people become autoreactive killers of autologous monocytes after treatment with DNA methylation inhibitors and this property can be inhibited by adding the perforin inhibitor concanamycin A to the cells.[91] In addition to PRF1 and CD11a, CD70 and CD40LG are also methylation-sensitive genes in SLE. CD70, also known as TNFSF7 or CD27L, is a member of the tumour necrosis factor (TNF) family. This protein is a B cell costimulatory molecule mainly synthesized by activated B and T cells.[93] The CD70 promoter is hypomethylated in $CD4^+$ T cells of SLE and SCLE patients and this loss of methyl groups causes an increase in transcription.[95-97] CD70 transfection of healthy $CD4^+$ T cells or treating $CD4^+$ cells with DNA methylation inhibitors also causes an increase in CD70 transcription and translation levels. Moreover, coculturing the treated or transfected cells with autologous B cells induces IgG overproduction, while the addition of antibodies against CD70 abrogates this increased production.[95] Similarly to CD70, CD40LG (also termed TNFSF5 or CD154) is also a B cell costimulatory molecule. Interestingly, it is encoded on the X-chromosome. For that reason, one copy in women is uniquely methylated and consequently silenced, while men have just one unmethylated copy. Indeed, the CD40LG regulatory region is hypomethylated in $CD4^+$ T cells of women with active SLE, promoting overexpression of the molecule.[98,99] This could explain the striking propensity for females to develop SLE. Several interleukins, such as IL-4 and IL-6, can also be overexpressed due to DNA demethylation, similarly to the previous examples.[100] Although T cells are the best studied and most frequently altered cell type in SLE, other genes may be susceptible to impaired DNA methylation in other cell types. For example, one study of white blood cells identified 49 genes that were differently methylated in five pairs of monozygotic twins discordant for SLE. Extending the study to a larger and more diverse population, the loss of methylation of eight gene promoters (IFNGR2, MMP14, LCN2, CSF3R, PECAM1, CD9, AIM2 AND PDX1) was confirmed and the first five of these genes were shown to have significantly reduced expression[67] (Figs. 1 and 3).

Mouse models are also used to study SLE pathogenesis and thereby detect genes that are deregulated by epigenetic mechanisms. For example, MRL/lpr mice share the impaired CD70 methylation profile with SLE patients, confirming an important role for this gene in lupus pathogenesis.[81] Conversely, the proto-oncogene c-myc is exclusively overexpressed due to gene promoter demethylation in this mouse model, unlike in humans[101,102](Fig. 4).

In conclusion, important genes are characterized by impaired DNA methylation in SLE, which gives rise to increased autoreactivity (ITGAL), osmotic lysis and apoptosis (PRF1, MMP14, LCN2) and inflammation (MMP14), as well as deregulating antigen presentation (CSF3R), B- T-cell interaction (CD70, CD40LG) and cytokine signalling (CSF3R, IL-4, IL-6, IFNGR2)(Fig. 1).

CHANGES IN HISTONE MODIFICATIONS ARE ALSO INVOLVED IN SLE PATHOGENESIS

The Epigenetic Contribution of Histone Modifications

Histones are nuclear proteins that associate with DNA to form nucleosomes, enabling it to be packaged into the nucleus and regulating its expression. Histones have tails that protrude from the nucleosome and can be modified by the covalent addition of chemical moieties such as methyl, phosphate or acetyl groups, among others.[103,104] The combination of these added modifications in different amino acids of the histone tails in part determines the affinity between DNA and histones and also generates or eliminates protein-binding sites, regulating accessibility to different regulatory proteins.[105,106]

Histone Modification Patterns Are Also Altered in SLE

As mentioned above, an increased apoptotic rate coupled with impaired clearance of apoptotic debris characterizes SLE.[56] During apoptosis, chromatin is cleaved by caspases, endonucleases and granzyme B as well as undergoing the addition or elimination of histone modifications. All of these changes can create new epitopes potentially recognized by the immune system, aggravating or inducing SLE development.[107] There is compelling evidence for specific binding of SLE autoantibodies to apoptotically modified antigens. For example, the lupus mouse-derived monoclonal antibody KM-2 mainly recognizes acetylated lysine 8, 12 and 16 in histone H4. Four hours after inducing apoptosis, the affected cells exhibit increased histone acetyl transferase (HAT) expression and reduced levels of histone deacetylases (HDAC), allowing H4 acetylation.[108] Moreover, the apoptosis-induced acetylation on lysine 12 of H2B is a target for predisease lupus mouse antibodies.[109]

