Blocking T_H17-polarizing cytokines by histone deacetylase inhibitors in vitro and in vivo

Daniela Bosisio,* Marisa Vulcano,† Annalisa Del Prete,†,‡ Marina Sironi,† Valentina Salvi,* Laura Salogni,* Elena Riboldi,* Flavio Leoni,§ Charles A. Dinarello,¶ Giampiero Girolomoni,|| and Silvano Sozzani*,[1]

*Section of General Pathology and Immunology, Department of Biomedical Sciences and Biotechnologies, University of Brescia, Brescia, Italy; †Istituto Clinico Humanitas (IRCCS), Rozzano, Italy; ‡Section of Clinical Biochemistry, Department of Medical Biochemistry, University of Bari, Bari, Italy; §Centro Ricerche Italfarmaco, Italfarmaco S.p.A., Cinisello Balsamo, Italy; ¶University of Colorado Health Sciences Center, Denver, Colorado, USA; and ||Section of Dermatology and Venereology, Department of Biomedical and Surgical Science, University of Verona, Verona, Italy

Abstract: Histone deacetylase (HDAC) inhibitors are small molecules inducing cell-cycle arrest, differentiation, and apoptosis, currently undergoing clinical trials as anticancer drugs. In addition, emerging evidence suggests HDAC inhibitors may have anti-inflammatory and immunomodulatory properties as well, although the molecular mechanisms remain poorly defined. Given the central role of dendritic cells (DC) in the induction and maintenance of the inflammatory and immune response, we investigated the effects of HDAC inhibitors on the maturation and activation of human monocyte-derived DC in the presence of LPS and IFN-γ. Our results show that the production of T_H1- and T_H17-inducing cytokines, namely IL-12 and IL-23, was inhibited by trichostatin A (72% and 52%, respectively) and suberoylanilide hydroxamic acid (86% and 83%). Strikingly, HDAC inhibitors were effective if added simultaneously as well as after the proinflammatory challenge, and their effect was not associated to a reduction of expression or function of LPS/IFN-γ receptors. These findings were confirmed in two different murine models. In addition, HDAC inhibitors selectively blocked the production of T_H1-attracting chemokines CXCL9, CXCL10, and CXCL11. The reduction of T_H1- and T_H17-inducing cytokines as well as T_H1-attracting chemokines may represent relevant mechanisms through which HDAC inhibitors at nonproapoptotic doses exert their immunomodulatory properties. *J. Leukoc. Biol.* 84: 1540–1548; 2008.

Key Words: *dendritic cells · T_H1/T_H2 cells · chemokines · autoimmunity*

INTRODUCTION

One of the most remarkable aspects of the innate immune system is its capability to recognize and discriminate the type of pathogen that is encountered in inflamed tissues. Based on this information, specific signals are produced to establish an effective antigen-specific adaptive response [1]. These signals include cell–cell contact and the production of soluble mediators determining the differentiation of naïve T lymphocytes into different types of mature effector cells (T_H1, T_H2, and T_H17). Deregulation of these events may lead to pathological situations characterized by defects in immune responses and increased susceptibility to pathogens or by an excessive response and autoimmunity [2–4].

Dendritic cells (DC) are professional APC and key regulators of effector T cell functions [5–11]. Of note, DC represent the most relevant source of IL-12, which promotes the differentiation of T_H1 effector T cells as well as the activation of NK cells and cytotoxic $CD8^+$ T cells. These latter cells in turn produce IFN-γ, a cytokine involved in the protective response against intracellular pathogens and transformed cells, as well as in allograft rejection [12]. IL-12 is a type I cytokine composed of two disulfide-linked proteins—p35 and p40. The p40 subunit also binds to p19 to form IL-23, another cytokine that similarly to IL-12, is mainly produced by DC [13] but at variance, is involved in the expansion of the T_H17 lineage of effector T lymphocytes [14]. Notably, IL-23 cannot drive the differentiation of this lineage, as naïve T cells do not express the specific receptor. Instead, IL-6 and TGF-β have been implicated in this process in the mouse, and recent studies highlight the relevance of the proinflammatory cytokine IL-1β in humans [15–19]. The significance of T_H17 cells in the immune response is possibly the defense against certain types of pathogens, particularly when endangering epithelial surfaces [20–22]. However, T_H17 cells also have a role into the pathogenesis of autoimmune diseases [23–27]. In humans, elevated levels of p19 were reported in individuals with multiple sclerosis [28], rheumatoid arthritis (RA) [29], psoriasis

[1] Correspondence: Section of General Pathology and Immunology, Department of Biomedical Sciences and Biotechnologies, University of Brescia, Viale Europa 11, 25123 Brescia, Italy. E-mail: sozzani@med.unibs.it

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[30], and Crohn's disease [31, 32]. In addition, IL-23 is upregulated in many human carcinomas [33] and has been suggested to provide a tumor-promoting environment.

At present, one of the most compelling issues in immunology is the selective modulation of the different branches of the T effector response, as this may lead to the development of novel therapeutic strategies for pathological states characterized by the expansion of T_H1 and/or T_H17 cells.

Histone deacetylase (HDAC) inhibitors are small molecules belonging to many different structural classes and nevertheless, all active on the same target enzymes, namely HDACs [34]. By blocking HDACs, these compounds induce cell-cycle arrest, cell differentiation, and apoptosis [35]. These promising anticancer properties are currently being tested in clinical trials [36–39]. In addition, increasing evidence indicates that HDAC inhibitors may be active in several murine models of autoimmune and inflammatory diseases, such as systemic lupus erythematosus [40], RA [41], Con A-induced hepatitis [42], and endotoxemia [42]. Similar properties were described in human diseases, such as ulcerative colitis [43, 44]. In all of these studies, the therapeutic effects of HDAC inhibitors were dependent on the reduction of circulating proinflammatory cytokines and on the possible immunomodulatory functions of these drugs [40, 42, 45–48]. However, the molecular basis of these effects remains elusive. The general paradigm is that HDAC inhibitors positively control transcription [34] as well as NF-κB activation [49]. Based on this, the inhibition of proinflammatory cytokines (that are also classical NF-κB targets) and the consequent anti-inflammatory effects might be unexpected. However, genome-wide studies have now clearly shown that these drugs can induce transcriptional repression and activation [50, 51] in an exquisitely gene- and cell type-specific manner (refs. [52, 53] and D. Bosisio, unpublished).

