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Modulation of Renal Disease in MRL/lpr Mice by Suberoylanilide Hydroxamic Acid

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Epigenetic regulation of gene expression is involved in the development of many diseases. Histone acetylation is a posttranslational modification of the nucleosomal histone tails that is regulated by the balance of histone deacetylases and histone acetyltransferases. Alterations in the balance of histone acetylation have been shown to cause aberrant expression of genes that are a hallmark of many diseases, including systemic lupus erythematosus. In this study, we determined whether suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor: 1) inhibits inflammatory mediator production in vitro and 2) modulates lupus progression in vivo. Mesangial cells isolated from 10-week-old MRL/lpr mice were stimulated with LPS/IFN-γ and incubated with SAHA. TNF-α, IL-6, NO, and inducible NO synthase expression were inhibited by SAHA. We then treated MRL/lpr mice with daily injections of SAHA from age 10 to 20 wk. The animals treated with SAHA had decreased spleen size and a concomitant decrease in CD4+CD8+ (double-negative) T cells compared with controls. Serum autoantibody levels and glomerular IgG and C3 deposition in SAHA-treated mice were similar to controls. In contrast, proteinuria and pathologic renal disease were significantly inhibited in the mice receiving SAHA. These data indicate that SAHA blocks mesangial cell inflammatory mediator production in vitro and disease progression in vivo in MRL/lpr mice.

HDI MODULATION OF LUPUS

HDIs inhibited LPS-induced TNF-α, IL-1β, IL-12, and IFN-γ in human PBMCs.

Mesangial cells are specialized cells within the glomerulus that are vasoactive as well as being active immunologically. Mesangial cells: 1) act as specialized smooth muscle cells engaged in the regulation of glomerular hemodynamics; 2) produce extracellular matrix and provide structural support to the glomerular tuft; and 3) through their phagocytic properties uptake and clear macromolecules such as immune complexes. Mesangial cells, similar to macrophages, produce NO, superoxide, and other inflammatory mediators in response to LPS, IFN-γ, and IL-1β (16, 26).

Because mesangial cells play a pivotal role in the initiation and resolution of the inflammatory response in the glomerulus in lupus nephritis, we sought to determine whether the administration of SAHA would block inflammatory mediator production in stimulated mesangial cells. Furthermore, we investigated whether targeting histone deacetylation in lupus pathogenesis by the administration of HDIs inhibited LPS-induced TNF-α, IFN-γ, and IL-1β (16, 26).

Materials and Methods

Mice

Eight-week-old female MRL/lpr mice were purchased from The Jackson Laboratory (Bar Harbor, ME), housed under specific pathogen-free conditions at the Ralph H. Johnson Veterans Administration Medical Center animal facility, and provided autoclaved food and sterile water ad libitum. Mice were randomly tested and were serologically negative for common murine pathogens.

Reagents

IFN-γ was purchased from BD Pharmingen (San Diego, CA); FCS, DMEM, and RPMI 1640 were from Invitrogen Life Technologies (Gaithersburg, MD); and the protein assay kit was from Bio-Rad (Hercules, CA). Anti-NO synthase 2 Ab was purchased from BD Transduction Laboratories (Lexington, KY). All other reagents, including LPS, were purchased from Sigma-Aldrich. SAHA was a gift from Tarrytown, NY.

Mesangial cell isolation

Because glomerulonephritis occurs in MRL/lpr mice beginning at 12–14 wk of age, glomeruli were isolated before disease onset at 8–10 wk of age. To accomplish this, mice were anesthetized with methofane and sacrificed by cervical dislocation. Renal tissue was surgically removed and kept on ice. Although sterile DMEM with 1% FCS. The kidneys were minced, and glomeruli were separated from the remaining renal tissue by sequential mechanical sieving (27). Glomeruli were incubated for 30 min at 37°C with collagenase and then plated in 75-cm² culture flasks in DMEM/F12 (50/50) medium containing 15% FCS, streptomycin (100 µg/ml), penicillin (100 U/ml), and 1-glutamine (2 mM). DMEM/F12 (50/50) medium containing 15% FCS, streptomycin (100 µg/ml), penicillin (100 U/ml), and 1-glutamine (2 mM). Media were replaced every 48 h, while adherent mesangial cells were retained. After cells reached confluence (≈2 wk), they were passaged using trypsin/EDTA. The cells exhibited typical morphologic characteristics of mesangial cells and tested uniformly positive for the presence of smooth muscle actin staining. Cells between passages 3 and 7 were used for subsequent experimental procedures.

