HDAC Inhibition in Lupus Models

Christopher M Reilly,[1,3] Nicole Regna,[1] and Nilamadhab Mishra[2]

[1]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, United States of America; [2]Wake Forest University Baptist Medical Center, Winston-Salem, North Carolina, United States of America; and [3]Virginia College of Osteopathic Medicine, Blacksburg, Virginia, United States of America

Systemic lupus erythematosus (SLE) is a prototypic autoimmune inflammatory disease characterized by the production of autoantibodies directed against nuclear antigens such as nucleosomes, DNA and histone proteins found within the body's cells and plasma. Autoantibodies may induce disease by forming immune complexes that lodge in target organs or by crossreacting with targeted antigens and damaging tissue. In addition to autoantibody production, apoptotic defects and impaired removal of apoptotic cells contribute to an overload of autoantigens that initiate an autoimmune response. Besides the well-recognized genetic susceptibility to SLE, environmental and epigenetic factors play a crucial role in disease pathogenesis as evidenced by monozygotic twins typically being discordant for disease. Changes in DNA methylation and histone acetylation alter gene expression and are thought to contribute to the epigenetic deregulation in disease. In SLE, global and gene-specific DNA methylation changes have been demonstrated to occur. Additionally, aberrant histone acetylation is evident in individuals with SLE. Moreover, histone deacetylase inhibitors (HDACi) have been shown to reverse the skewed expression of multiple genes involved in SLE. In this review, we discuss the implications of epigenetic alterations in the development and progression of SLE, and how therapeutics designed to alter histone acetylation status may constitute a promising avenue to target disease.

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INTRODUCTION

There are approximately 80 different known autoimmune diseases affecting at least 5% of the population in the Western world (1). SLE is a prototypic autoimmune disease of unknown etiology in which a genetic predisposition coupled with environmental agents trigger disease. SLE is characterized by the production of antibodies against its own cells and various components within the cell. SLE targets many organs, including the joints, skin, kidneys, heart, lungs, blood vessels and brain. People with SLE have many different symptoms; the most common ones include extreme fatigue, painful or swollen joints (arthritis), unexplained fever, skin rashes and kidney dysfunction. The severity of these problems increases with age and disease duration (2–5). In SLE, the major cause of morbidity and mortality is lupus nephritis (LN), which affects over half of all SLE patients. Traditional treatment for lupus has included the use of cyclophosphamide and corticosteroids, both of which improve renal function but have other deleterious side effects (6). Although most patients respond to this treatment, it is estimated that 35% suffer at least one episode of renal relapse and 5% to 20% develop end-stage renal disease (7). Furthermore, treatment with cyclophosphamide and corticosteroids is non-specific and inhibits normal cellular function.

Extensive research has focused on identifying genes and inflammatory mediators involved in disease. Over the last decade, great strides have been made in understanding the complex pathogenesis underlying LN, and our increased understanding of molecular immunology has led the way for the development of new immunomodulatory therapies (8–10). The use of specific immunomodulators, including monoclonal antibody therapy, has shown promising results, although these agents are still somewhat nonspecific and target groups of cells (6,11). To target lupus more effectively, it is imperative that we uncover the underlying mechanisms that trigger disease activity.

EPIGENETICS AND LUPUS

While environmental as well as genetic factors are associated with SLE, there is no specific biomarker to indicate disease pathogenesis, despite decades of extensive work in the understanding of the etiopathogenesis of SLE. Additionally, wide-ranging analysis of both genetics and environmental pathogens has yet to reveal a common mechanism for the initiation of disease. The term "epigenetics" describes heritable alterations of phenotypic traits that are not based on changes in DNA sequence (12). Epigenetics may explain many aspects of the transmission of genetic information ranging from the

Address correspondence and reprint requests to *Christopher M Reilly, 2265 Kraft Drive, Blacksburg, VA 24060. Phone: 540-231-5345; Fax: 540-231-5252; E-mail: chreilly@vcom.vt.edu.*
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silencing of the whole chromosome to specific gene activation, as well as nutritional and environmental effects on gene expression patterns. One important epigenetic regulator of gene expression is the modification of histone proteins. Histones are alkaline proteins that package and order the DNA into structural chromatin units. Core histone proteins are classified as H2A, H2B, H3 and H4. Two of each of the core histones assemble to form one octameric nucleosome core around which the DNA wraps. A linker histone (H1) binds the nucleosome, thus locking the DNA into place. The discovery of histone modification has initiated a flurry of investigations into how histone proteins may regulate gene expression (13).