The aim of the present study was to investigate the ability of two different HDAC inhibitors, namely trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA), to modulate human DC functions and particularly, the production of T_H1- and T_H17-inducing cytokines when used at nonproapoptotic concentrations. Our findings substantiate the immunomodulatory effects postulated on the bases of animal models and propose HDAC inhibitors as a new pharmacological strategy for the control of autoimmune diseases.

MATERIALS AND METHODS

DC preparation and culture

Buffy coats were obtained through the courtesy of the Centro Trasfusionale, Spedali Civili (Brescia, Italy). Monocytes were purified from PBMC by immunomagnetic separation using anti-CD14-conjugated magnetic microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany) and DC generated in vitro as described previously [11]. DC maturation (10^6 DC/ml) was induced by incubation with 100 ng/ml LPS (*Escherichia coli* 055:B5, Sigma Chemical Co., St. Louis, MO, USA) plus 500 U/ml IFN-γ (Roussel Uclaf, Paris, France). TSA was purchased by Upstate (Lake Placid, NY, USA), solubilized in EtOH at 1 mg/ml, and kept at −80°C. SAHA was from Alexis Biochemicals (San Diego, CA, USA), solubilized in DMSO at 20 mg/ml, and kept at −80°C. Stock solutions and EtOH/DMSO controls were diluted in RPMI prior to experiments. Peripheral blood DC were obtained from PBMC by magnetic sorting with the blood DC antigen-1 kit (Miltenyi Biotech), according to the manufacturer's instructions. The cell culture medium routinely used for primary cells was RPMI 1640 (Gibco-BRL, UK) supplemented with 10% FCS (Hyclone, Logan, UT, USA), 2 mM glutamine, antibiotics, Na-pyruvate, nonessential amino acids, and β-ME (all from Gibco-BRL). All reagents were endotoxin-free as assessed by *Limulus* assay (BioWhittaker Inc., Walkersville, MD, USA).

FACS analysis

Cell staining was performed using the indicated FITC-conjugated mouse mAb or the isotype-matched, irrelevant Ig (all from BD PharMingen, San Diego, CA, USA). Samples were read on a particle analyzing system (PAS; Partec GmbH, Muenster, Germany) and analyzed with FlowJo (Tree Star Inc., Ashland, OR, USA).

MLR

Irradiated control or treated DC were added in graded doses to 1×10^5-purified, allogenic T cells (from cord blood) in 96-well round-bottom microtest plates. Each group was performed in triplicate. [^{3}H] Thymidine incorporation was measured on Day 5 after 18 h pulse (5 Ci/μmol; Amersham, Buckingham, UK).

FITC-dextran uptake

Endocytosis was measured as the cellular uptake of FITC-dextran (Sigma Chemical Co.) as described [54]. Approximately 2×10^5 cells/sample were incubated in media containing FITC-dextran (1 mg/ml) for 60 min. Uptake of the label was determined by flow cytometry using a PAS.

Real-time PCR

RNA was extracted using TRIzol reagent, according to the manufacturer's instructions. After RNA purification, samples were treated with DNase to remove contaminating genomic DNA (DNaseI amplification grade). Reverse transcription was performed using random hexamers and Superscript II RT. All reagents were from Invitrogen (Carlsbad, CA, USA). Gene-specific primers were purchased from NBS Biotech Scrl (Milan, Italy; sequences and detailed amplification protocols are available upon request). The iQ™ SYBR Green Supermix (Bio-Rad Laboratories Inc., Hercules, CA, USA) was used to run relative quantitative real-time PCR of the samples according to the manufacturer's instructions. Reactions were run in triplicate on an iCycler™ (Bio-Rad Laboratories Inc.) and generated products analyzed with the iCycler™ iQ Optical System Software (Version 3.0a, Bio-Rad Laboratories Inc.). Gene expression was normalized routinely based on GAPDH mRNA and 18S rRNA content with similar results. Data are displayed as $2^{-\Delta \text{comparative threshold (Ct)}}$ values (unless specified differently) and are representative of at least three independent experiments.

In vitro cytokine and chemokine production

Control or treated DC were plated at 10^6/ml and incubated for 18 h with the indicated treatments. Cell-free supernatants were harvested and tested in sandwich ELISA using specific Duo-Set kits (R&D Systems, Minneapolis, MN, USA) with the exceptions of IL-12p70 (Endogen, Pierce Biotechnology Inc., Rockford, IL, USA) and IL-23 (eBioscience Inc., San Diego, CA, USA).

Intracellular signaling

DC (3×10^6 cells per sample) were preincubated in the presence of the two HDAC inhibitors (300 nM TSA and 1000 nM SAHA) at 37°C for 1 h and then stimulated with LPS and IFN-γ (100 ng/ml and 500 U/ml, respectively). Total cell lysates (20 μg) and nuclear extract (5 μg) were analyzed by 8% SDS-PAGE followed by Western blotting with antibodies against phosphorylated (p)-STAT1, p-ERK1/2 (Cell Signaling Technology, Beverly, MA, USA), NF-κB p50, NF-κB p65, or ERK2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

In vivo stimulation of DC function and measurement of IL-12 secretion

Female C57Bl6N mice (8–10 weeks of age) were purchased from Charles River (Calco, Italy). Mice were housed in the specific pathogen-free animal facility. Procedure involving animals and their care conformed with institutional guidelines in compliance with national (4 D.L. N.116, G.U., Suppl. 40,

18-2-1992) and international [European Economic Community (EEC) Council Directive 86/609, OJ L 358,1,12-12-1987; National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996] law and policies.

