Histone Deacetylase Inhibitors Affect Dendritic Cell Differentiation and Immunogenicity

Alessio Nencioni,1,2 Julia Beck,3 Daniela Werth,3 Frank Grünewab,3 Franco Patrone,1 Alberto Ballestrero,1 and Peter Brossart3

Abstract

Purpose: Histone deacetylases (HDAC) modulate gene transcription and chromatin assembly by modifying histones at the posttranscriptional level. HDAC inhibitors have promising antitumor activity and are presently explored in clinical studies. Cumulating evidence in animal models of immune disorders also suggests immunosuppressive properties for these small molecules, although the underlying mechanisms remain at present poorly understood. Here, we have evaluated the effects of two HDAC inhibitors currently in clinical use, sodium valproate and MS-275, on human monocyte-derived DCs.

Experimental Design: DCs were generated from monocytes through incubation with granulocyte macrophage colony-stimulating factor and interleukin-4. DC maturation was induced by addition of polyinosinic-polycytidylic acid. DC phenotype, immunostimulatory capacity, cytokine secretion, and migratory capacity were determined by flow cytometry, mixed leukocyte reaction, ELISA, and Transwell migration assay, respectively. Nuclear translocation of RelB, IFN regulatory factor (IRF)-3, and IRF-8 were determined by immunoblotting.

Results: HDAC inhibition skews DC differentiation by preventing the acquisition of the DC hallmark CD1a and by affecting the expression of costimulation and adhesion molecules. In addition, macrophage inflammatory protein-3α/chemokine, motif CC, ligand 19–induced migration, immunostimulatory capacity, and cytokine secretion by DCs are also profoundly impaired. The observed defects in DC function on exposure to HDAC inhibitors seem to reflect the obstruction of signaling through nuclear factor-κB, IRF-3, and IRF-8.

Conclusions: HDAC inhibitors exhibit strong immunomodulatory properties in human DCs. Our results support the evaluation of HDAC inhibitors in inflammatory and autoimmune disorders.

Histone deacetylases (HDAC) are enzymes that, in concert with their counterpart histone acetyltransferases, regulate the acetylation status of histones and other intracellular substrates (1, 2). Histone acetylation, together with other posttranslational modifications, such as methylation, phosphorylation, and ubiquitination, contributes to regulate chromatin architecture and DNA accessibility for transcription. Namely, histone acetylation by histone acetyltransferases favors the open chromatin structure, whereas deacetylation by HDACs induces a more condensed and repressed chromatin state. Drugs that inhibit HDACs (HDAC inhibitors) produce an increase of histone acetylation within hours of treatment and thereby help maintain DNA in a more open and transcriptionally active state (1, 2).

Increased HDAC activity is almost invariably observed in cancer cells leading to changes in local chromatin structure, altered gene transcription, and impaired differentiation. Importantly, virtually all the HDAC inhibitors available show some degree of preclinical activity in tumor cell lines and in animal cancer models (1, 2). In acute leukemias, HDAC inhibitors exert their antitumor effects by relieving transcriptional repression and thereby reversing the differentiation arrest induced by chimeric oncogenes, such as PML-RARα, PLZF-RARα, or AML-ETO (1, 2). In other types of malignancies, other mechanisms seem to come into play, including BAX relocalization to the mitochondria, up-regulation of p21/WAF and of other tumor suppressors, or, vice versa, HDAC-induced repression of tyrosine kinases, such as BCR/Ab1 or HER-2/neu. In addition, HDAC inhibitors induce promoter hyperacetylation and subsequent up-regulation of different members of the surface death receptor pathway, including Fas, Fas ligand, tumor necrosis factor (TNF)–related apoptosis-inducing ligand, and the TNF–related apoptosis-inducing ligand receptor DR5, and cooperatively enhance TNF-related...
apoPTosis-inducing ligand–induced apoptosis in leukemic cells (1, 2). Several HDAC inhibitors are now in clinical trials for solid and hematologic malignancies with initial promising results obtained in T-cell lymphomas and certain subtypes of acute myelogenous leukemia (1 – 4).