Substantial evidence exists that histone proteins may play a critical role in the regulation of a wide variety of normal cellular functions and pathologies (14). For genes to be transcribed, DNA must uncoil from around the histone proteins. This is accomplished with the assistance of histone acetylases (HAT), which acetylate the lysine residues in core histones leading to transcriptionally active chromatin. Conversely, HDAC removes the acetyl groups from the lysine residues, condensing the chromatin and silencing gene expression. HDAC inhibitors (HDACi) block this action and can result in hyperacetylation of histones, thereby affecting gene expression. Owing to their conserved catalytic domain, HDACs have been actively targeted as a therapeutic target. The reversible nature of these epigenetic maintainer enzymes provides a plausible treatment for skewing the differentiation of a cell type to a different phenotype and may provide viable treatment options to ameliorate disease (15,16). Indeed, the vast majorities of studies with pan HDACi in cell culture systems and *in vivo* disease models of cancers have resulted in FDA approval of these classes of drugs for the treatment of cancer (17). The present review will discuss the current knowledge of histone proteins' modification for the treatment of SLE as well as the molecular mechanisms by which HDACi may modulate disease.

HDAC INHIBITORS

In the human genome, there have been approximately 2,000 genes that have been identified as transcription factors. To date, only 18 genes have been identified as HDAC and these are subdivided into four classes based on structural and functional similarities (18). Class I HDACs (HDAC1, -2, -3, -6, and -8) are expressed in a wide range of tissues and found exclusively in the nucleus (19,20,21). In contrast, class II HDACs (HDAC4, -5, -7, and -9) have a more limited cellular expression, including immune cells, and are found both in the nucleus and the cytoplasm (21–25). Class III HDACs, known as sirtuins or SIRTs (silent mating type information regulation 2 homolog), are thought to play important roles in the regulation of metabolism and apoptosis via deacetylation of cytosolic and mitochondrial enzymes. Sirt1-Sirt7 are homologues of yeast Sir2 and form a structurally distinct class of nicotinamide adenine dinucleotide (NAD)-dependent enzymes and have been reviewed previously (26,27). HDAC1, -2 and -3 are ubiquitously expressed in the various immune tissues (28) and their expression in human peripheral blood mononuclear cells (PBMCs) is increased by polyclonal activators (phorbol ester and α-CD3 antibodies) and HDACi, but not by lipopolysaccharide (29). Currently, several HDACi, including hydroxylbutyrate, suberoylanilide hydroxamic acid (SAHA), Trochostain A (TSA), MS275, PXD 101, FR901228, ITF2357 and others designed to inhibit specific classes of HDAC proteins, are in various stages of development (30–32).

GENETICS, EPIGENETICS AND LUPUS

In regard to SLE, studies between monozygotic and dizygotic twins have provided insight into the role of genetic components and epigenetics on identifying specific susceptible genes. In SLE, several alleles have been identified as risk factors including major histocompatibility complex region, IFN-signaling molecules, transduction signaling genes, components of the complement cascade and others (33–36). Many of the SLE susceptibility genes also are associated with pathways regulating other autoimmune disorders, including rheumatoid arthritis (3,16,37). Direct comparison of identical twins provides an excellent model for identifying epigenetic factors that may play a role in disease pathogenesis. Studies in monozygotic twins have revealed differences with DNA methylation and histone acetylation, and may explain the existence of genome-wide epigenetic differences that result in altered phenotypes (38–40). Javierre *et al*. recently published data concerning DNA methylation status collected from identical twins discordant for SLE and two other related systemic autoimmune diseases. They found only SLE samples exhibited significant changes in DNA methylation status at both the global and sequence-specific levels in comparison with their healthy discordant twins and compared with unrelated matched normal controls (39). Their studies support the hypothesis for epigenetic alterations playing a critical role in SLE pathogenesis.