Experimental conditions

Confluent cells in triplicate six-well cell culture dishes were washed twice with DMEM devoid of phenol red, as phenol red interferes with nitrate/nitrite (N/N) measurements. After the final wash, DMEM plus 10% FCS without phenol red was added, containing various reagents described for each experiment. At the end of each incubation, supernatants were collected and analyzed for N/N production. Cellular protein was isolated for Western analysis.

N/N analysis

NO is metabolized rapidly to N/N; these stable metabolites are accepted measures of in vivo and in vitro NO production and were measured in 50 µl of supernatant, as previously described (27). Briefly, supernatants were filtered using Centricon ultrafiltration tubes (Millipore, Beverly, MA). Nitrate was converted to nitrite using nitrate reductase (Boehringer Mannheim, Indianapolis, IN), and total N/N was determined by measuring nitrite via the Greiss reaction. Amounts of N/N in PBS were used to generate a standard curve.

Western blot analysis

Immunoblots were performed, as previously described (27). Briefly, mesangial cells were lysed with distilled water containing proteinase inhibitors (BD Pharmingen) and then sonicated. After using a Bio-Rad rapid Coomassie kit to determine total protein concentration, 20 µg of protein was loaded into each well. The proteins were transferred onto a polyvinylidene difluoride membrane, blocked with 5% milk solids, and incubated with a mouse anti-N/N polyclonal antibody (1/2500 dilution) mAb, washed, and then exposed to a secondary goat anti-mouse HRP conjugate. Chemiluminescence (ECL; Amersham, Piscataway, NJ) in conjunction with video densitometry (NIH Images) was used to quantitate inducible NO synthase (iNOS) protein.

Flow cytometry

Splenoocytes were surgically removed from MRL/lpr mice at the time of sacrifice, and single cell suspensions were prepared. Cells (10⁶) were double labeled with monoclonal FITC-labeled hamster anti-mouse CD3 and monoclonal PE-labeled rat anti-mouse CD4 or CD8. All Abs and isotype controls were obtained from BD Transduction Laboratories and analyzed by flow cytometry (BD Biosciences, Sunnyvale, CA).

Mesangial cells (10⁶) were stimulated for 24 h with LPS/IFN-γ with or without various concentrations of SAHA (100, 10, 1, and 0.1 µM) added 2 h before stimulation. MHC II expression was determined using FITC-conjugated anti-mouse I-A subunit mAb (BD Biosciences). As an isotype control, we used FITC-conjugated IgG2b (BD Biosciences). Cell viability was determined using the annexin V-FITC assay, according to the manufacturer’s protocol (R&D Systems, Minneapolis, MN).

ELISA

IFN-γ and IL-6 in supernatants were quantified by ELISA, as per the manufacturer’s instructions (R&D Systems).

In vivo treatment

Female MRL/lpr mice (n = 8) 10 wk of age were treated with i.p. injections of SAHA (25 or 50 mg/kg BW) in 40 µl of DMSO 5 days/wk over 10 wk. Control MRL/lpr mice (n = 10) were treated with 40 µl of DMSO alone. Cyclophosphamide-treated mice (n = 10) received 25 mg/kg injections i.p. once per week as a positive therapeutic control. Results were not noted as the absorbance (A290) at a 1/100 serum dilution, 0.1 M citrate buffer (pH 4) containing 0.015% H2O2 and 3,3′,5,5′-tetramethylbenzidine (Sigma-Aldrich) was added. Results were de-
without prior knowledge of the treatment modality. Renal pathology was graded by previously described methods for glomerular inflammation, proliferation, crescent formation, and necrosis. In addition, interstitial changes and vasculitis were also noted. Scores from 0 to 3 were assigned for each of the features, and then added together to yield a final renal score (29). Scores for necrosis and crescent formation were doubled before adding. For example, glomerular inflammation was graded, as follows: 0, normal; 1, few inflammatory cells; 2, moderate inflammation; and 3, severe inflammation.