Several studies have shown that, besides their antitumor properties, HDAC inhibitors have anti-inflammatory effects. Administration of these small molecules to mice was found to ameliorate the autoimmune manifestations of graft-versus-host disease, systemic lupus erythematosus, concanavalin A–induced hepatitis, experimental autoimmune encephalomyelitis, rheumatoid arthritis, and colitis (5 – 11). Besides, both in vivo and in vitro, the HDAC inhibitors suberoylanilide hydroxamic acid, trichostatin A, and sodium valproate (VPA) were reported to block secretion of proinflammatory cytokines, such as TNF-α, interleukin (IL)-1β, IL-6, and IL-12 (5, 6, 10, 11). How the immune effects of HDAC inhibitors are exactly mediated is presently unclear. In T lymphocytes, HDAC6-mediated tubulin deacetylation is involved in CD3 and LFA-1 orientation and in the organization of the immune synapse (12). However, separate studies ruled out major effects of HDAC inhibition on T-cell proliferative and cytotoxic responses and on IFN-γ production (5, 6, 10), thus leaving open the possibility that other immune cell types may be mostly affected by HDAC inhibitors and thereby contribute to the immunomodulatory properties of these drugs. In this context, recent experiments have shown the capacity of a selective HDAC inhibitor, LAQ824, to specifically modulate gene expression in macrophages and dendritic cells (DCs) leading to impaired chemokine production and to preferential stimulation of Th2 versus Th1 T lymphocytes (13).

DCs are a leukocyte population with antigen-presenting cell function that plays a central role in the initiation of immune responses (14, 15). Bone marrow–derived DC precursors, including circulating monocytes, home to the peripheral tissues and here undergo differentiation probably under the influence of locally produced cytokines and prostaglandins. DCs become alerted of the presence of pathogens when Toll-like receptors correspondingly pathogen-derived product [i.e., lipopolysaccharide (LPS)], polyinosinic-polycytidylic acid [poly(I–C)], and double-stranded RNA; ref. 16] are recognized. On activation, DCs migrate to the afferent lymphoid tissues driven by the chemotactant, chemokine, motif CC, ligand 19 (CCL19)/macrophage inflammatory protein-3β and here they stimulate antigen-specific T lymphocytes (14). DC immunogenicity is believed to be one of the main factors controlling the outcome of an immune response and indeed deregulated DC function and survival have been shown recently to lead to autoimmune manifestations in the mouse model (17).

In the present study, we have evaluated the effects of HDAC inhibition on human monocyte-derived DC differentiation and function. As different HDAC inhibitors can have markedly different effects on biological pathways, we have made use of two structurally unrelated compounds, MS-275 and VPA (1, 2), to confirm the specificity of our findings.

Materials and Methods

Reagents. The medium used for cell cultures was RPMI 1640 supplemented with 10% inactivated FCS, 50 mmol/L L-2-mercaptoethanol, and antibiotics, all purchased from Life Technologies. Human recombinant granulocyte macrophage colony-stimulating factor (GM-CSF; molgramostim, Leucogam) was from Novartis. Human recombinant IL-4 was purchased from R&D Systems. poly(I–C), VPA, and MS-275 were from Sigma-Aldrich.

DC generation from adherent monocytes. DCs were generated from adherent monocytes as described previously (18 – 22). Buffy coat preparations from healthy volunteers were obtained from the blood bank of the S. Martino Hospital (Genoa, Italy) and of the University of Tübingen (Tübingen, Germany). Peripheral blood mononuclear cells were isolated by Ficoll-Paque (Biochrom) density gradient centrifugation. Cells were resuspended in serum-free X-VIVO 20 medium (Cambrex) and seeded (1 x 10⁵ cells per well) for 2 h in six-well plates at 37°C. Afterward, nonadherent cells were removed by extensive washing with PBS (Life Technologies). DCs were generated by culturing the adherent monocytes in RPMI 1640–based medium supplemented with 100 ng/mL GM-CSF and 20 ng/mL IL-4 with or without addition of VPA or MS-275. The medium was replenished with cytokines and HDAC inhibitors every other day. At day 6 of culture, cells were stimulated with poly(I–C) (50 μg/mL) or left unstimulated. Cells were harvested for further experiments at day 7 of culture. HDAC inhibition was detected by monitoring the levels of acetylated H3 histone in cell lysates by immunoblotting.