DRUG-INDUCED SLE AND HDAC

Nearly 30 years ago, a study by Lintwin *et al*. showed that decreased acetylation status resulted in a higher incidence of autoantibodies (41). Indeed, drug-induced lupus has been reported by patients taking pharmacologic agents such as procanamide (a pharmaceutical antiarrhythmic agent used for the medical treatment of cardiac arrhythmias) if the patients showed decreased acetylation (42,43). Conversely, reports by Portanova *et al*. found that acetylation did not induce a lupus phenotype or alter antibody production (44). In larger prospective studies, Mongey *et al*. (45) reported patients receiving procanamide showed a persistently higher frequency of developing autoantibodies, although the majority of the patients did not develop drug-related lupus. Furthermore their

studies suggested that although acetylation status correlated with IgG antibodies to the H2A-H2B dimer complex, this was not a risk factor for the development of procanamide-related lupus.

LUPUS MODELS FOR HDAC INHIBITION

Studies of human lupus have been aided by the availability of animal models that in many ways resemble the human disease, including autoantibody production and glomerulonephritis. Additionally, congenic strains have allowed the study into the genetics of SLE at both polygenic and at the single-gene levels. There are several different strains of mice that develop various aspects of disease similar to human lupus. Two such strains are the MRL/MPJ-*Fas*^{lpr} (MRL/*lpr*) and the NZB/NZW F1 mouse strain. The common immunological and clinical manifestations of SLE in both of these strains include hyperactive B and T cells, high titers of several autoantibodies directed against nuclear antigens, a defect in the clearance of immune complexes, and immune complex–mediated glomerulonephritis that eventually leads to renal failure and death (46–49). MRL/MPJ mice with a mutation in the *lpr* gene alters the *Fas* receptor and prevents autoreactive T cells from undergoing deletion in the thymus (50). Subsequently these animals develop systemic autoimmunity, massive lymphadenopathy associated with proliferation of aberrant T cells, arthritis and immune complex glomerulonephritis similar to human lupus and die at around 24 weeks from renal disease. The composite genome distribution of autoantibodies produced by these mice are similar in spectrum to those seen in human lupus including anti–double-stranded DNA antibodies and anti–Sm antibodies (51,52). New Zealand Black × New Zealand White (NZB/W) F1 female mice develop autoimmunity characterized by high levels of antinuclear antibodies, hemolytic anemia, proteinuria and progressive immune complex glomerulonephritis and have served as a model for autoimmune disease since the early 1960s (51).

In the MRL/*lpr* murine model of lupus, $CD4^+$ T cells play an essential role in disease pathogenesis in the kidney and facilitate polyclonal B-cell activation, autoantibody production, immune complex formation and direct infiltration by production of inflammatory mediators in the kidney (53–55). The effector T cells including Th1, Th2 helper cells and follicular helper T (Tfh) cells are largely responsible for B-cell activation, class switching and induction of long-lived plasma and memory cells which are thought to play a major role in the pathogenesis of lupus (56,57). Conversely, regulatory T (Treg) cells are key mediators of peripheral tolerance by suppressing T effector cells and are known to be decreased either in number or function in lupus (58–62). Therefore, it may be argued that aberrant differentiation of multipotential naive $CD4^+$ T cells into distinct lineages including Th1, Th2, Tfh and Treg cells are responsible for the pathogenesis of lupus.