For the quantification of cytokines in sera, SAHA (50 mg/kg) was administered to mice ($n=6$) by oral gavage [42]. Vehicle-treated mice received water. After 1 h, mice were injected with LPS (1.3 mg/kg, i.p.) and killed after 3 h, and serum was obtained. IL-12p70 and IL-23 were determined using commercial kits (R&D Systems and eBioscience Inc., respectively).

For ex vivo experiments, mice ($n=3$–5) were injected i.v. with 20 μg soluble tachyzoite antigen (STAg) or an equivalent volume of PBS as described [55, 56]. STAg was a kind gift from Alan Sher (NIH, Bethesda, MD, USA). SAHA was administered as above 1 h before STAg injection. $CD11c^+$ immunomagnetically selected cells were prepared from spleens harvested 4 h after the indicated STAg injection. Cells were plated at $2–6 \times 10^5$ per well in 96-well plates in RPMI-1640 medium supplemented with 10% FCS, penicillin-streptomycin, and β-ME. Cultures were then incubated in the presence or absence of STAg (5 μg/ml), and supernatants were collected at 20 h for IL-12p70 determination using a commercial kit (R&D Systems). Statistical analysis was performed using unpaired Student's *t*-test (GraphPad Prism Version 4.00 for Windows, GraphPad Software, San Diego, CA, USA).

RESULTS

Lack of effect of HDAC inhibitors on DC maturation

To elucidate the effect of TSA and SAHA on the maturation of monocyte-derived DC, we initially investigated the effect of these drugs on the up-regulation of costimulatory molecules. Day 6 GM-CSF/IL-4-differentiated DC were treated for 24 h with increasing concentrations of TSA and SAHA in the presence of 100 ng/ml LPS plus 500 U/ml IFN-γ. Under these experimental conditions, toxicity could be detected starting from 1000 nM TSA and 5000 nM for SAHA, as assessed by Annexin V/propidium iodide staining (not shown). When used at lower concentrations, neither of the two compounds affected the maturation-dependent up-regulation of the tested surface markers. **Figure 1A** shows representative data obtained with 300 nM TSA, and similar results were obtained with SAHA (not shown) in at least three different DC preparations for each inhibitor. Figure 1B also shows that neither of the two HDAC inhibitors (300 nM TSA or 1000 nM SAHA) decreased the ability of DC to induce allogeneic T cell proliferation, a function that is increased dramatically with maturation. Similarly, FITC-dextran uptake was comparably reduced in untreated, mature DC and in TSA- and SAHA-treated cells (Fig. 1C). Altogether, these data demonstrate that neither TSA nor SAHA, even when used at high (although nontoxic) concentrations, affects the up-regulation of maturation markers as well as DC function.

HDAC inhibitors inhibit the production of T_H1- and T_H17-inducing cytokines

Mature DC acquire the capability to secrete cytokines that are required for the polarization of the adaptive immune response. To investigate the immunomodulatory potential of HDAC inhibitors, DC were pretreated for 1 h with 100 nM TSA, stimulated with LPS and steady-state levels of cytokine mRNA analyzed by quantitative PCR. **Figure 2** shows that TSA dramatically reduced the production of IL-12p35, IL-12/23p40, IL-23p19, IL-6, and IFN-β during the entire time-course. In the case of TNF-α, a reduction in the mRNA levels was detected occasionally (two out of five donors), as in the case of the donor shown in the picture. IFN-α and TGF-β were undetectable under the experimental conditions used (not shown).

Consistent with these findings, TSA reduced the secretion of IL-12p70 and IL-23 in a dose-dependent manner with a similar IC_{50} ranging between 50 and 100 nM TSA, depending on the donor (**Fig. 3A**). These two cytokines were also similarly reduced in the presence of SAHA (Fig. 3B). The production of