Changes in histone modifications occur not only during apoptosis but in other situations as well. Histone alterations are also detected in SLE cells and correlate with aberrant gene expression patterns that may be associated with lupus pathogenesis. The first evidence for impaired histone modifications in lupus emerged from the use of epigenetic drugs in SLE mouse models. In vivo administration of HDAC inhibitors, such as trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA) and others, ameliorates kidney disease in MRL/lpr mice without changing autoantibody titers.[110,111] Administration of HDAC inhibitors to MRL/lpr splenocytes reduces the expression of various cytokines, including IL-6, IL-10, IL-12, or TNF-α.[110,111] Indeed, a mouse model characterized by an HDAC p300 mutation exclusively in B cells develops lupus-like disease.[112] A recent study has also analyzed H3 and H4 methylation and acetylation levels in splenocytes of MRL/lpr mice. Global hypoacetylation and hypermethylation (excluding H3K4 methylation) relative to the control MRL/MPJ mouse model has been reported and novel

histone modifications characterize the SLE model. Administration of TSA reverts the impaired histone modifications and improves the disease course in this model[113](Fig. 4).

Histone modifications have also been studied in people with SLE. Similar to MRL/lpr splenocytes, $CD4^+$ T cells of SLE patients with active disease are characterized by global H3 and H4 hypoacetylation and disease activity correlates inversely with H3 acetylation.[114] This T cell subset overexpresses IL-10 and CD154 and underproduces IFN-γ. Further, treating SLE $CD4^+$ cells with TSA reverses the skewed gene expression.[115] Monocytes also develop changes in histone modifications in SLE. Using ChIP on chip analysis, 179 genes have been characterized as likely to undergo H4 hyperacetylation in monocytes of SLE patients. The acetylation-enriched genes mainly affect macrophage activation, cell proliferation, central nervous system toxicity and antiviral immunity and they also have potential IRF1 binding sites within the 5 Kb upstream region. Although many genes are hyperacetylated, only twelve of them are known to exhibit expression changes. Moreover, treating these macrophages with IFNα increased expression of the 179 selected genes and induced acetylation of histones located in 199 promoter genes.[116] These results not only confirm the role of altered histone modifications in SLE but also suggest the possibility of using them in epigenetic treatments (Fig. 3).

POTENTIAL USE OF EPIGENETIC DRUGS FOR SLE TREATMENT

Progress in the field of epigenetics has been faster than in any other discipline, reflecting its extensive involvement in different diseases. Compelling evidence demonstrating a role for epigenetic dysregulation in SLE emerged several years ago, is now widely accepted and holds promise for therapeutic applications. One of the most important aspects of epigenetic regulation is the possibility of reversion through the use of drugs that inhibit the epigenetic machinery. In fact, some of these compounds are already being used in preclinical and clinical phases for the treatment of haematological malignancies following their approval by the US Food and Drug Administration.[117] The effects of DNMTs and histone modification enzyme inhibition summarised above support the potential for using these inhibitors for disease amelioration. Indeed, studies based on treatments with HDAC inhibitors highlight the ability of these drugs to reverse the skewed gene expression associated with lupus and to modulate immune system activity and reduce inflammation.[118,119] Before designing a therapeutic approach, an in-depth understanding is required of the epigenetic alterations of each cell type associated with the disease. To this end, we need to develop and use SLE animal models as well as create cell lines in which to test the agents. Problems associated with human studies can be resolved using in vitro and animal models, although one must always bear in mind their limitations and corroborate the results in SLE patients. In addition, a more exhaustive study of the relationship between cancer and autoimmunity will help us extrapolate our extensive knowledge of epigenetic deregulation in cancer to SLE.[120] Further, a new gene expression regulator, known as microRNA (miRNA), is attracting attention because of its involvement in many disorders.[121,122] miRNAs are noncoding RNA molecules, around 22 nucleotides long, that regulate the expression of target genes through various posttranscriptional mechanisms.[123] The current finding that a set of miRNAs are differentially expressed in lupus patients and normal controls together with a recent description of their epigenetic regulation (including DNA methylation-dependent regulation of miRNA expression) suggests a potential role for epigenetic dysregulation of miRNA in SLE.[124-126] For this

reason, miRNAs are potential players in SLE pathogenesis as well as potential therapeutic targets and diagnosis biomarkers.[121]

CONCLUSION

In conclusion, a deeper and more specific understanding of the epigenetic alterations at the level of DNA methylation and histone modifications of gene promoters, repetitive sequences and miRNAs that occur in lupus and the exhaustive study of the epigenetic connections between cancer and autoimmunity are the new epigenetic aims in the fight against SLE in the immediate future.

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