T CELLS AND HDAC INHIBITORS

An epigenetic switch occurs when a stable cell type changes to another stable cell type without changes in DNA sequences (63,64). It has been proposed recently that three categories of signals (epigenerator, epigenetic initiator and epigenetic maintainer) are required in the establishment of a stable cell (65). Epigenerator signals generated by self-antigen or environmental factors may induce naïve $CD4^+$ T cells to differentiate into specific effector cell types. These cellular processes depend on modulation of lineage specific master transcription factors (epigenetic initiators) for T effector and Treg cells (i.e. Foxp3 for Tr, for example, BCL6 for Tfh, T bet for Th1 and GATA-3 for Th2) (62). Subsequently, the epigenetic maintainers including chromatin modifying enzymes (that is, histone acetyltransferase, deacetylases, methyltransferase, demethylase, DNA methylation enzymes and microRNAs) establish a chromatin landscape by changing DNA methylation, histone modification, histone variants and nucleosome positioning, resulting in terminally differentiated cell types (65). Among different epigenetic maintainers, histone acetylation is one of the most widespread modifiers of histones and serves as a key regulator of chromatin structure and gene transcription, and thus may play a key factor in the differentiation of $CD4^+$ T cells (66).

Using two HDACi (TSA and SAHA), we have demonstrated that these agents decrease kidney disease in both the MRL/*lpr* and NZB/NZW F1 mice lupus mouse models (67–69). In MRL/*lpr* mice, we demonstrated that TSA and SAHA decreased inflammatory cytokine (IL-12, IFN-γ, IL-6 and IL-10) production in isolated splenocytes while increasing accumulation of acetylated histones H3 and H4 in total cellular chromatin. Although we did not find decreased production of IL-1β in splenocytes in our studies treated with HDACi, Susick *et al*. showed TSA decreased IL-1β-induced metabolic dysfunction in pancreatic cells (70). Inhibition of IL-1β could have therapeutic effects in SLE as deletion of IL-1β in lupus mice has been shown to ameliorate disease (71). We demonstrated that HDACi decreased inflammatory mediator production in renal mesangial cells both *in vitro* and *in vivo* with reduced disease as demonstrated by decreased inflammatory mediator production, proteinuria and glomerulonephritis. The decrease in disease was in part by upregulation of $Foxp3^+CD4^+CD25^+$ (Treg) cells (69). This may be important in the pathogenesis of SLE, as human studies have demonstrated the numbers of Treg cells in SLE patients was related inversely to both the serum level of anti-dsDNA and disease activity (72,73).

Several studies have supported a role for HDAC inhibition in inflammatory diseases (74,75) and have focused on Treg cells as they have been reported to be crucial in the mediation of inflammatory bowel disease (IBD) (24). It was reported that HDAC inhibition *in vivo* increased Foxp3 gene expression, as well as the production and suppressive function of Treg cells. Of particular interest is

the role of HDAC9 on Treg cell function. Studies have suggested that HDAC9 proved particularly important in regulating Foxp3-dependent suppression (24,76). Optimal Treg cell function required acetylation of several lysines in the forkhead domain of Foxp3, and Foxp3 acetylation enhanced binding of Foxp3 to the IL-2 promoter and suppressed endogenous IL-2 production (24). Moreover, recent studies have demonstrated that overexpression of HDAC9 in Treg cells decreased their suppressive capacity (72). We currently are examining the role of HDAC9 in lupus. We found the expression levels of HDAC9 were decreased in MRL/*lpr* mice compared with MRL/MJP mice (unpublished data). This increase in HDAC9 in Treg cells may explain the decrease in suppressive activity of Treg cells in MRL/*lpr* mice consistent with previously published studies (77).

