Histone Hyperacetylation Is Associated with Amelioration of Experimental Colitis in Mice

Rainer Glauben, Arvind Batra, Inka Fedke, Martin Zeitz, Hans A. Lehr, Flavio Leoni, Paolo Mascagni, Giamila Fantuzzi, Charles A. Dinarello and Britta Siegmund

J Immunol 2006; 176:5015-5022; doi: 10.4049/jimmunol.176.8.5015
http://www.jimmunol.org/content/176/8/5015

References
This article cites 30 articles, 8 of which you can access for free at:
http://www.jimmunol.org/content/176/8/5015.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Histone Hyperacetylation Is Associated with Amelioration of Experimental Colitis in Mice

Rainer Glauben,* Arvind Batra,* Inka Fedke,* Martin Zeitz,* Hans A. Lehr, ‡ Flavio Leoni,§ Paolo Mascagni,§ Giamila Fantuzzi, † Charles A. Dinarello, † and Britta Siegmund2*†

Inhibitors of histone deacetylases (HDAC) are being studied for their antiproliferative effects in preclinical cancer trials. Recent studies suggest an anti-inflammatory role for this class of compounds. Because inflammatory bowel disease is associated with an increased risk of malignancies, agents with antiproliferative and anti-inflammatory properties would be of therapeutic interest. HDAC inhibitors from various classes were selected and evaluated for their in vitro capacity to suppress cytokine production and to induce apoptosis and histone acetylation. Valproic acid (VPA) and suberylanilide hydroxamic acid (SAHA) were chosen for further studies in dextran sulfate sodium- and trinitrobenzene sulfonic acid-induced colitis in mice. In vitro, inhibition of HDAC resulted in a dose-dependent suppression of cytokine synthesis and apoptosis induction requiring higher concentrations of HDAC inhibitors for apoptosis induction compared with cytokine inhibition. Oral administration of either VPA or SAHA reduced disease severity in dextran sulfate sodium-induced colitis. The macroscopic and histologic reduction of disease severity was associated with a marked suppression of colonic proinflammatory cytokines. In parallel to the beneficial effect observed, a dose-dependent increase in histone 3 acetylation at the site of inflammation was shown under VPA treatment. Furthermore, SAHA as well as VPA treatment resulted in amelioration of trinitrobenzene sulfonic acid-induced colitis, which was associated with an increase of apoptosis of lamina propria lymphocytes. Inhibitors of HDAC reveal strong protective effects in different models of experimental colitis by inducing apoptosis and suppressing proinflammatory cytokines, thereby representing a promising class of compounds for clinical studies in human inflammatory bowel disease. The Journal of Immunology, 2006, 176: 5015–5022.

Inhibitors of nuclear histone deacetylases (HDAC)1 have been described for their suppression of cancer cell proliferation in vitro and reduction of experimental tumor growth in vivo (1–3). Several HDAC inhibitors are in clinical trials for a variety of solid and hemological cancers (4). The classes of compounds that are identified as HDAC inhibitors now include short-chain fatty acids, hydroxamic acids, cyclic tetrapeptides, and benzamides (2).

A common mechanism of HDAC inhibitors is alteration in gene transcription. Chromatin remodeling following histone acetylation or deacetylation appears to play a central role in the regulation of several genes. Acetylation of core nucleosomal histones is a posttranscriptional modification mediated by opposing activities of histone acetyltransferases and HDAC (5). HDAC inhibitors modify gene expression positively or negatively in a cell- and gene-specific manner (6). HDAC inhibitors increase the accumulation of hyperacetylated histones H3 and H4, directly influencing chromatin structure and, thereby, the relationship of the nucleosome and the gene promoter elements (6).

Suberylanilide hydroxamid acid (SAHA)—the classic member of the class of hydroxamic acids—has potent anti-inflammatory activities, both in vitro and in vivo (7). HDAC inhibition was associated with a significant suppression of proinflammatory cytokines (7). These anti-inflammatory properties could be confirmed by studies demonstrating that treatment with SAHA resulted in a significant reduction of disease severity in the murine model of systemic lupus erythematosus, the MLR-lpr/lpr mouse (6, 8). The anti-inflammatory effects described for HDAC inhibitors are so far limited to SAHA and trichostatin A, both members of the class of hydroxamic acids. Thus, it remains to be clarified whether the anti-inflammatory effects of HDAC inhibition are restricted to this class or whether inhibition of HDAC in general results in suppression of cytokine production.