**Immunofluorescence staining**

The other kidney was snap frozen, cut into 4-μm-thick sections, and stained with FITC-conjugated rabbit anti-mouse IgG (Sigma-Aldrich) and sheep anti-mouse C3 (Sigma-Aldrich). The deposition of IgG and C3 in frozen sections was graded 0–3⁺ (29).

**Statistical analysis**

The unpaired Student’s t test or ANOVA, followed by post hoc analysis, was used to test for significant differences between groups. Table I was analyzed by Wilcoxon rank sum test.

**Results**

**SAHA inhibits inflammatory mediator production from stimulated mesangial cells**

Because mesangial cells are the principal resident immunoregulatory cells in the kidney, we sought to determine whether SAHA inhibits inflammatory mediator production from stimulated mesangial cells. We, and others, previously showed that MRL/lpr mesangial cells exhibit a heightened response to inflammatory stimulation compared with mesangial cells from control strains (7, 27).

We treated mesangial cells with various concentrations of SAHA 2 h before and during stimulation with LPS (1 μg/ml) and IFN-γ (50 U/ml). Twenty-four hours after the addition of LPS/IFN-γ, supernatants were collected to determine cytokine production, and cellular supernatants were assayed for N/N production (stable metabolites of NO) in the supernatant. Our results demonstrate that N/N, similar to iNOS, was enhanced with LPS/IFN-γ stimulation, but was significantly decreased when the stimulated mesangial cells were treated with SAHA 2 h before the addition of LPS/IFN-γ (Fig. 1B). The concentrations of SAHA required to block NO production paralleled the concentrations required to block iNOS expression.

Once we determined that SAHA inhibited NO production, we sought to determine whether SAHA pretreatment of stimulated mesangial cells inhibited other inflammatory mediators associated with disease. This was accomplished by measuring the production of IL-6 and TNF-α by ELISA in the supernatants of stimulated mesangial cells with and without various concentrations of SAHA (Fig. 2). Similar to our findings that NO was concentration dependently inhibited by SAHA, we observed decreased production of IL-6 and TNF-α in stimulated mesangial cells treated with SAHA. IL-6 and TNF-α production were both completely inhibited with a concentration of SAHA of 10 μM. Taken together, these results indicate that SAHA significantly decreased IL-6, TNF-α, and NO production by stimulated MRL/lpr mouse mesangial cells.

**In vivo administration of SAHA**

Based on our in vitro data, we tested the hypothesis that SAHA would delay the progression of lupus disease activity and reduce disease manifestations. At 10 wk of age, we began daily i.p. injections of SAHA (25 or 50 mg/kg body weight (BW) in 40 μl of DMSO, five times per week) into female MRL/lpr mice. As a positive treatment control, we injected 25 mg/kg cyclophosphamide weekly. For a negative control, MRL/lpr mice were given daily injections of 40 μl of DMSO, 5 days per week.

**FIGURE 1.** Effect of different concentrations of SAHA on NO production by MRL/lpr mesangial cells. A, Mesangial cells were treated with increasing concentrations of SAHA 2 h prior to stimulation with LPS (1 μg/ml) and IFN-γ (50 U/ml). After 24 h of stimulation, total cellular protein was isolated and iNOS expression was determined by Western blot. B, Supernatant from MRL/lpr mesangial cells treated with increasing concentrations of SAHA 2 h prior to stimulation with LPS (1 μg/ml) and IFN-γ (50 U/ml). After 24 h of stimulation, cellular supernatants were assayed for N/N production. The bar represents the mean ± SD of triplicates.