It is established that the effector T-cell lineage shows great plasticity. While Th17 cells play a crucial role in the immunologic response to microbial infection, they also are induced in SLE (78,79). When TSA was added to cultures of human T cells *in vitro*, profound negative effects on the emergence of IL-17-producing cells from Treg cells were observed, implying that Treg cell differentiation into IL-17-producing cells depends on histone/protein deacetylase activity (80). In this regard, the addition of HDACi to lupus mice might skew the T-cell population to that of a Treg-cell population. Both SAHA and TSA inhibited the production of IL-12 and IL-23. Furthermore, the HDACi selectively blocked the production of Th1-attracting chemokines CXCL9, CXCL10 and CXCL11 (80). Therefore, it can be concluded the reduction of Th1- and Th17-inducing cytokines as well as Th1-attracting chemokines may represent relevant mechanisms through which HDACi exert their immunomodulatory effects.

In our investigations to understand further the interplay between various histone modifications including acetylation, methylation and lupus disease, we performed differential expression histone modification analysis in splenocytes from the MRL/*lpr* mouse model of lupus. Using stable isotope labeling in combination with mass spectrometry, we found global site-specific hypermethylation (except H3 K4 methylation) and hypoacetylation in histone H3 and H4 in MRL/*lpr* mice compared with control MRL/MPJ mice (66). Moreover, histone modifications such as H3 K18 methylation, H4 K31 methylation and H4 K31 acetylation were expressed differentially in MRL/*lpr* mice compared with controls. We also observed *in vivo* administration of the HDACi TSA corrected the site-specific hypoacetylation states on H3 and H4 in MRL/*lpr* mice with improvement of disease phenotype showing that histone modifications can therefore be reset with histone deacetylase inhibition *in vivo* (66).

Within the Th subpopulation, there also is an imbalance between Th1 and Th2 cytokine production in SLE (81-83). Activated Th2 cells overproduce IL-6, IL-12, IL-10 and tumor necrosis-α, whereas Th1 cells under produce IL-2, IFN-γ and transforming growth factor-β (TGF-β) (84–86). Moreover, activated Th cells overexpress cell-surface CD40 ligand (CD154) that persists over an extended time interval compared with activated normal T cells (87). The ongoing interaction between CD154 on T cells and CD40 on B cells, in the presence of high levels of IL-6 and IL-10 and in the absence of effective regulatory $CD8^+$ T cytotoxic/suppressor cells, persistently activates B cells, resulting in high-output immunoglobulin production. This altered homeostasis ultimately leads to polyclonal hypergammaglobulinemia. Recent studies have shown the HDACi ITF2357 inhibits IL-6 receptor signaling (88) and inflammation in colitis (89) and traumatic brain injury (90) by decreasing inflammatory mediator production triggered by glial cell activation (91). IL-6 receptor inhibition currently is being pursued in clinical trials as a means to ameliorate SLE (92), possibly through the inhibition of plasmacytoid dendritic cells (93). Reddy and coworkers recently have shown the HDACi SAHA and ITF2357 directly decrease dendritic cell function through increased expression of the dendritic cell inhibitor indoleamine 2,3-dioxygenase (94). This would suggest that, in addition to dampening receptor responsiveness to inflammatory molecules, HDACi may upregulate immunosuppressive genes.

Several other reports have shown TSA to exert a dampening effect on T cell activation with stimulation. Nambiar and coworkers showed TSA reduced the expression of the T cell receptor zeta chain gene. Additionally, TSA upregulated the expression of its homologous gene Fc (epsilon) receptor I γ chain (95). These effects were associated with a decreased intracytoplasmic-free calcium response and altered tyrosine phosphorylation patterns of cytosolic proteins. Along with these effects, the expression of the IL-2 gene was suppressed. The effects of TSA on human T cells were predominantly immunosuppressive and reminiscent of the signaling aberrations that have been described in patients with systemic lupus erythematosus (95). Further evidence supporting a role for HDAC inhibition of T-cell activation was demonstrated in the graft versus host disease (GVHD) model. This model has been used to elucidate complications that may arise from allergenic bone marrow transplantation which is widely viewed as a T cell–mediated response. After bone marrow transplantation, T cells present in the graft, either as contaminants or intentionally introduced into the host, attack the tissues of the transplant recipient after perceiving host tissues as antigenically foreign. Recent strategies to prevent GVHD have included the ablation of T cells by an anti-CD3 conditioning regime for the recipient. However, anti-CD3 administration often has been associated with cytokine storm syndrome (96). Li *et al*. reported that SAHA reduced the cytokine storm associated with anti-CD3 preconditioning (97). Furthermore, they demonstrated in NZB/W lupus mice that anti-CD3 and SAHA precondi-