Immunostaining and cell viability assay. Cells were stained using FITC- or phycoerythrin-conjugated mouse monoclonal antibodies against CD11c, CD80, and CD54 (Becton Dickinson); CD40 and CD86 (PharMingen); CD83 (Immunotech); DC–specific intercellular adhesion molecule 3-grabbing nonintegrin (V5–R&D Systems); and mouse IgG isotype control (Becton Dickinson). Cells were analyzed on a FACSCalibur cytometer (Becton Dickinson). A rate of 1% false-positive events was accepted in the negative control samples throughout.

For cell viability determination, cells were stained with 5 μg/mL propidium iodide and propidium iodide–positive cells were quantified by flow cytometry.

Mixed leucocyte reaction assay. Responding cells (10⁵) from allogeneic peripheral blood mononuclear cells were cultured in 96-well flat-bottomed microplates (Nunc) with 10⁵ stimulator cells. Thymidine incorporation was measured on day 5 by a 16-h pulse with [3H]thymidine (0.5 μCi/well; Amersham Life Science).

Migration assay. Cells (2 x 10⁵) were seeded into a Transwell chamber (8 μm; BD Falcon) in a 24-well plate and migration to CCL19 (CCL19/macroage inflammatory protein-3β; 100 μg/mL; R&D Systems) was analyzed after 3 h by counting gated DCs for 60 s on a FACSCalibur cytometer.

Cytokine determination. DCs were incubated at 1 x 10⁶ per well in 2 mL medium and treated with different stimuli as indicated. Supernatants were collected and stored at -70°C until use for cytokine determination. Cytokine concentrations were measured with commercially available two-site sandwich ELISAs for IL-6, IL-10, IL-12, and TNF-α (Immunotech) according to the manufacturer’s instructions.

PAGE and Western blotting. Nuclear extracts were prepared from DCs as described previously (22). For the preparation of whole-cell lysates, cells were lysed in a buffer containing 1% Igepal, 0.5% sodium deoxycholate, 0.1% SDS, 2 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 2 μg/mL aprotinin, and 1 mmol/L sodium orthovanadate. Protein concentrations of protein lysates were determined by bicinchoninic acid assay (Pierce, Perbio Science). For the detection of nuclear localized RelB, IFN regulatory factor (IRF)-3, and IRF-8, 20 μg of nuclear extracts were separated on a 10% SDS-polyacrylamide gel and transferred onto nitrocellulose membrane (Schleicher & Schuell). Penone S staining of the membrane was done to confirm that equal amounts of protein were present in every lane. The membrane was probed with antibodies for RelB (C-19, rat polyclonal), IRF-3 (C-20, goat polyclonal), or IRF-8 (C-19, goat polyclonal), all from Santa Cruz Biotechnology. Total PU.1, Bcl-6, and glyceraldehyde-3-phosphate dehydrogenase levels were determined by separating 20 to 30 μg...
whole-cell lysates on a 12% SDS-polyacrylamide gel and subsequent blotting to nitrocellulose membranes (22). These were probed with antibodies specific for PU.1 (H-135, rabbit polyclonal) and Bcl-6 (C-19, rabbit polyclonal), both from Santa Cruz Biotechnologies, and for glyceraldehyde-3-phosphate dehydrogenase (6C5, HyTest).

**Results**

**HDAC inhibitors affect monocyte differentiation to DCs.** We were first interested in evaluating whether HDAC inhibition would affect the differentiation of monocytes to DCs that is induced by culture in the presence of GM-CSF and IL-4. To this purpose, we made use of two synthetic HDAC inhibitors, MS-275 and VPA (1, 2), that were added to the culture medium all throughout the differentiation process. For our study, we chose concentrations of HDAC inhibitors in the pharmacologic range, where therapeutic VPA concentrations are between 50 and 100 μg/mL (23) and the reported serum concentrations of MS-275 following oral administration range between 1 ng/mL (2.7 nmol/L) and 10 ng/mL (27 nmol/L; ref. 24).