### Table I. Measurement of proteinuria in MRL/lpr mice by dipstick analysis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proteinuria (mg/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>10/30/100</td>
</tr>
<tr>
<td>25 mg/kg (n = 8)</td>
<td>8/0/0</td>
</tr>
<tr>
<td>50 mg/kg (n = 8)</td>
<td>8/0/0</td>
</tr>
<tr>
<td>Cyclophosphamide (n = 10)</td>
<td>9/1/0</td>
</tr>
</tbody>
</table>

* Mice received daily injections of either SAHA (25 or 50 mg/kg BW in DMSO), vehicle (DMSO), or cyclophosphamide (25 mg/kg BW) for 10 wk beginning at 10 wk of age. Data are the number of mice with each level of proteinuria at a given time point (*, p < 0.05 at 18 wk of age compared with controls).
Animals were monitored throughout the study for general health. We monitored animals for diarrhea and weight loss due to drug treatment or disease progression. At the concentrations of SAHA used in this study, there were no general adverse effects noted in the animals. Although previous reports have shown that SAHA administration to animals at concentrations from 25 to 100 mg/kg/day has resulted in antitumor effects, in our studies we observed adverse effects of SAHA when given at the 100 mg/kg/day dose or above (22). Throughout the duration of the study, the untreated controls, and cyclophosphamide-treated, and 25 and 50 mg/kg BW SAHA-treated animals gained weight consistently with no differences in weight gain between any of the treatment groups (data not shown). There was also no difference in the development of skin rash between the treated and control mice.

SAHA had no effect on autoantibody production

As MRL/lpr mice age, they develop hypergammaglobulinemia and exhibit high concentrations of autoantibodies directed against several nuclear and extracellular autoantigens, including dsDNA and GBM. To determine whether treatment with SAHA modified autoantibody production, serum levels of anti-dsDNA and anti-GBM autoantibodies were quantified. Compared with vehicle-treated controls and cyclophosphamide-treated animals, the levels of both anti-dsDNA and anti-GBM autoantibodies in the serum of SAHAtreated mice were similar at ages 10, 14, 18, and 20 wk (Fig. 3, A and B). Thus, SAHA treatment did not affect autoantibody production. Cyclophosphamide, however, also had minimal effect on autoantibody production in this experiment.

SAHA decreases spleen weight

At age 20 wk, the animals were sacrificed, and the spleen weights were determined. Compared with vehicle-treated controls, the SAHA (25 and 50 mg/kg BW)-treated mice had significantly smaller spleens (p < 0.05) (Fig. 4), although there was no change in splenic architecture after SAHA treatment. The cyclophosphamide-treated animals also had spleen weights that were significantly decreased compared with the untreated controls (p < 0.01).

SAHA decreases CD3+ CD4−CD8− cells in spleen

To investigate the mechanism for the lower spleen weights in the SAHA-treated animals, we analyzed B and T cells’ splenic composition by flow cytometry. The flow cytometry results showed that the predominant phenotype of T cells in the untreated control MRL/lpr mice was CD3+CD4+CD8−, double-negative T cells (DN T cells), as expected (Fig. 5). The cyclophosphamide-treated animals had a significant decrease in the number of T cells that were DN. Interestingly, the SAHA-treated animals also showed a significant decrease in the number of DN T cells compared with control untreated animals. However, there was no further decrease of DN T cells with the 50 mg/kg dose of SAHA as compared with the 25 mg/kg dose. The proportion of CD4+ and CD8+CD3−
positive cells was not different between the groups. Additionally, there were no differences between the B cell populations in any of the groups, as judged by staining for CD19 and CD21 (data not shown). These results indicate that one effect of SAHA treatment in MRL/lpr mice is inhibiting the proliferation of DN T cells.

SAHA decreases MHC II expression in stimulated mesangial cells

In addition to LPS/IFN-γ stimulation increasing the production of inflammatory mediators, LPS/IFN-γ enhances MCH class II expression (30). To evaluate whether SAHA administration inhibits the up-regulation of MHC II, stimulated mesangial cell cultures (10⁶ cells) were treated with various concentrations of SAHA (Fig. 6; Table II). Our results showed that MHC II expression was significantly up-regulated in mesangial cells treated with LPS/IFN-γ for 24 h as compared with unstimulated (control) mesangial cells (p < 0.05). The addition of SAHA to the stimulated cultures caused a dose-dependent decrease in MHC II expression that was observed in the stimulated cells. SAHA treatment alone had no effect on MHC II expression as compared with unstimulated mesangial cells.