Inflammatory bowel disease represents a chronic disease accompanying patients throughout their life (9). The etiology and pathogenesis have not been definitely clarified. Various studies have led to the consensus hypothesis that, in genetically predisposed individuals, exogenous and host factors together result in a chronic state of dysregulated mucosal immune function that is further modified by specific environmental factors (9). Ulcerative colitis and Crohn’s disease are the two major types of inflammatory bowel disease. Crohn’s disease is regarded as a Th1-polarized disease; ulcerative colitis is considered as Th2-polarized disease (10). Thus, in Crohn’s disease, disease severity can be ameliorated by Th1 inhibitory strategies, such as the neutralizing anti-TNF-α Ab, infliximab (11). For ulcerative colitis, anti-Th2 strategies such as IL-13-neutralizing compounds are efficacious in animal models and are currently under clinical investigation (12). Long-term complications of inflammatory bowel diseases include an increased incidence of malignancies. Therefore, a therapeutic compound...
which includes antiproliferative and anti-inflammatory properties would open new therapeutic avenues.

The present study aims to investigate the colon-specific anti-inflammatory effects of HDAC inhibitors. To approach this question, we first evaluated whether the anti-inflammatory effect of HDAC inhibition is limited to the class of hydroxamic acids or whether this can be observed in general during inhibition of HDAC. In the next step, the effect of HDAC inhibition in dextran sodium sulfate (DSS)-induced colitis was investigated using two different HDAC inhibitors from two classes selected after in vitro experiments. In addition, to further evaluate the effects in a Th1-specific model of intestinal inflammation, both compounds were evaluated in the model of Th1-dependent trinitrobenzenesulfonic acid (TNBS)-induced colitis.

Materials and Methods

Reagents

RPMI 1640 and FCS were obtained from Invitrogen Life Technologies. RPMI 1640 was supplemented with 2% FCS, 2 mM l-glutamine (Invitrogen Life Technologies), 50 U/ml penicillin, and 50 μg/ml streptomycin (both from Sigma-Aldrich). LPS (Escherichia coli 055:B5) and Con A (type IV-S) were obtained from Sigma-Aldrich.

Mice

Animal protocols were approved by the animal studies committee of the University of Colorado Health Sciences Center or by the regional animal study committee of Berlin, Germany. Six- to 8-week-old female C57BL/6J were obtained from The Jackson Laboratory or Harlan Winkelmann. The animals were housed at controlled temperature with light-dark cycles, fed ad libitum and acclimatized before being studied. Upon the end of an experimental period, mice were killed by cervical dislocation under CO2 anesthesia.

Compound preparation

SAHA was prepared by reacting suberoyl dichloride with one equivalent of aniline and then by hydrolysis of the reaction product to form the monoamide monocacid. Activation of the carboxyl group of the latter and its reaction with hydroxylamine yielded SAHA. The synthesis was performed by the Chemical Department of Ialfarmaco as described in WO 95/31977 PTC/US95/08454 and WO 95/31977 PTC/US95/06554 (13). After synthesis, the structure of SAHA was confirmed by mass spectrometry and proton nuclear magnetic resonance spectroscopy. The compound was ≥99% pure as assessed by HPLC. SAHA was added to water at 10 mg/ml, heated to 95°C until dissolved and kept at ~7°C. Valproic acid (Orifril i.v.) was purchased from Desitin; trichostatin A and apicidin were from Sigma-Aldrich.

Induction of acute DSS-induced colitis

C57BL/6J mice were fed 3.5% DSS (molecular mass 40 kDa; ICN) dissolved in sterile, distilled water ad libitum from days 1 to 5, followed by 2 days of regular drinking water, resulting in a 8-day experimental period (14).

Induction of TNBS-induced colitis

To induce TNBS colitis, 2.0 mg of TNBS (pH 1.5–2.0; Sigma-Aldrich) in 50% ethanol was administered per rectum through a 3.5 F catheter inserted into the rectum. The catheter tip was ~3–4 cm proximal to the anal verge, 150 μl of fluid (TNBS/ethanol or 50% ethanol alone) was slowly instilled into the colon, and the mouse was held in a vertical position for 30 s.