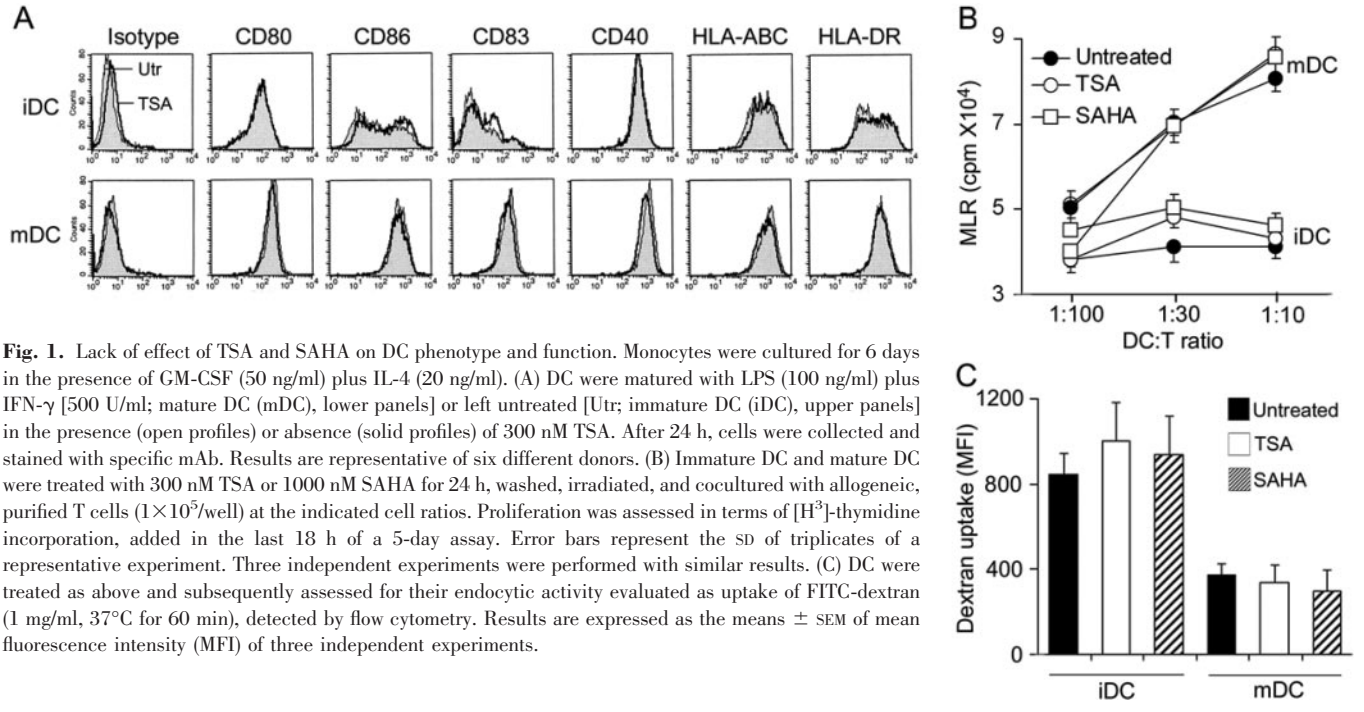

Fig. 1. Lack of effect of TSA and SAHA on DC phenotype and function. Monocytes were cultured for 6 days in the presence of GM-CSF (50 ng/ml) plus IL-4 (20 ng/ml). (A) DC were matured with LPS (100 ng/ml) plus IFN-γ [500 U/ml; mature DC (mDC), lower panels] or left untreated [Utr; immature DC (iDC), upper panels] in the presence (open profiles) or absence (solid profiles) of 300 nM TSA. After 24 h, cells were collected and stained with specific mAb. Results are representative of six different donors. (B) Immature DC and mature DC were treated with 300 nM TSA or 1000 nM SAHA for 24 h, washed, irradiated, and cocultured with allogeneic, purified T cells (1×10^5/well) at the indicated cell ratios. Proliferation was assessed in terms of [H^3]-thymidine incorporation, added in the last 18 h of a 5-day assay. Error bars represent the SD of triplicates of a representative experiment. Three independent experiments were performed with similar results. (C) DC were treated as above and subsequently assessed for their endocytic activity evaluated as uptake of FITC-dextran (1 mg/ml, 37°C for 60 min), detected by flow cytometry. Results are expressed as the means ± SEM of mean fluorescence intensity (MFI) of three independent experiments.

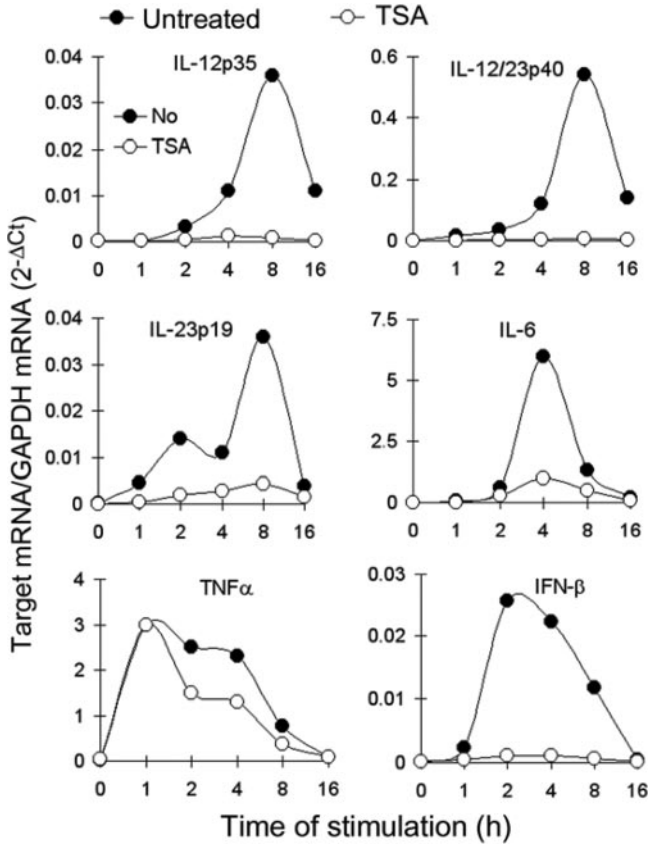

Fig. 2. TSA decreases steady-state levels of T_H1- and T_H17-inducing cytokine mRNA by DC, which at Day 6 of culture, were pretreated for 1 h with 100 nM TSA (○) or vehicle (●) and then stimulated with 100 ng/ml LPS plus 500 U/ml IFN-γ. At the indicated time-points, cells were collected, and the amount of the specified cytokine mRNAs was evaluated by quantitative PCR. Data are displayed as $2^{-\Delta Ct}$ values from one experiment, representative of at least three independent experiments. Samples were run in triplicates; the variability among replicates was always lower than 0.5 cycle. Gene expression was normalized routinely based on GAPDH content.

IL-12p70 was also inhibited in LPS plus IFN-γ-stimulated, primary-circulating myeloid conventional DC with a similar effective concentration reaching maximum inhibition at 100 nM (not shown). Of interest, IL-12 production was also inhibited when HDAC inhibitors were added several hours after the proinflammatory challenge. In particular, a strong inhibition was observed when TSA or SAHA was added 4 h after LPS plus IFN-γ, but it was still detectable when given after 8 h (Fig. 3C). Consistent with the quantitative PCR data, TSA also reduced the secretion of IL-6 by 40–50%, whereas TNF-α protein release was only affected slightly. Similar results were obtained with SAHA, although SAHA was consistently less effective than TSA in suppressing IL-6 secretion (Fig. 3D).

Based on these observations, we conclude that TSA or SAHA reduces the T_H1- as well as the T_H17-inducing potential of DC in vitro by decreasing the production of IL-12/IFN-β and IL-6/IL-23, respectively.