We next sought to determine whether the inhibition of inflammatory mediator production and decrease of MHC II expression by SAHA treatment were due to a nonspecific toxic effect or by affecting cellular apoptosis. Once again, mesangial cell cultures were treated with various concentrations of SAHA for 2 h before and during stimulation of LPS/IFN-γ for 24 h (Fig. 7; Table II). In unstimulated mesangial cells, the amount of cell death attributed to either apoptosis, as judged by annexin V staining, or necrosis, as determined by propidium iodide (PI) uptake, was low. The administration of LPS/IFN-γ did not induce a significant increase in cell death by either apoptosis or necrosis. However, when the cells were given SAHA at 100 μM concentration, there was a significant increase in the number of cells that died by necrosis. SAHA administration at 10 μM resulted in slight increase in necrosis, but it was not significant. Furthermore, at a 1 μM concentration of SAHA, there was no increase in cell death as compared with the unstimulated controls.

Measurement of protein excretion

To determine whether SAHA altered the progression of renal disease, we quantified protein excretion in the urine that was collected over 24-h intervals at 10, 12, 14, 16, and 18 wk of age. At 10 wk, the protein levels in each group were low, but as the mice aged, protein concentrations, as measured by protein dipstick and albumin ELISA in the urine, began to increase. By 18 wk of age in the control mice, 7 of the 10 animals had urinary protein concentrations of 30 mg/dl or greater compared with 3 of 8 in the 25/mg/kg SAHA group and 2 of 10 in the cyclophosphamide group (Table I). The levels of proteinuria in the 25 mg/kg BW SAHA-, 50 mg/kg BW SAHA-, and cyclophosphamide-treated animals were consistently lower compared with the untreated controls. These results were confirmed by ELISA analysis of 24-h urinary albumin excretion (data not shown).

Renal pathology

To assess further the effect of SAHA on renal disease, kidney sections were stained with H&E and scored by a pathologist that was blinded to the treatment groups. The cyclophosphamide-treated mice had significantly reduced renal scores compared with the untreated controls (Fig. 8). The SAHA-treated mice (25 and 50 mg/kg BW) also had renal scores that were significantly less than the untreated control mice. These results indicate that SAHA administration inhibited proliferative renal disease in MRL/lpr mice. In addition to H&E staining, one kidney was frozen, sectioned, and stained with fluorescein-conjugated anti-mouse IgG or C3. There were no differences in staining intensity or staining pattern in the various treatment groups for either Ig or complement renal deposition (data not shown).
Discussion

The goals of these experiments were 2-fold: first, to determine whether an HDI (SAHA) can inhibit the inflammatory mediators produced by stimulated mesangial cells from MRL/lpr mice, and second, to determine whether treating MRL/lpr mice with SAHA would abrogate renal disease. In the first set of studies, we used mesangial cells from 10-wk-old (predisease) mice. Mesangial cells from MRL/lpr mice are hyperresponsive to inflammatory stimulation (27). Our in vitro studies demonstrated that SAHA blocked NO, TNF-α, and IL-6 production by stimulated MRL/lpr mesangial cells in a concentration-dependent manner. We next sought to translate our in vitro findings into an in vivo setting. MRL/lpr mice were treated with either vehicle or two concentrations of SAHA (25 or 50 mg/kg daily). As a positive control, one group of mice received cyclophosphamide (25 mg/kg weekly). The results from our in vivo studies demonstrated that SAHA inhibited DN T cell proliferation similar to cyclophosphamide and reduced renal pathology scores as compared with vehicle-treated control. Neither cyclophosphamide nor SAHA significantly inhibited renal deposition of Ig or complement nor blocked autoantibody production during the time course of these experiments.