Monocyte differentiation to DCs in the presence of VPA or MS-275 was accompanied by CD14 down-regulation as it is typically observed in monocyte-derived DCs (data not shown). However, DCs grown in the presence of the HDAC inhibitors lacked expression of CD1a, a DC and Langerhans cell hallmark (Figs. 1 and 3). On exposure to VPA and MS-275 (data not shown), DCs exhibited reduced levels of the costimulation molecule CD80 (B7.1) and of the activation marker CD83 (14), whereas the expression of CD86 (B7.2), another costimulatory protein, was minimally affected (Fig. 1). HDAC inhibition also affected adhesion molecules on DCs, as shown by CD54 and DC-specific intercellular adhesion molecule 3-grabbing nonintegrin expression. Finally, we detected CD11a up-regulation and CD11c and CD33 down-regulation in DCs treated with MS-275, indicating that HDAC inhibition skew multiple myeloid markers on these antigen-presenting cells (Fig. 2).

Subsequently, we evaluated the effects of HDAC inhibition on DC activation in response to the TLR-3 ligand poly(I:C). This stimulus normally induces functional and phenotypic changes in DCs that include CD40, CD80, CD86, and CD83 up-regulation. DCs that had been grown in the presence of VPA or MS-275 showed reduced CD40, CD80, and CD83 levels compared with the control cells, whereas, once again, the effect on CD86 was much less pronounced (Figs. 1 and 3). DC-specific intercellular adhesion molecule 3-grabbing nonintegrin expression was lower in DCs generated with HDAC inhibitors, whereas CD54 levels were comparable with the control after poly(I:C) stimulation. Figure 3 shows a titration experiment with MS-275 that shows the concentration dependence of the reported effects on CD1a, CD83, and CD86 in immature (Fig. 3A) and poly(I-C)-stimulated, mature (Fig. 3B), DCs.

Importantly, we did not observe any decrease in cell viability in response to the concentrations of inhibitors used for these experiments (Fig. 4). Thus, the observed HDAC inhibitor–induced phenotype modifications seem to be only functional.

**HDAC inhibitors blunt DC allostimulatory capacity and cytokine secretion.** The effect of HDAC inhibitors on DC immunostimulatory capacity was evaluated in mixed leukocyte reaction. Treatment of differentiating DCs with the HDAC inhibitor MS-275 was found to reduce their capacity to stimulate allogeneic lymphocyte proliferation (Fig. 5A), this effect being detectable with immature DCs as well as with DCs that were activated by exposure to poly(I:C). Comparable results were obtained with VPA (Fig. 5B).

We subsequently tested whether MS-275 would also affect cytokine secretion by DCs and indeed found that poly(I:C)-induced release of TNF-α, IL-6, and IL-12 was profoundly reduced by the HDAC inhibitor (Table 1). These results are consistent with previous reports, which showed that HDAC

---

**Fig. 1.** Phenotypic changes in DCs via VPA. Adherent monocytes were cultured in the presence of GM-CSF (GM) and IL-4 with or without 100 μg/mL VPA for 6 d. At day 6, cells were stimulated with 50 μg/mL poly(I:C) or left unstimulated. Cells were harvested at day 7, washed, stained, and analyzed by flow cytometry. Solid histograms, matched isotype controls. Mean fluorescence intensity of each surface marker is reported inside the plot.
inhibitors prevent cytokine secretion in the mouse and in human peripheral blood mononuclear cells (5, 6, 10, 11). Given the importance of DC-derived cytokines for the stimulation of lymphocyte responses, inhibition of cytokine production by HDAC inhibitor is likely to contribute to impair DC immunostimulatory capacity as detected in mixed leukocyte reaction. It is noteworthy that IL-10 secretion in response to poly(I-C) was also reduced by MS-275, which rules out IL-10-mediated suppression of lymphocyte proliferation.

**Impaired migratory capacity in DCs exposed to HDAC inhibitors.** Mature DCs acquire the capacity to migrate in response to CCL19/macrophage inflammatory protein-3α due to the expression of the cognate receptor chemokine, CC motif, receptor 7 (14). This effect is believed to be crucial to ensure that DCs reach the afferent lymphoid tissues. Here, we found that exposure of DCs to increasing concentrations of the HDAC inhibitors MS-275 and VPA reduces their capacity to migrate toward CCL19 after stimulation with poly(I-C) (Fig. 6A and B).

**Fig. 2.** Effect of MS-275 on myeloid markers expressed on monocyte-derived DCs. Adherent peripheral blood monocytes were cultured in GM-CSF and IL-4 with or without 20 nmol/L MS-275. Cells were harvested at day 7, washed, stained, and analyzed by flow cytometry.