Treatment schedule

SAHA, valproic acid (VPA), or the vehicle was administered via oral gavage in 200 μl once daily starting at day 1 of colitis induction. The first administration of either SAHA or VPA was performed 1 h before the first exposure to DSS or induction of TNBS colitis, respectively. In the therapeutic model of DSS-induced colitis, DSS was added to drinking water for days 1–5 followed by regular drinking water. Treatment with SAHA (once daily) was started at the end of day 5 and continued until the end of experiment.

Clinical assessment of colitis

Body weight, the presence of occult or gross blood per rectum, and stool consistency were determined daily. These parameters were assessed by trained individuals blinded to the treatment groups. Baseline body weight, stool consistency, and rectal bleeding were determined before the initiation of DSS or TNBS exposure. Weight change during the experimental time course was calculated as change in percent of the weight at baseline. For stool consistency, 0 points were assigned for well-formed pellets, 2 points for pasty and semiformal stools which did not adhere to the anus, and 4 points for liquid stools that did adhere to the anus. Bleeding was scored 0 for no blood using hemoccult (Beckman Coulter), 2 points for positive hemoccult, and 4 points for gross bleeding. Mice were sacrificed by cervical dislocation; the entire colon was removed from the cecum to the anus and colon length was measured as a marker of inflammation.

Histological scoring

Post mortem, the entire colon was excised and two segments of the ascending, transversing, and descending part of the colon were collected and fixed in 10% buffered formalin for histological analysis. Paraffin sections were stained with H&E. Four to six colon rings were obtained from each 1-cm colon segment and were thus available for histological examination. Histological scoring was performed in a blinded fashion by a pathologist (H. A. Lehr) as a combined score of inflammatory cell infiltration (0–3) and tissue damage (0–3) resulting in a score ranging from 0 to 6, 0 representing no signs of inflammation and 6 maximum disease, as described previously in detail (15, 16). The mean histologic score of the three parts of the colon was calculated and is shown.

Colon organ culture

The total colon culture was performed as described previously in detail (14). Briefly, a segment of the colon was removed, cut open longitudinally, and washed in PBS containing penicillin and streptomycin. The colon was then further cut into strips of ~1 cm2 and placed in 24 flat-bottom well culture plates containing 1 ml of fresh RPMI 1640 supplemented with penicillin and streptomycin. Strips were incubated at 37°C for 20 h. Culture supernatants were harvested and assayed for cytokines. Protein concentration of the supernatant was quantified using the Bio-Rad protein assay (Bio-Rad).

Cytokine measurement

The murine cytokines TNFα, IFN-γ, IL-6, and IL-10 were measured using a specific ELISA (BD Pharmingen). The range of quantification is 20 pg/ml to 10 ng/ml.

Isolation of lamina propria mononuclear cells (LPMC)

Mice were killed by isoflurane inhalation and cervical dislocation and the colon was trimmed of fat, mesenteric tissue, and Peyer’s patches. LPMCs were subsequently isolated as described previously (14). Briefly, the colon was opened longitudinally and cut into 5-mm cross-wise pieces. The tissue was incubated in calcium- and magnesium-free HBSS containing 1 mmol/L EDTA for 30 min under vigorous vortexing at room temperature; this step was repeated once. Supernatants of the EDTA steps were discarded and the remaining tissue was washed twice and subsequently incubated for 60 min at 37°C in calcium- and magnesium-free HBSS supplemented with 5% FCS and 100 U/ml collagenase types II and VIB and 300 U/ml hyaluronidase (Sigma-Aldrich). Cells were separated from tissue debris by filtration through a 100-μm cell strainer (BD Pharmingen) followed by purification through a discontinuous 44/67% Percoll gradient (Sigma-Aldrich) for 20 min at 600 × g. For stimulation experiments, LPMCs were incubated in the presence or absence of Con A (5 μg/ml) for 24 h.