HDAC inhibitors modulate the production of T_H1- but not T_H17-attracting chemokines

As HDAC inhibitors reduce the production of T_H1- and T_H17-inducing cytokines, we asked whether TSA or SAHA could also affect the secretion of chemokines that are important for the recruitment of previously polarized T cells. **Figure 4** shows that mRNA levels of CXCL9, CXCL10, and CXCL11 were reduced dramatically by TSA over the 16 h time-course. This was paralleled by a consistent reduction of the secreted protein in the presence of TSA and SAHA (**Fig. 5**, and not shown for CXCL10). Similar results were obtained for CCL19, a chemokine active on naïve T cells and mature DC (Figs. 4 and 5). In contrast, the secretion of T_H17-attracting chemokines (CCL5, CCL17, CCL20, CCL22) and that of the prototypic neutrophil chemokine, namely CXCL8, was insensitive to TSA or SAHA (Figs. 4 and 5, and not shown). These results

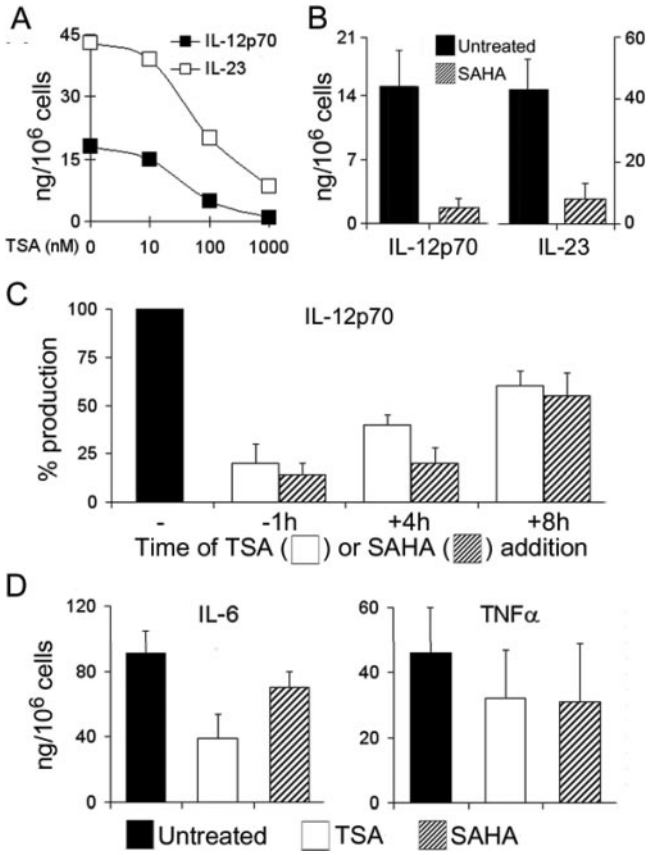

Fig. 3. TSA and SAHA decrease the production of T_H1- and T_H17-inducing cytokines by DC (A), which were matured as in Figure 2 in the presence of the indicated concentrations of TSA (1 h pretreatment). After 18 h, supernatants were collected, and the production of IL-12p70 (■) and IL-23 (□) was evaluated by ELISA. Two different donors were analyzed with similar results. (B) DC were matured as above in the absence (black bars) or presence of 1000 nM SAHA (1 h pretreatment, hatched bars). After 18 h, supernatants were collected, and the production of IL-12p70 (left panel) and IL-23 (right panel) was evaluated by ELISA. Results are expressed as mean ± SE of four donors for IL-12p70 and three donors for IL-23. (C) DC were stimulated with LPS as above in the absence (black bar) or presence of 100 nM TSA (open bars) or 1000 nM SAHA (hatched bars). HDAC inhibitors were added at the indicated time-points before or after LPS/IFN-γ stimulation. After 18 h, supernatants were collected, and the production of IL-12p70 was evaluated by ELISA. Results are expressed as percentage of production compared with cells without HDAC inhibitors and represent the mean ± SE of three independent experiments. (D) DC prepared as in A were pretreated for 1 h with 100 nM TSA or 1000 nM SAHA, and the production of IL-6 (left panel) and TNF-α (right panel) was assessed by ELISA. Results are expressed as means ± SEM of at least three donors.

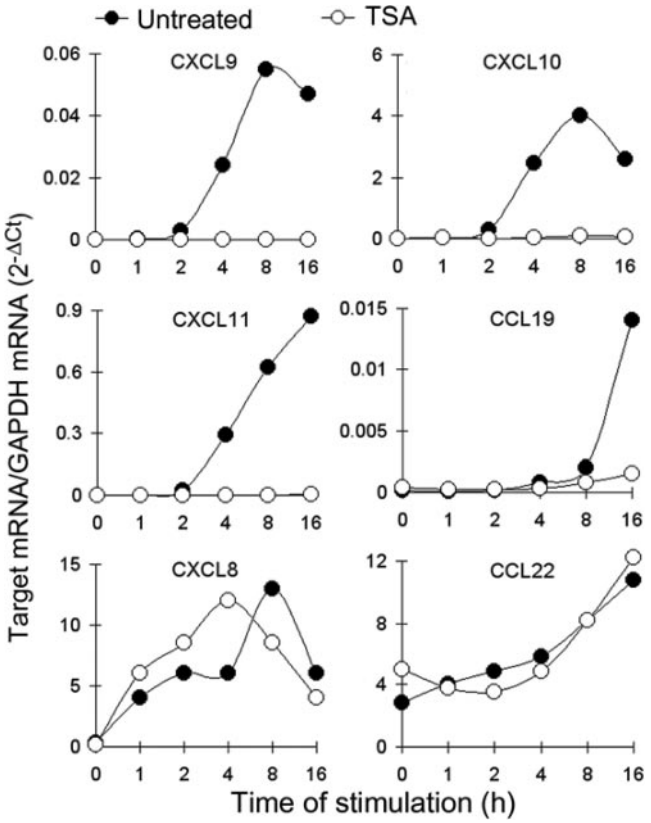

Fig. 4. TSA decreases the production of T_H1-attracting chemokines mRNA by DC, which were matured with LPS (100 ng/ml) plus IFN-γ (500 U/ml) in the presence (○) or absence (●) of 100 nM TSA (1 h pretreatment). At the indicated time-points, cells were collected, and the amount of the indicated chemokine mRNAs was evaluated by quantitative PCR. Data are displayed as $2^{-\Delta Ct}$ values from a single experiment, representative of at least three independent experiments. Samples were run in triplicates, and differences among replicates were lower than 0.5 cycle. Gene expression was routinely normalized based on GAPDH contents.

indicate that HDAC inhibitors selectively target the recruitment of T_H1 cells, whereas that of other cell types, such as T effectors of the T_H17 and T_H2 lineages (CCL17 and CCL22) and neutrophils, is not impaired (see Discussion).