**Fig. 3.** MS-275 modulates DC markers in a concentration-dependent fashion. A, adherent peripheral blood monocytes were cultured in GM-CSF and IL-4 with or without 20 nmol/L MS-275. Cells were harvested at day 7, washed, stained, and analyzed by flow cytometry.

**Fig. 4.** Effect of MS-275 on myeloid markers expressed on monocyte-derived DCs. Adherent peripheral blood monocytes were cultured in GM-CSF and IL-4 with or without 20 nmol/L MS-275. Cells were harvested at day 7, washed, stained, and analyzed by flow cytometry.
which indicates another crucial DC function that is affected by HDAC inhibition.

**HDAC inhibition affects nuclear factor-κB and IRF signaling in DCs.** We finally investigated the effects of HDAC inhibition on intracellular signaling pathways that play a key role in controlling DC differentiation and function. HDAC inhibitors have been reported to cause a down-regulation of the hematopoietic transcription factor PU.1, which contributes to the development of thymic and myeloid DCs and of Langerhans cells in the mouse (25–27). We evaluated herein whether PU.1 expression was affected by VPA in monocyte-derived DCs; however, no significant reduction in protein levels could be detected (Fig. 7). Therefore, our data rule out PU.1 shortening as a mechanism responsible for the functional defects observed in DCs that were exposed to HDAC inhibitors.

Similarly, no significant effect of HDAC inhibitors was detected on Bcl-6, another known target of HDAC inhibitors that, besides its role in lymphomas, has also been linked to the regulation of cytokine and chemokine release in macrophages (28–30).

In subsequent experiments, we tested whether HDAC inhibition affects the activation, as detected by nuclear translocation, of the nuclear factor-κB (NF-κB) subunit RelB and of IRF-3 and IRF-8 in DCs. The RelA NF-κB subunit is regulated by acetylation and HDAC inhibitors promote its localization to the nucleus and thereby its transcriptional activity (31, 32). Conversely, no information is available on the effect of HDAC inhibitors on RelB and c-Rel, where RelB is especially known for its importance in DC generation and function (33–36). Similarly, hints exist that HDAC inhibitors may prevent activation of the IFN pathway and IFN-γ secretion, although this evidence is challenged by data obtained in osteoclasts (37, 38). Our data indicate that exposure of DCs to VPA results in reduced levels of RelB protein in nuclear extracts (Fig. 8). Plus, VPA also blocked RelB nuclear relocalization in response to poly(I-C) in a concentration-dependent manner. On the other hand, IL-1 receptor-associated kinase-1 (IRAK-1) down-regulation in response to poly(I-C) was not affected by VPA, suggesting that the effects of HDAC inhibition on RelB are probably mediated downstream of MyD88 and IRAK-1 (39). Finally, IRF-3 and IRF-8, which have been involved in DC differentiation, IL-12 production, and DC migration (40–42), were also affected by HDAC inhibition, as VPA reduced their nuclear levels, this effect being most evident in poly(I-C)-activated DCs (Fig. 8).

**Discussion**

We show here that HDAC inhibitors have profound effects on human DCs in that, when given to DCs during the differentiation process from monocytes, they lead to the acquisition of a skewed phenotype with impaired immunostimulatory capacity. Our data therefore confirm that HDAC inhibition could lead to immunosuppression possibly by acting at the antigen-presenting cell level.