Cell culture

Splenocytes were isolated from freshly collected C57BL/6J spleens using standard procedures. CD4+ T cell cultures were isolated from murine spleens using the mouse CD4+ T cell isolation kit as described in the protocol provided by Miltenyi Biotec. Murine monocytes were isolated from murine spleens using the CD11b MACS isolation kit (Miltenyi Biotec). The respective cells were preincubated with increasing concentrations of the various HDAC inhibitors for 2 h and subsequently stimulated with LPS (1 μg/ml) or Con A (5 μg/ml) as indicated for 48 h (ELISA) or overnight for Western blot analysis.

Analysis of apoptosis by annexin binding

Exposure of phosphatidylinerine at the outer plasma cell membrane of apoptotic cells was quantified by surface annexin V staining. Briefly, cells were washed in staining buffer, resuspended in 200 μl of binding buffer (10 mmol/L HEPES (pH 7.4), 2.5 mmol/L CaCl2, 140 mmol/L NaCl), and incubated with 0.5 μg/ml Annexin V-FITC (BD Pharmingen) and propidium
iodide (PI; 5 μg/ml) for 15 min in the dark. Cells were washed again and resuspended in binding buffer. Flow cytometric analysis was performed on a FACSCalibur (BD Pharmingen) using the CellQuest analysis program (BD Pharmingen).

Western blot analysis

For liver homogenates, 200 mg of liver were homogenized in radioimmunoprecipitation buffer and the supernatants were applied for Western blot analysis. For LPMC and splenocytes, 2 million cells per condition were used as indicated. At the end of the incubation period, cells were lysed in radioimmunoprecipitation buffer. Anti-acetyl histone (Lys9; Cell Signaling) was used in a 1/1000 dilution to detect acetyl histone 3 and anti-β-actin (Sigma-Aldrich) was used in a 1/2000 dilution. As secondary Ab, an HRP-labeled polyclonal goat anti-rabbit Ab in a dilution of 1/2000 (DakoCytomation) was used. For detection, the ECL light detecting kit was applied (Amersham Biosciences).

Statistical analysis

Data are expressed as mean ± SEM. Statistical significance of differences between treatment and control groups were determined by factorial ANOVA and a Bonferroni-Dunn procedure as a posthoc test. Statistical analyses were performed using Stat-View 4.51 software (Abacus Concepts).

Results

Dose-dependent comparison of apoptosis induction, cytokine suppression, and histone hyperacetylation

To evaluate the effect of HDAC inhibitors on cytokine production, apoptosis, and histone hyperacetylation, splenocytes were isolated from C57BL/6J mice and incubated in the absence or presence of increasing concentrations of different HDAC inhibitors. Besides the reference substance SAHA (hydroxamic acid), three additional HDAC inhibitors from various classes were evaluated, namely trichostatin A (TsA; hydroxamic acid), apicidin (cyclic tetrapeptide), and VPA (short-chain fatty acid). The preincubation period of 2 h with one selected HDAC inhibitor was followed by LPS stimulation (1 μg/ml). After 24 h, apoptosis and IFN-γ synthesis were
A dose-dependent increase in suppression of IFN-γ synthesis as well as apoptosis induction was observed for all HDAC inhibitors tested. However, a 10 times higher concentration was required for apoptosis induction when compared with suppression of IFN-γ synthesis (Fig. 1). IL-1β, IL-10, and TNF-α suppression went in parallel with the IFN-γ suppression while MIP-1α remained unchanged (data not shown). To characterize whether HDAC inhibitors lead to a dose-dependent increase in histone acetylation, acetylation of histone 3 was analyzed by Western blot analysis. As shown in Fig. 1, a dose-dependent increase in acetylation of histone 3 was observed in LPS-stimulated cells in the presence of increasing concentrations of the various HDAC inhibitors. Similar data were obtained stimulating murine splenocytes with Con A (5 μg/ml) or anti-CD3/CD28 (data not shown).

To evaluate whether HDAC inhibition is directly affecting T cells as well as monocytes, CD4+ T cells as well as monocytes were isolated from murine spleens as described in Materials and Methods. Both cell populations were preincubated for 2 h with either SAHA or VPA (Fig. 1C). This preincubation period was followed by either LPS (1 μg/ml) or Con A (5 μg/ml) stimulation as indicated (Fig. 1C). In monocytes, HDAC inhibition resulted in a profound suppression of TNF-α synthesis after LPS stimulation. In parallel, in CD4+ T cells, HDAC inhibition was followed by a significant decrease in IFN-γ synthesis after Con A stimulation. The pattern for apoptosis induction went in parallel with the results shown in Fig. 1A (data not shown). Thus, one can conclude that inhibition of HDAC exerts an anti-inflammatory potency in CD4+ T cells as well as monocytes.