tioning not only prevented GVHD disease when given cells from nonautoimmune donor cells, but also reversed glomerulonephritis in the NZB/W diseased mice. Their studies showed an effective dosing regimen of SAHA of δ 200 μg/g. In our studies, we have shown SAHA administration of 50 μg/g decreased lupus disease progression in NZB/W animals while adverse effects with prolonged administration of SAHA greater than 50 μg/g were noted (67). Taken together, these results indicate that conditioning with anti-CD3 and HDACi represent a radiation-free treatment regime for the prevention of GVHD which may have clinical applications for treating cancers and rheumatic disorders. T-cell differentiation and activation may be modulated by HDAC inhibitors as they may act at various stages of naïve T-cell development (Figure 1).

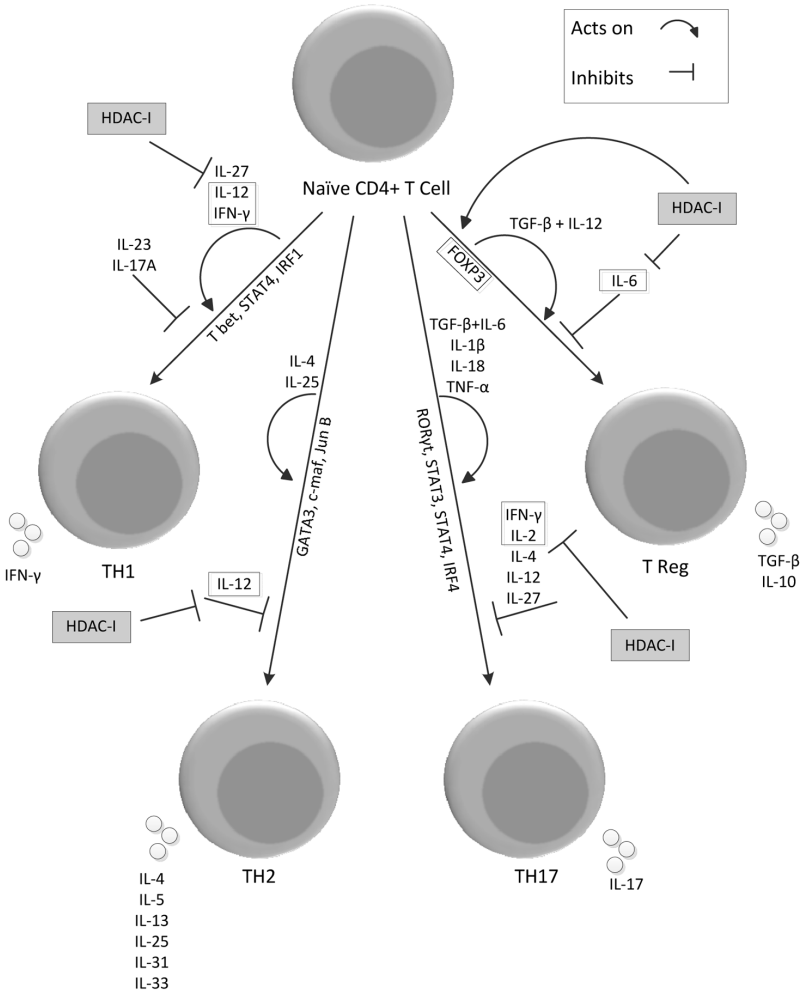

Figure 1. Reported mechanisms by which histone deacetylase inhibitors modulate naïve T-cell differentiation to that of a T-regulatory cell phenotype.