In a recent report, Brogdon et al. (13) did genome-wide gene expression analysis to detect the effects of the HDAC inhibitor LAQ824 in human macrophages and monocyte-derived DCs stimulated with lipopolysaccharide. They found that HDAC inhibition selectively affects a group of genes involved in stimulation and chemotaxis of Th1 lymphocytes, such as the costimulation molecules CD40 and SLAM, the polarization cytokines IL-12, IL-15, and EBI3, and the chemokines IP10 and MIG. Conversely, signals mediating Th2 cells activation, such as CD86, MDC, and I-309, were unaffected. Accordingly, IFN-γ production and migration of Th1 cells were impaired, whereas no inhibition of IL-4 secretion and migration by Th2 effectors was detected. Our results are in line with these observations in that we also found reduced CD40 expression and IL-12 secretion in DCs exposed to HDAC inhibitors. Moreover, our experiments also indicate that the costimulatory molecule CD86 is preferentially expressed by these cells. However, compared with Brogdon et al. observations, we found more dramatic effects of HDAC inhibitors on DC differentiation and function. In our hands, these compounds profoundly impaired the expression of CD1a, a DC hallmark, CD80, a key...
costimulation signal, and CD83, which is typically expressed on mature DCs (14). DC secretion of the proinflammatory cytokines TNF-α and IL-6 was blunted by HDAC inhibition, as was IL-10 production. Finally, DCs grown in the presence of VPA or MS-275 had impaired immunostimulatory capacity and migration to CCL19. Although some of these aspects were not evaluated in the previous report, it is possible that the prolonged exposure times we used in our experiments (7 days) over those used by Brogdon et al. may also play a role in determining how strongly HDAC inhibitors will affect DC function.

It is of interest that previous studies evaluating the immunologic effects of HDAC inhibitors on acute myelogenous leukemia cells found that these drugs would actually favor the acquisition of DC features and enhance the expression of costimulation molecules, such as CD40, CD80, and CD86 (43, 44). Similar effects have also been described for multiple myeloma and melanoma cells (45). The reasons underlying the discrepancy between these studies and the effects observed with primary DCs are unclear. It can be reasoned that HDACs may have different roles in cancerous compared with normal tissues. In tumors, HDAC activity is frequently deregulated leading to skewed gene expression and possibly to phenotypes that favor immune escape. HDAC inhibitors could then relieve these effects. Conversely, under physiologic conditions, HDACs may come into play by fine-tuning the expression of genes involved in DC differentiation and/or by directly intervening in important proinflammatory signaling cascades, including those mediated by NF-κB and the IRFs.

NF-κB is a transcription factor that controls the expression of numerous inflammatory mediators, such as inducible nitric oxide synthase, IL-6, IL-8, IL-10, and IL-12. The effects of HDAC inhibitors on NF-κB seem to be complex and may also vary depending on the cell type and on the experimental context. Blockade of NF-κB transcriptional activity via HDAC inhibitors has been reported, and the proposed mechanism for this effect

**Table 1.** MS-275 effect on cytokine secretion by DCs

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>poly(I-C)</th>
<th>poly(I-C)+MS 1</th>
<th>poly(I-C)+MS 10</th>
<th>poly(I-C)+MS 20</th>
<th>poly(I-C)+MS 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>130.9</td>
<td>&gt;20,000</td>
<td>10,347.4</td>
<td>10,737.16</td>
<td>3,242.4</td>
<td>838.2</td>
</tr>
<tr>
<td>IL-6</td>
<td>48.2</td>
<td>&gt;20,000</td>
<td>17,830.4</td>
<td>12,657.6</td>
<td>4,658.5</td>
<td>2,730.2</td>
</tr>
<tr>
<td>IL-10</td>
<td>86</td>
<td>1,270</td>
<td>150</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>IL-12</td>
<td>&lt;0.1</td>
<td>349.3</td>
<td>85</td>
<td>72</td>
<td>85.6</td>
<td>24.7</td>
</tr>
</tbody>
</table>

NOTE: Adherent monocytes were cultured with GM-CSF and IL-4, with or without addition of MS-275 at the indicated concentrations (nmol/L) for 6 d. Subsequently, cells were stimulated for 24 h with poly(I-C) (50 μg/mL). Cytokine concentrations (picograms per milliliter) in the supernatants were determined using commercially available ELISAs.

Abbreviation: MS, MS-275.
includes impaired NF-κB nuclear translocation, IκBα stabilization, and proteasome inhibition (46, 47). On the other hand, HDAC inhibitor–induced NF-κB activation has also been described as a result of RelA hyperacetylation, which in turn promotes RelA relocation to the nucleus (31, 32). In our study, we focused on RelB given its importance in DC biology (33–36). Our results indicate that DC exposure to HDAC inhibitors leads to reduced nuclear levels of RelB under basal conditions and after stimulation with the TLR ligand poly(I-C), indicating an important inhibitory effect that may well account for the impaired cytokine production and the reduced immunostimulatory capacity in DCs that were exposed to these drugs. NF-κB activation in response to TLR triggering is believed to be dependent on signal transduction through MyD88 and IRAK-1, where prolonged TLR stimulation was reported to lead to IRAK-1 down-regulation (39). Therefore, the observation that IRAK-1 levels become normally down-regulated in response to poly(I-C) even in the presence of the HDAC inhibitor suggests that TLR signaling upstream of RelB may not be affected by HDAC inhibition. Conversely, RelB function may more critically depend on HDAC activity.