Since the discovery of HDACs, 11 mammalian HDACs have been reported. The mechanisms by which HDACs and HDIs alter gene transcription are currently an area of active investigation. Individual HDACs associate with specific target DNA. Recently, Yu et al. (31) reported that primarily HDAC2, but also other HDAC isoforms, are recruited to, and interact with NF-κB p65, leading to the induction of the iNOS enzyme in response to inflammatory stimuli. The addition of TSA, another HDI, inhibited cytokine induction and iNOS transcription without a change in NF-κB nuclear translocation, suggesting that the trans activation potential of NF-κB or interactions with other coregulatory proteins was inhibited by TSA (32). Furthermore, overexpression of HDAC2, 4, 5, and 6 resulted in heightened activity of an iNOS promoter construct and a NF-κB promoter construct. They also demonstrated that HDAC2 and NF-κB p65 cocommunoprecipitated (32).

In addition to the ability of HATs to modify histones, HATs may also affect gene transcription through acetylation of transcription factors such as p53, E1A, GATA-1, and Myc (33–35). HDAC inhibitors may affect gene transcription through p53 or independent of p53 activation (36, 37). Greenberg et al. (38) showed HDAC inhibition repressed the growth of anaplastic thyroid carcinoma cells independent of p53, and increased expression of the cyclin-dependent kinase inhibitors p21waf1 and p27Kip1. Similarly, Huang et al. (39) showed that SAHA activated the cyclin-dependent p21waf1 independent of the p53 promoter. Conversely, Kim et al. (39) demonstrated that TSA up-regulated p53 in the Lewis lung carcinoma model. Furthermore, Balomenos et al. (40) demonstrated that p21waf1-deficient mice develop Abs against dsDNA, lymphadenopathy, and glomerulonephritis, suggesting that p21 inhibition may worsen disease. Taken together, these studies indicate that the ability of HDIs to inhibit inflammatory mediator production in lupus may be multifactorial. We are currently pursuing studies to address the mechanisms for the inhibition of inflammatory mediators by MRL/lpr mesangial cells by SAHA.

Our in vivo studies were performed to determine whether SAHA would prevent or delay the onset of renal disease in MRL/lpr mice. The concentrations of SAHA used were based on studies by Butler et al. (22) that showed an inhibition of a prostate cancer xenograft in nude mice with 25 and 50 mg/kg BW of SAHA injected daily. Butler et al. showed the concentration needed to inhibit acetylation of both H3 and H4 in vitro was 2 μM. Furthermore, the authors observed robust acetylation of H3 and H4 at 5 μM. In our previous studies, we described acetylation of H3 and

Table II. Expression of MHC II and cell death in mesangial cellsa

<table>
<thead>
<tr>
<th>SAHA (μM)</th>
<th>Control</th>
<th>With LPS/IFN-γ</th>
<th>Without LPS/IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>MHC II %</td>
<td>45.9 ± 2.7</td>
<td>55.8 ± 1.9*</td>
<td>51.6 ± 3.4</td>
</tr>
<tr>
<td>Annexin V</td>
<td>2.0 ± 0.9</td>
<td>1.8 ± 0.5</td>
<td>1.8 ± 0.6</td>
</tr>
<tr>
<td>PI</td>
<td>5.6 ± 3.2</td>
<td>6.5 ± 2.7</td>
<td>7.3 ± 2.3</td>
</tr>
<tr>
<td>Annexin + PI</td>
<td>5.3 ± 2.0</td>
<td>4.7 ± 2.6</td>
<td>5.3 ± 3.1</td>
</tr>
</tbody>
</table>

a Mesangial cells from MRL/lpr mice were treated with increasing concentrations of SAHA (0.1–100 μM) 2 h prior to stimulation with LPS (1 μg/ml) and IFN-γ (50 U/ml). After 24 h of stimulation, mesangial cells were analyzed by FACS after staining with FITC-conjugated MHC II or FITC-conjugated annexin V. Results are expressed as mean percentage of expression of triplicates. The MHC II data show the mean percentage of expression ± SD of cells that express MHC II (*, p < 0.05 vs control).
H4 using concentrations of 7.5 and 10 μM (25). Additionally, Butler et al. showed, in vivo, that a dosage of 25 or 50 mg/kg SAHA increased total acetylation of H3 and H4 (22). Therefore, we believe it can be concluded that a 5 μM concentration of SAHA administered in vitro is equivalent to a 25 mg/kg dose of SAHA in vivo.