B CELLS AND HDAC INHIBITION

B cells have been believed to play a critical role in SLE, including the loss of B-cell tolerance and hyperresponsiveness of B cells to immune stimulation in lupus-prone strains. Autoreactive B cells have been recognized for over 50 years as a central component of disease. Using B-cell deficient progeny of SLE-prone MRL/*lpr* mice bred to mice with the Jh (B-cell deficient) mutation, Schmoclik *et al*. reported no signs of autoimmune kidney destruction or vasculitis. In contrast, wild type littermates with intact B cells had severe nephritis and vasculitis as well as autoantibodies (49,98). Similar findings have been reported in NZB/W F1 animals (99). Although in our studies we found little effect of TSA on splenic B-cell profiles in MRL/*lpr* mice, Lu *et al*. reported TSA strongly inhibited germline and post-switch immunoglobulin transcripts in primary splenic B cells from MRL/*lpr* mice (100). Furthermore, they observed by chromatin immunoprecipitation assays that HDAC1 was recruited to the 3′-IgH enhancer. Overexpression of HDAC1 increased the activity of IgH enhancers, especially 3′-IgH enhancers. These findings implicate HDAC in IgH gene transcription via activation of 3′-IgH enhancers.

Autoantibodies are a hallmark of lupus nephritis; studies by van Bavel *et al*. reported that elevated autoantibody production was observed in acetylated apoptotic cells compared with non-acetylated apoptotic cells (101). This would suggest acetylation may increase autoantibody production in lupus; we found in our studies that lupus mice treated with TSA did not show a decreased production of autoantibodies (69). It may be somewhat surprising that autoantibody production was not decreased with HDAC inhibition. It has been established that, although B cells are required for lupus nephritis (49,102), autoantibody generation is not necessarily indicative of active disease (103).

One significant advance made in identifying lupus susceptibility genes in the last decade is the discovery of the role of IFN-α in lupus. In 2003, Baechler *et al*. reported an increase in IFN-α inducible genes in the peripheral blood from patients with severe lupus (104). This has been coined the "interferon signature" and several later studies have verified these results and further clarified the role of IFN-α-inducible genes in SLE. Several IFN-regulated genes have been shown to be induced in patients with SLE (21,35, 105). Plasmacytoid dendritic cells represent a major cell type that is responsible for IFN-α production. TSA has been

shown to block IFN-α production by plasmacytoid dendritic cells cultured *in vitro* in the presence of serum obtained from SLE patients (93).

TRANSCRIPTION FACTORS AND HDAC INHIBITION

Class III group of HDACs, collectively called sirtuins (SIRTs), have been implicated in chromatin silencing, cell survival and aging (106). Unlike the class I and II HDACs, the deacetylase activity of SIRTs is dependent on the NAD-to-NADH ratio of the cell and is considered a sensor of redox signaling (107). During oxidative stress, cells alter their NAD-to-NADH ratio to increase the NAD, which elevates the deacetylase activity of SIRTs. Conversely, NADH and nicotinamide act as inhibitors of this family. In a recent study, Hu *et al*. showed SIRT-1 expression was decreased with SIRT-1 siRNA injection in MRL/*lpr* lupus mice (108). Additionally, transcription of P300, PCAF and HDAC7 were decreased as compared with noninjected animals. Moreover, serum anti-dsDNA antibody levels, renal IgG deposition and renal pathological scores, particularly tubulointerstitial scores, decreased significantly, showing SIRT1 overexpression and decreased histone acetylation is implicated in lupus in MRL/*lpr* mice. In human lupus, reports also suggest a role for SIRT1 in disease (109). Lupus patients with active disease showed a global histone H3/H4 hypoacetylation in the $CD4^+$ T cells compared with controls (109). The degree of histone H3 acetylation correlated negatively with increased disease activity in lupus patients as measured by the systemic lupus erythematosus disease activity index.