The IRFs are a family of transcription factors involved in the expression of type I IFNs in cells that are exposed to viruses and to pathogen-derived products of bacterial origin. Importantly, IRFs are activated not only by TLR triggering but also by endogenous signals, such as CD40L and cytokines. The IFN pathway is presently attributed great importance in antigen-presenting cell function because different IRFs, including IRF-3 and IRF-8/ICSBP1, turned out to be required for DC generation, migration, and cytokine production (40–42). Previous experiments indicated that HDAC activity plays a role in the IFN pathway (37, 38, 48, 49). However, the exact function of HDACs in this context and how HDAC inhibitors ultimately affect the activity of IRFs is presently poorly understood. Nusinzon et al. (37) showed that virus-induced expression of the IFN-β gene is blocked by the HDAC inhibitor trichostatin A. These same authors went on to show that whereas activation, nuclear translocation, and DNA binding of IRF-3 were not impaired by HDAC inhibition, HDAC activity was required downstream of IRF-3 DNA binding by the IRF-responsive positive regulatory domain. Finally, this study shows that different HDACs have different effects on IRF-3 and NF-κB, where HDAC6 activates IRF-3, and HDAC1 and HDAC8 repress both IRF-3 and NF-κB. However, this model has been challenged by another report showing that HDAC inhibitors augment INF-β secretion by osteoclasts (38). We found here that the HDAC inhibitor VPA reduces IRF-3 and IRF-8 levels in nuclear extracts, this effect being more evident in DCs that were stimulated with poly(I-C). Our data thus indicate a profound effect of HDAC inhibitors on these IRFs and suggest that HDAC activity is required for the nuclear translocation of IRFs, at least in this cell model. Because both IRF-3 and IRF-8 have a role in the control of IL-12 production and IRF-8/ICSBP1 is required for DC and Langerhans cell migration to the lymph nodes (40–42), obstruction of these IRFs by HDAC inhibitors will add to RelB inhibition and further undermine DC–induced immune responses.

Fig. 6. MS-275 prevents DC migration toward CCL19/macrophage inflammatory protein-3. A, adherent monocytes were incubated for 7 d in medium containing GM-CSF and IL-4, with or without MS-275 at the indicated concentrations. poly(I-C) (50 μg/mL) was added at day 6. DCs were harvested at day 7 and migration toward CCL19 was analyzed using transwell chambers. B, DCs were generated from adherent monocytes with GM-CSF and IL-4 with or without addition of VPA. poly(I-C) (50 μg/mL) was added at day 6 and cells were harvested at day 7 and used in the migration assay.
In summary, our data indicate that HDAC inhibition affects DC differentiation and reverts these antigen-presenting cells to a less stimulatory mode. This effect is possibly achieved by interference with signaling pathways that are directly involved in immune responses and in DC biology, including NF-κB and the IRFs. HDAC inhibitors may weaken antimicrobial and antitumor immune responses. On the other hand, HDACs seem promising therapeutic targets for inflammatory and immune disorders where a control of DC immunogenicity is desired.

Acknowledgments
We thank Sylvia Stephan and Bruni Schuster for the excellent technical assistance.

References
25. Lanibee RN, Klembsz MJ. Loss of PU.1 expression and glyceroldehyde-3-phosphate dehydrogenase levels were detected by immunoblotting.


Histone Deacetylase Inhibitors Affect Dendritic Cell Differentiation and Immunogenicity

Alessio Nencioni, Julia Beck, Daniela Werth, et al.


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/13/13/3933

Cited articles  This article cites 49 articles, 29 of which you can access for free at: http://clincancerres.aacrjournals.org/content/13/13/3933.full#ref-list-1

Citing articles  This article has been cited by 18 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/13/13/3933.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.