TSA does not affect the expression and function of LPS and IFN-γ receptors

To address the mechanism by which TSA exerts the described effects on cytokine production, we examined the expression of LPS and IFN-γ receptors at the mRNA level. **Figure 6A** shows that TSA did not substantially change the expression of the two chains of the IFN-γ receptor along the kinetics investigated (2, 4, and 8 h). Similarly, TLR4 mRNA levels were not changed by the action of the inhibitor, with only a minor decrease observed at the earliest (2 h) time of stimulation. A similar regulation was observed at the protein level by FACS analysis (data not shown). Next, we investigated the effect of TSA on the early signaling events induced by LPS/IFN-γ. TSA pretreatment did not affect the phosphorylation of STAT1 (Fig. 6B) and ERK1/2 (Fig. 6C) and the nuclear translocation of NF-κB p65 and p50 (Fig. 6D), induced by 30 min stimulation of DC with LPS/IFN-γ. Similar results were obtained with SAHA (data not shown). Taken together, these results indicate that HDAC inhibition does not have a major negative effect on LPS/IFN-γ receptor expression and signaling in DC.

SAHA inhibits the production of IL-12p70 in vivo

To assess the potential therapeutic application of the above findings, HDAC inhibitors were tested for the capability to reduce the production of IL-12p70 and IL-23 after LPS administration. Of note, we had shown previously that HDAC inhibitors can efficiently reduce the serum levels of the LPS-induced circulating cytokines TNF-α, IL-1β, IL-6, and IFN-γ [42], each of which plays key roles in the differentiation of the T_H1 and T_H17 lineages [15]. Here, we additionally show that SAHA, at previously demonstrated, active doses [42], reduces the production of circulating IL-12p70 and IL-23 (45% and 46% inhibition, respectively; **Fig. 7A**). However, as expected, the overall levels of circulating IL12-p70 were low. Therefore, to confirm this finding further, we performed the ex vivo experiments shown in Figure 7B. Again, SAHA efficiently reduced (55% inhibition) the secretion of STAg-induced IL-12p70 released by the ex vivo culture of splenic $CD11c^+$ cells. As expected on the basis of the reversibility of the effect of these drugs, IL-12 inhibition was even stronger when SAHA was added again in vitro (89% inhibition). These experiments show that HDAC inhibitors can be used efficiently in vivo to control the production of T cell-polarizing cytokines.

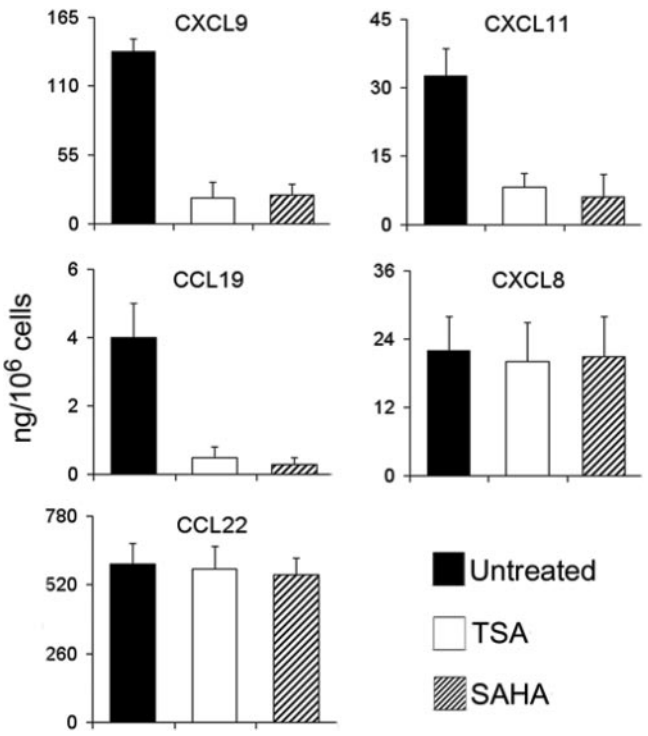

Fig. 5. TSA and SAHA decrease the production of T_H1-attracting chemokines by DC, which were matured as in Figure 4 in the absence (black bars) or presence of 100 nM TSA (open bars) or 1000 nM SAHA (hatched bars). HDAC inhibitors were added 1 h before the stimulation. After 18 h, supernatants were collected, and the production of the indicated chemokine was evaluated by ELISA. Results are expressed as means ± SEM of at least three independent donors.