Apoptosis induction and cytokine suppression mediated by HDAC inhibition in LPMC

To confirm that inhibition of HDAC induces similar effects in LPMC, LPMC were isolated from healthy mice as described in Materials and Methods. Cells were stimulated with Con A (5 μg/ml) for 24 h and IFN-γ production as well as apoptosis were evaluated subsequently. Apoptosis was evaluated by flow cytometry via Annexin V/PI staining, IFN-γ production was determined by ELISA as described in Materials and Methods. Bars indicate mean ± SEM; n = 5/group; *, p < 0.05; **, p < 0.01 vs Con A-stimulated cells.

FIGURE 2. In vitro effect of HDAC inhibition in LPMC. LPMC were isolated from healthy mice as described in Materials and Methods. Cells were stimulated with Con A (5 μg/ml) for 24 h and IFN-γ production as well as apoptosis were evaluated subsequently. Apoptosis was evaluated by flow cytometry via Annexin V/PI staining, IFN-γ production was determined by ELISA as described in Materials and Methods. Bars indicate mean ± SEM; n = 5/group; *, p < 0.05; **, p < 0.01 vs Con A-stimulated cells.

FIGURE 3. Local hyperacetylation during treatment with VPA in DSS-induced colitis. Mice were exposed to 3.5% DSS for 6 days followed by 2 days of regular drinking water. Mice were treated with either vehicle or VPA at increasing concentrations (40, 200, or 400 mg/kg/day). A, Weight was determined daily and expressed as a percentage of initial body weight. Data are mean ± SEM; n = 10/group; *, p < 0.05 vs DSS vehicle, in vivo experiments were performed twice. B, At the end of the experiment, LPMC were isolated and Western blot analysis for histone 3 acetylation was performed as described in Materials and Methods. Vehicle-treated as well as VPA-treated non-DSS controls were summarized and a representative band is shown in the control lane because there was no difference between these experimental groups. Equal loading was confirmed by β-actin staining. The blot shown is representative of four independent blots performed. C, At the end of the experiment, liver homogenates, splenocytes, and LPMC of DSS-exposed animals treated or untreated with 400 mg/kg/day VPA were compared via Western blot analysis for histone 3 acetylation. Equal loading was confirmed by β-actin staining. The blot shown is representative of four independent blots performed.
either SAHA or VPA as indicated, and subsequently stimulated with Con A (5 μg/ml). As shown in Fig. 2, in the presence of 1 μM SAHA or VPA a significant increase in apoptosis was observed when compared with Con A-stimulated LPMC. In parallel, SAHA as well as VPA pretreatment was followed by a significant decrease in IFN-γ synthesis. Remarkably, as observed for the splenocytes, 0.1 μM SAHA already resulted in a marked suppression of IFN-γ while 1 μM was required to detect a significant increase in apoptosis. Thus, the effects observed during HDAC inhibition in stimulated LPMC were comparable to the ones described above for splenocytes or purified CD4+ T cells as well as monocytes. To further explore the anti-inflammatory potency of HDAC inhibition in intestinal inflammation, the effect of SAHA or VPA treatment in different models of colitis was evaluated.