Although several studies have shown inhibition of HDAC being beneficial for autoimmune diseases, reports by Kuwatsuka *et al*. suggest decreased levels of antibodies against HDAC3 actually may be associated with the severity of autoimmune disease (110). They found in systemic sclerosis (SSc) patients, the production of autoantibodies against HDACs, IgG and IgM anti-HDAC3 were significantly lower than in normal controls. Furthermore, they reported the decreased levels of IgG anti-HDAC3 antibodies were specific to SSc, while IgG anti-HDAC3 antibody levels in SLE were similar and slightly increased relative to normal controls. These data suggest an increase in anti-HDAC3 antibodies may be protective in SSc, while anti-HDAC3 autoantibodies may be pathogenic in SLE patients.

FUTURE DIRECTIONS FOR SLE

The genetic studies in human and murine lupus suggest that polymorphisms in different genes with different combinations contribute to the lupus pathogenesis (111). Several gene specific knockout studies in mice have shown different ways to elicit the lupus phenotype, perhaps by the creation of specific pathways and networks of aberrant gene expression (112). Positive or negative feedback loops involving transcriptional regulatory proteins are the fundamental principle of epigenetic inheritance of gene expression in a cell-specific context (113). The change in the epigenetic landscape generated by a specific class or subclass of HDACi may dampen the positive inflammatory feedback loop initiated to induce production of cytokines and chemokines, which favors infiltration of inflammatory cells to target organs and facilitates autoantibody deposition in kidney tissue in lupus. To this end, recent studies showed the HDACi ITF2357 selectively inhibited function of the mutated JAK2 (V617F) gene associated with polycythemia vera (114). Furthermore, ITF2357 has shown efficacy in treating juvenile idiopathic arthritis (115).

Several studies using microarray analyses have demonstrated that 1% to 3% of genes are either upregulated or downregulated with lupus pathogenesis (116,117). Depending upon the cell type and context, HDACi also affect 1% to 5% genes (66). It may be argued that HDACi represent a better approach than targeted therapy because it may affect aberrant expression in specific genes to alter the global inflammatory landscape. It has been reported that pan-HDACi may elicit side effects similar to lupus symptoms such as fatigue, low platelet count, thromboembolism and others. It is highly likely that the development of isoform-specific HDACi to target specific abnormal genes may dampen the hyperresponsive lupus phenotype leading to better efficacy with fewer side effects. Indeed, selective therapeutics targeting HDAC may reverse the expression of several genes in the spleen and kidney that show altered expression in lupus. Delineating the mechanisms by which specific HDACi treat lupus remains in its early stages of investigation.

Epigenerator signals, including environmental cues or niches (such as viral, bacterial infection, self antigen, etc.) induce intracellular signals that activate or decrease epigenetic initiators (transcription factors, non-coding RNAs, etc.). Once induced, an epigenetic maintainer (histone acetylases and deacetylases) coordinates the overall cell response. Once the stable chromatin landscape change occurs as a result of aberrant expression of chromatin-modifying enzymes in a particular cell type, it will differentiate the cell terminally without the requirement of other additional initiating signals. The persistence of chromatin modifying enzymes requires the cooperation between both the initiator and maintainer signals for terminal differentiation of effector cells. This may be where epigenetic modulators such as HDACi have the efficacy to alter the cell phenotype.

HDACi have been considered for the treatment of systemic lupus erythematosus based upon *in vitro* cell culture and *in vivo* studies in murine models of lupus, but clinical efficacy remains to be established. Although there is a great need for new lupus therapies, progress has been slow. Recently, Belimumab was the first therapeutic approved by the FDA for the treatment of SLE although its efficacy in treating patients with active renal disease was not studied. Furthermore, African Americans with SLE did not significantly respond to treat-

ment. Lupus is a complex disease without a large enough patient base to attract the attention of most pharmaceutical companies. This, coupled with the expenses associated with lupus trials and the recent unsuccessful study outcome for other lupus therapeutics, illustrates the need for new strategies for targeting lupus. Modifying specific histone proteins using HDACi may prove to be a successful therapeutic approach.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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