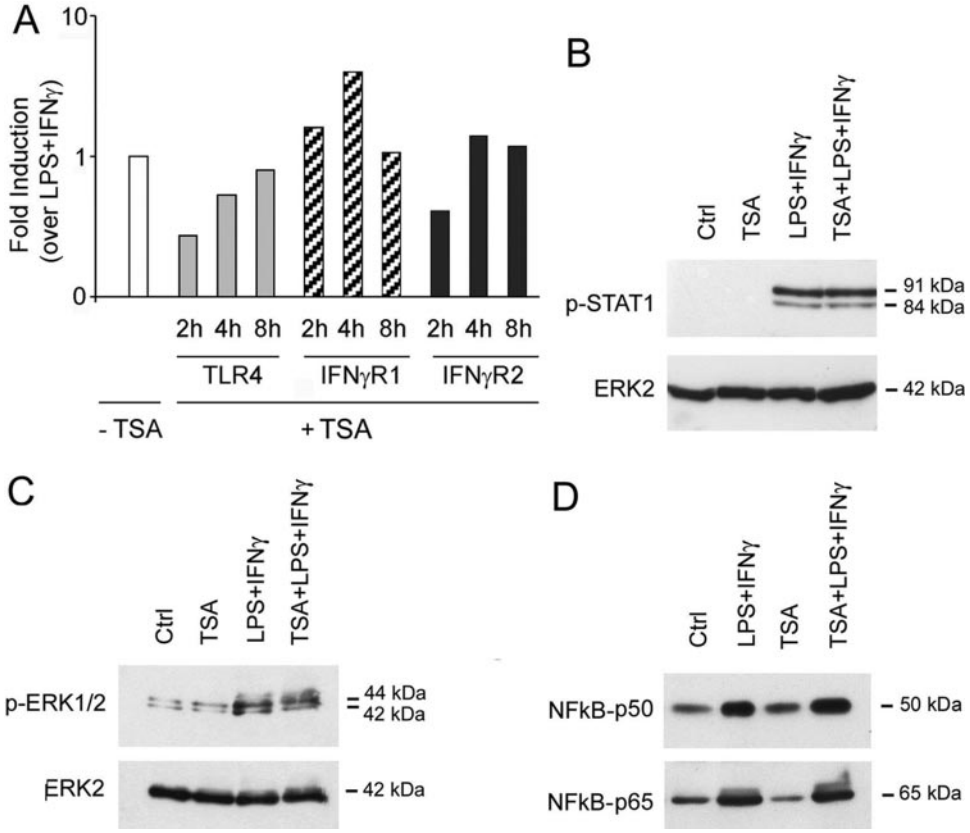

Fig. 6. TSA does not affect the expression and function of LPS and IFN-γ receptors. (A) DC at Day 6 of culture were pretreated for 1 h with 300 nM TSA and then stimulated with 100 ng/ml LPS and 500 U/ml IFN-γ. At the indicated time-points, cells were collected, and mRNA was evaluated by quantitative PCR. Data are presented as fold of induction over control values (LPS+IFN-γ) and are the mean of two similar independent experiments. Samples were run in triplicates, and differences among replicates were lower than 0.5 cycle. Gene expression was normalized routinely based on GAPDH content. (B–D) DC were stimulated with 100 ng/ml LPS/500 U/ml IFN-γ for 30 min in the presence or absence of 300 nM TSA (1 h pretreatment). Cells were lysed, subjected to SDS–PAGE, and blotted with anti-p-STAT1, anti-p-ERK1/2, and anti-NF-κB p65 and p50 mAb. Anti-ERK2 antibody was used as loading control. The figure shows one experiment representative of three.

DISCUSSION

This study reports that two different HDAC inhibitors, namely TSA and SAHA, when used at concentrations that are nonpro-apoptotic, inhibit in a selective manner the ability of DC to produce the T_H1- and T_H17-polarizing cytokines IL-12, IL-23, and IL-6 (Figs. 2 and 3) with no effect on DC differentiation or maturation and the production of many chemokines (e.g., CXCL8, CCL5, CCL17, CCL20, and CCL22; Fig. 1). HDAC inhibitors were active also when added several hours after the proinflammatory challenge (Fig. 3C). In addition, TSA and SAHA efficiently reduced the production of LPS-stimulated IL-12 and IL-23 in vivo and STAg-dependent production of IL-12 ex vivo (Fig. 7). Therefore, these data suggest that HDAC inhibition may be exploited as a therapeutic strategy to control pathological conditions characterized by a functional expansion of T_H1 and T_H17 cells such as autoimmune diseases [23–25, 27–30, 32].

The mechanism responsible for the selective gene regulation by HDAC inhibitors is not fully understood. In this study, we show that TSA does not change the expression of LPS and IFN-γ receptors and does not regulate the proximal receptor signaling events induced by LPS/IFN-γ in human DC, such as STAT-1 and MAPK phosphorylation and NF-κB nuclear translocation (Fig. 6). These results, in addition to the evidence that TSA and SAHA do not affect LPS/IFN-γ-induced DC maturation and do not induce a general inhibition of chemokine production, indicate that the two HDAC inhibitors investigated in this study do not have a profound, suppressive activity of LPS/IFN-γ responses. These results, which are in agreement with a recent report performed with bone marrow-derived human macrophages [57], support the hypothesis that TSA and SAHA may act specifically on certain genes by affecting promoter occupancy by transcription factor, nucleosoma remodeling, and/or formation of the transcription initiation complex. Conversely, other studies have found previously that HDAC inhibitors may block NF-κB, MAPK activation, and the IFN-γ-STAT1-induced signaling pathway [58–60]. As discussed below, this apparent discrepancy may rely on the type of inhibitor used and on cell-specific effects (see below).

Previous studies [46, 61] reported that other HDAC inhibitors (i.e., *n*-butyrate, sodium valproate, and MS-275) affect DC differentiation and maturation. These latter results differ from the present data, possibly as a result of the diverse activity of the different molecules used. It is worth noting that although it is well known that single HDAC inhibitors possess cell-specific effects (discussed below), a comparison of different HDAC inhibitors on a single cell type has never been performed.

In the present study, we report that TSA and SAHA also inhibit the production of TNF-α and IL-6 in DC (Figs. 2 and 3). We and others have reported previously that TSA and SAHA affect the production of proinflammatory cytokines by mononuclear cells in vitro [42] and in vivo [40–42]. However, the same drugs were also described to enhance the secretion of these cytokines in microglia and lung epithelial cells [62, 63]. Similarly, the present observations (Figs. 4 and 5) and previous reports [48, 62] show that TSA and SAHA do not affect CXCL8 production in DC, macrophages, or lung epithelial cells, which is in contrast to the findings obtained in Caco-2 cells [64]. These discrepancies emphasize the concept that HDAC inhibitors possess cell-specific effects.