Comparison of SAHA and VPA in DSS-induced colitis

In the subsequent experiments, SAHA and VPA—two HDAC inhibitors from distinct classes—were compared in DSS-induced colitis. Two inhibitors from various classes were chosen to distinguish between class-specific effects and effects mediated by HDAC inhibition. Mice were treated for the entire experimental period with either vehicle, 200 mg/kg VPA, or 50 mg/kg SAHA once daily via oral feeding. The treatment dose for SAHA was chosen based on previous in vivo studies (7), whereas for VPA, preliminary dose-response studies were performed based on the published literature, the results of which will be described in further detail in the following paragraph (17). A dose of 200 mg/kg VPA was chosen for additional experiments (Fig. 3A). SAHA as well as VPA treatment resulted in a significant protection from weight loss (94.1 ± 0.9 and 88.4 ± 2.1% of initial body weight) when compared with DSS-fed vehicle-treated mice (78.9 ± 3.1% of initial body weight). This was confirmed by a significant reduction in histological signs of inflammation (Fig. 4B). Because no histological difference was detected between SAHA- and VPA-treated mice, one representative section for both groups is shown (Fig. 4B). In addition, colon shortening, an indicator of inflammation in this model, was prevented in mice treated with either SAHA or VPA (Fig. 4C). The non-DSS control mice were treated with either vehicle, SAHA (50 mg/kg) or VPA (200 mg/kg) and were summarized in the figure as non-DSS control because, with the concentrations used in our experiments, no side effects were observed.

Cytokine release from whole colon tissue cultures at the site of inflammation was compared as shown in Fig. 4D. Both SAHA and VPA treatment resulted in a profound suppression of IFN-γ as well as IL-6 production compared with vehicle-treated DSS-exposed mice (Fig. 4D). Treatment with either SAHA or VPA did not influence IL-10 concentrations (data not shown).

Local hyperacetylation after VPA treatment in DSS-induced colitis

Inhibition of HDAC is associated with histone hyperacetylation. To confirm that local hyperacylation occurs during therapy with an HDAC inhibitor in experimental colitis, mice were treated with increasing concentrations of VPA (40, 200, and 400 mg/kg) as indicated (Fig. 3). In the absence of DSS, treatment with VPA (400 mg/kg) did not result in an increase in acetylation as shown in Fig. 3. In DSS-treated mice, 200 mg/kg was the most effective concentration with regard to weight loss (Fig. 3A) as well as colon shortening, cytokine production, and histologic scores (data not shown). Administration of 400 mg/kg VPA to healthy mice results in weight loss of ~10% body weight over the 10-day period. At the end of the experiment, LPMC were isolated and histone 3 acetylation was evaluated by Western blot analysis. As demonstrated in

![FIGURE 4](http://www.jimmunol.org/)

Effect of HDAC inhibitors in DSS-induced colitis. Mice were exposed to 3.5% DSS for 6 days followed by 2 days of regular drinking water. Mice were treated either with vehicle, VPA (200 mg/kg body weight), or SAHA (50 mg/kg body weight) once daily starting on the first day of the experiment. A, Weight was determined daily and expressed as percentage of initial body weight. B, The histological score was evaluated as described in Materials and Methods. C, On day 8, colon length was measured. D, At day 8, colon culture was performed as described in Materials and Methods and IFN-γ as well as IL-6 was determined in the supernatant. Data are mean ± SEM, n = 10/group; **, p < 0.01; and ***, p < 0.001 vs DSS vehicle; in vivo experiments were performed twice.
At day 10 colon culture was performed as described in vehicle; in vivo experiments were performed twice.

Materials and Methods

B of initial body weight.

Mice were exposed to 3.5% DSS for 5 consecutive days followed by 5 days of regular drinking water. SAHA treatment (50 mg/kg body weight) once every day with either 200 mg/kg VPA or 50 mg/kg SAHA orally. As shown in Fig. 6A, both SAHA- and VPA-treated mice regained weight starting from days 5 and 6, respectively, while vehicle-treated mice continued losing weight until the end of experiment. 

Therapeutic efficacy of SAHA treatment in DSS-induced colitis

To evaluate whether inhibition of HDAC exerts equal anti-inflammatory potency even after induction of colitis, SAHA treatment was initiated at the end of day 5 of DSS-induced colitis. As demonstrated in Fig. 5, SAHA treatment resulted in a significantly faster weight recovery when compared with vehicle-treated mice (Fig. 5A). This went in parallel with the histological signs of colitis (Fig. 5B) as well as less colon shortening in the SAHA-treated group (Fig. 5C). In addition, the local production of proinflammatory cytokines was suppressed in SAHA-treated mice when compared with DSS vehicle-treated animals (Fig. 5D).