We observed a decrease in the spleen weight primarily due to a decrease in the number of CD3⁺CD4⁺CD8⁻ (DN) T cells. DN T cells increase significantly in MRL/lpr mice as they age and produce inflammatory mediators including IFN-γ (41). However, it remains controversial which immune factors, including IFN-γ, are implicated in lymphoaccumulation in MRL/lpr mice. MRL/lpr mice that lack IFN-γ have reduced lymphadenopathy and splenomegaly (42). Further studies suggested that mice lacking IFN-γ exhibited reduced lymphadenopathy due to a decrease in DN T cells (43, 44). Conversely, Haas et al. (45) found that MRL/lpr mice lacking the IFN-γ receptor exhibited no decrease in the expansion of DN T cells compared with WT MRL/lpr mice. Thus, the mechanism for the decrease in DN T cells in MRL/lpr mice treated with SAHA is unclear, but is most likely secondary to decreased production of inflammatory mediators such as IFN-γ.

In patients with SLE, purified T cells treated with TSA showed a significant reduction in gene expression of CD40L and IL-10 stimulated by phorbol esters and ionomycin, while gene expression and synthesis of IFN-γ were up-regulated (46). In our studies, we found that SAHA blocks the increase in MHC II expression in stimulated mesangial cells. Inhibiting MHC II expression, but not MHC I, has been shown to effect glomerulonephritis (47–49). In this respect, SAHA may regulate disease by decreasing MHC II. Contrary to our results, Magner et al. (50) demonstrated TSA up-regulated MHC I, MHC II, and CD40 gene expression. The differences in our findings may be attributed to the different cell type, time stimulated, or concentrations used or to differential effects of TSA and SAHA.

The circulating levels of anti-dsDNA and anti-GBM serum autoantibodies were not reduced, nor was IgG or complement deposition in the kidney with treatment with SAHA, yet renal disease was reduced (51). The uncoupling of autoantibody production, immune complex deposition, and proliferative renal disease has been reported for a number of therapeutic interventions and genetic manipulations in MRL/lpr mice (3). These studies indicate that therapies targeted at postimmune complex deposition in lupus can be effective in preventing pathologic outcomes such as renal disease.

One surprising outcome of these studies was that the cyclophosphamide treatment had no significant effect on autoantibody production contrary to previously published reports. We did, however, observe a trend toward decreased autoantibody production in the cyclophosphamide-treated mice. We believe that as the mice aged, the trend in autoantibody production would have continued, eventually reaching significance, as has been the case in previous studies by our laboratory and others in which cyclophosphamide inhibited autoantibody production and proteinuria when started before disease expression. We were encouraged that SAHA was as effective as cyclophosphamide and TSA (25); however, the lack of a more profound effect of cyclophosphamide in this experiment emphasizes the disease and treatment response variability inherent in experiments using MRL/lpr mice. We did not assess survival in this study, but chose to sacrifice the mice before the time (24–26 wk) when MRL/lpr mice typically begin to die. Choosing this early time point allowed us to assess the effects of HDIs on pathologic renal disease. Additional treatment trials are planned to determine whether SAHA treatment affords a survival benefit in MRL/lpr mice.
In summary, the studies reported in this work demonstrate that SAHA inhibits inflammatory mediator production in vitro by MRL/lpr mesangial cells, providing evidence for a local renal effect of SAHA on lupus nephritis. We also demonstrated modulation of renal disease and splenomegaly in MRL/lpr mice by SAHA. Further studies are needed to delineate the most effective therapeutic regimen and the precise mechanisms of the anti-inflammatory properties of SAHA in lupus.

References