Inhibition of HDAC in Th1-mediated TNBS-induced colitis

We next investigated the possible anti-inflammatory effects of HDAC inhibitors in a model of T cell-mediated intestinal inflammation, the model of TNBS-induced colitis. Mice were treated once daily with either 200 mg/kg VPA or 50 mg/kg SAHA orally. As shown in Fig. 6A, both SAHA- and VPA-treated mice regained weight starting from days 5 and 6, respectively, while vehicle-treated mice continued losing weight until the end of experiment. This rapid recovery was associated with a significant reduction in the spontaneous production of IL-6 and IFN-γ from colon cultures (Fig. 6B). A similar reduction was observed for IL-1β as well as MIP-2 (data not shown). There was no induction of IL-10 in this model (data not shown). As shown in Fig. 6C, SAHA treatment resulted in a significant reduction of the histological score, whereas a tendency toward reduction of histologic signs of inflammation was achieved by VPA treatment. As indicated in Fig. 6D, amelioration of disease was associated with a significant increase in the rate of apoptosis in LPMC obtained from TNBS SAHA- or VPA-treated mice when compared with LPMC from TNBS vehicle-treated animals.

Discussion

The present study demonstrates that inhibition of HDAC in vitro results in a dose-dependent suppression of proinflammatory cytokines, induction of apoptosis, as well as a local increase in histone acetylation. In vivo, treatment with either SAHA or VPA resulted in amelioration of disease in DSS-induced colitis as indicated by a significant reduction in weight loss and histological signs of inflammation as well as suppression of IFN-γ as well as IL-6, IL-1β, and MIP-2 (IL-1β and MIP-2 are not shown) in proinflammatory cytokines. In agreement with the in vitro results, increasing doses of VPA led to a parallel increase in histone 3 acetylation. Furthermore, both VPA and SAHA proved to be efficacious in the T cell-dependent TNBS-induced colitis model. In this model, disease amelioration was associated with prevention from weight loss, a decrease in histological signs of inflammation, suppression of the proinflammatory cytokines IFN-γ and IL-6, and, in addition, an increase in the rate of apoptosis in LPMC.

A previous study from our group demonstrated that the HDAC inhibitor SAHA has broad anti-inflammatory properties via suppression of cytokines (7). These anti-inflammatory properties were demonstrated in vitro, by inhibiting the secretion of proinflammatory cytokines in LPS- and cytokine-stimulated human PBMC as well as cytokine-induced NO in mouse macrophages (7). In addition, SAHA reduced circulating cytokine concentrations during endotoxemia in mice and prevented hepatocellular damage in Con A-injected mice (7). Remarkably, in vitro as well as in vivo, SAHA suppressed production of proinflammatory cytokines at significantly lower concentrations compared with those required to achieve antiproliferative effects on tumor cells (1, 7). In the present study, these findings were confirmed using different HDAC inhibitors from various classes, thus strengthening the concept of that HDAC inhibition is a relevant mechanism mediating anti-inflammatory effects rather than a class-specific effect of SAHA. Furthermore, inhibition of HDAC was also efficacious, with regard to cytokine suppression and apoptosis induction, in in vitro-stimulated LPMC.

In addition, HDAC treatment resulted in a dose-dependent increase of histone 3 acetylation in in vitro-stimulated splenocytes, as well as in LPMC isolated from DSS-exposed VPA-treated mice (Fig. 3). However, histone 3 hyperacetylation was only observed at the site of inflammation, because no histone 3 hyperacetylation could be detected in either liver homogenates or splenocytes (Fig. 5), thus suggesting the requirement of cell activation in the presence of an HDAC inhibitor to achieve hyperacetylation. Butler et al. (1) previously demonstrated, using a prostate cancer model in mice, that a concentration of 25 or 50 mg/kg SAHA in vivo increased total acetylation of histone 3 and 4. Recent data indicate that T cell polarization is associated with a characteristic
promoter-specific acetylation pattern (18). The precise mechanisms involved will require further investigations.

The observed amelioration of disease in different models of experimental colitis was associated with a significant suppression of proinflammatory cytokines in the colon. We first used the model of DSS-induced colitis because this model has been frequently used for evaluation of various pharmacological agents (19, 20). The second model used in our study is the model of TNBS-induced colitis. It is hypothesized that ethanol used as vehicle in the rectal administration of TNBS disrupts the mucosal barrier, enabling this hapten to bind covalently to proteins of colonic epithelial cells and modify surface proteins. Fragments of these altered cells can be taken up by macrophages, and the subsequent presentation of Ag to T cells by macrophages and dendritic cells results in a Th1-dominated colitis. Previous studies have demonstrated that neutralizing IL-12 or IL-18, and therefore blockade of the Th1 pathway, is protective (16, 21–23). A suppression of this pathway is indicated in our studies by a profound suppression of IFN-γ both models investigated.

Noncytokine-related properties of HDAC inhibitors might contribute to the anti-inflammatory effects. By altering the structure of nucleosomes, HDAC permit access of the transcriptional machinery to chromatin-complexed DNA. HDAC inhibitors, by decreasing the levels of histone acetylation, can lead to a local alteration in the structure of chromatin, which facilitates gene-specific repression of transcription (2). Transcription factors are also targeted by HDAC. HDAC have been found in complexes with proteins involved in the regulation of cell-cycle progression and apoptosis (2). Several HDAC inhibitors reduce tumor growth in animals (2, 24). The proapoptotic properties of HDAC inhibitors, such as SAHA or VPA, are of particular interest for the in vivo models used in the present study. In both the DSS and TNBS colitis models, the administration of proapoptotic agents have been proven to be beneficial (14, 15, 25). For instance, administration of anti-IL-12 and anti-IL-6R Abs, which is protective in models of colitis, affects survival of CD4⁺ T cells mediating inflammation (reviewed in Ref. 10). In healthy humans, LPMC exhibit a high susceptibility to Fas-mediated apoptosis, whereas LPMC from patients with Crohn’s disease are resistant to multiple apoptotic pathways (10). In addition, treatment with the anti-TNF-α Ab (infliximab), which is highly effective in patients with steroid-refractory Crohn’s disease, results in monocyte and caspase-3-dependent T cell apoptosis (26, 27). As indicated in Fig. 6D, both VPA and SAHA treatment results in a significant increase in LPMC apoptosis, thus contributing to the anti-inflammatory action of HDAC inhibitors. Furthermore, in a previous study, patients were treated with butyrate enemas resulting in a beneficial effect which was associated with a reduction of NF-κB translocation in lamina propria macrophages (28). These results further indicate that the inhibition of the NF-κB pathway presents one mechanism responsible for the effects observed. These results together with our cytokine data strongly suggest that the anti-inflammatory properties of HDAC inhibitors in models of inflammatory bowel disease depend on the suppression of proinflammatory cytokines as well as the induction of apoptosis.

FIGURE 6. Effect of VPA and SAHA on TNBS-induced colitis. Colitis was induced by administration of TNBS as described in Materials and Methods. A. Weight was measured daily and expressed as percent of initial body weight, determined at day 1 before TNBS exposure. B. At the end of the experiment, colon cultures were performed and spontaneous cytokine production determined in the culture supernatant. C. The histological score was evaluated as described in Materials and Methods. D. At the end of the experiment, LPMC were isolated and stained for annexin V and PI as described in Materials and Methods. The mean percentage of Annexin V/PI-positive cells from five independent samples per group is shown as well as one representative staining. Data are expressed as mean ± SEM; n = 10, *, p < 0.05; **, p < 0.01; in vivo experiments were performed twice.
For patients with chronic inflammatory bowel diseases, ulcerative colitis more than Crohn’s disease is associated with an increased risk of colon cancer (29, 30). HDAC inhibitors have primarily been investigated and developed for cancer therapy, and are currently tested in clinical trials for a variety of solid and hematological cancers (4). Although the current study demonstrates that HDAC inhibitors have potent anti-inflammatory effects in experimental colitis, it remains to be studied whether the anti-inflammatory efficacy would also reduce the incidence of gastrointestinal malignancies.

In conclusion, the present study indicates that inhibition of HDAC provides a novel anti-inflammatory concept in experimental colitis. Considering the advanced clinical trials for SAHA in phase II clinical trials and the fact that low concentrations as well as oral administration are sufficient to achieve anti-inflammatory efficacy, inhibitors of HDAC represent a promising new class of compounds for clinical trials in patients with chronic inflammatory bowel disease.

Disclosures

The authors have no financial conflict of interest.

References