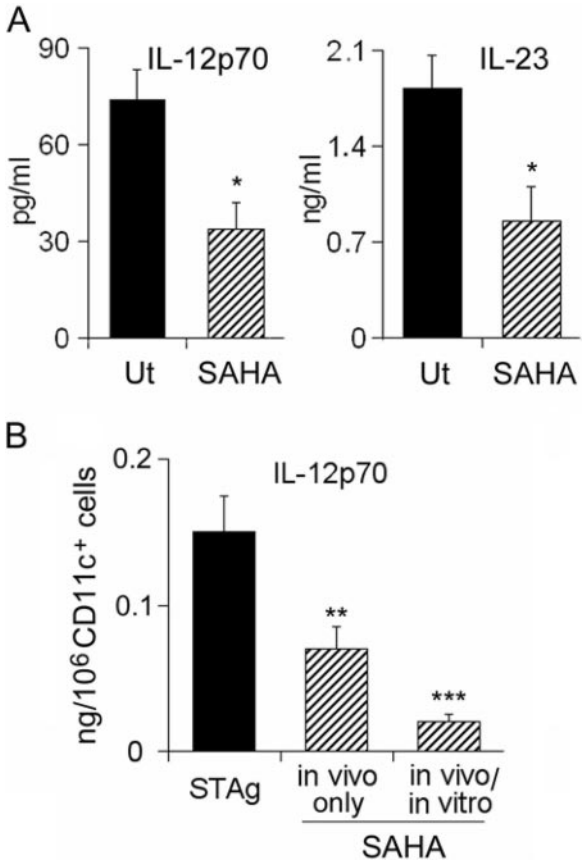

Fig. 7. SAHA inhibits the production of IL-12p70 and IL-23 in vivo. (A) Mice were pretreated with 50 mg/kg SAHA by gavage. Control mice [untreated (Ut)] received vehicle. After 1 h, LPS 1.3 mg/kg was injected i.p. Serum was taken after 3 h, and cytokines were measured by ELISA. The means ± SEM are indicated ($n=6$; *, $P<0.05$). (B) The production of IL-12p70 was investigated in a subpopulation of splenic DC as described previously [55, 56]. Mice were i.v.-injected with STAg (20 μg/mouse), with 1 h pretreatment with SAHA or vehicle. Four hours after STAg injection, mice were killed, spleens removed, and splenic $CD11c^+$ cells immunomagnetically selected and cultured in the presence of STAg (5 μg/ml, black bars) and in the presence (hatched bar, right: "in vivo/in vitro") or absence (hatched bar, middle: "in vivo only") of SAHA (1000 nM, hatched bars). Supernatants were collected 24 h later and assayed for IL-12p70 production by ELISA. Bars represent the mean ± SEM of triplicates (**, $P=0.004$; ***, $P=0.0003$). The experiment was repeated twice with similar results.

Previous reports have shown that IL-12 is inhibited by HDAC inhibitors in mononuclear cells [42, 46, 48, 65]. The present study confirms and extends these findings, showing for the first time that HDAC inhibitors also affect IL-23 transcription and secretion. Inhibition of IL-23 production, in association with that of the proinflammatory cytokines, may profoundly affect the efficiency of T_H17 differentiation. In addition, it is tempting to speculate that neutralization of IL-23 may suppress tumor-associated inflammation and improve tumor penetration by cytotoxic cells [33]. Although this mechanism needs to be demonstrated, it might contribute further to the anti-tumor activity of this class of drugs.

This report also describes a selective inhibition of T_H1-attracting chemokines (i.e., CXCL9, CXCL10, and CXCL11) [66] by TSA- and SAHA-treated DC (Figs. 4 and 5). Therefore, TSA and SAHA may not only impair the differentiation of T_H1 effectors but also the recruitment of previously activated cells. The repertoire of chemokine receptors in human T_H17 cells is currently under investigation [20, 25, 67]. Although it is not clear whether CCR6 can be considered a specific T_H17 lineage marker, these cells have been shown to respond to CCL20, its cognate ligand [25]. In addition, T_H17 cells were reported to express CCR4 and CCR2 [20, 67]. Our results show that the ligands for these receptors were not affected by TSA or SAHA treatments (Figs. 4 and 5, and not shown). Thus, we conclude that the two drugs do not impair the recruitment of differentiated T_H17 effectors.

DC are professional APC for naïve T cells as well as among the major producers of proinflammatory cytokines [11, 12, 14, 66]. DC also play a key role in instructing T cells to mount an effective antigen-specific response [6, 9]. Therefore, DC represent a promising target for pharmacological therapies aimed at the control of adaptive immunity. This study shows that TSA and SAHA have the peculiar ability to inhibit T_H1- and T_H17-polarizing cytokines in the absence of a general inhibition of DC function that might be detrimental for host immune protection. In fact, most of the chemokines active on innate effector cells were not affected by the two inhibitors. In addition, the selective inhibition of only certain genes emphasizes the low toxicity of the treatment, in keeping with the results coming from ongoing clinical trials in the context of anticancer therapies [38, 68, 69]. Although the biological relevance of IL-23 suppression needs to be addressed further in vivo, altogether, these findings provide the rational basis for the use of HDAC inhibitors in the modulation of autoimmune disorders.

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REFERENCES

1. Iwasaki, A., Medzhitov, R. (2004) Toll-like receptor control of the adaptive immune responses. *Nat. Immunol.* **5,** 987–995.
2. Fieschi, C., Casanova, J. L. (2003) The role of interleukin-12 in human infectious diseases: only a faint signature. *Eur. J. Immunol.* **33,** 1461–1464.
3. Uhlig, H. H., McKenzie, B. S., Hue, S., Thompson, C., Joyce-Shaikh, B., Stepankova, R., Robinson, N., Buonocore, S., Tlaskalova-Hogenova, H., Cua, D. J., Powrie, F. (2006) Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. *Immunity* **25,** 309–318.
4. Alaibac, M., Berti, E., Chizzolini, C., Fineschi, S., Marzano, A. V., Pigozzi, B., Riboldi, E., Sozzani, S., Kuhn, A. (2006) Role of cellular immunity in the pathogenesis of autoimmune skin diseases. *Clin. Exp. Rheumatol.* **24,** S14–S19.
5. Banchereau, J., Steinman, R. M. (1998) Dendritic cells and the control of immunity. *Nature* **392,** 245–252.
6. Kapsenberg, M. L. (2003) Dendritic-cell control of pathogen-driven T-cell polarization. *Nat. Rev. Immunol.* **3,** 984–993.

7. Reis e Sousa, C. (2001) Dendritic cells as sensors of infection. *Immunity* **14,** 495–498.
8. Reis e Sousa, C. (2004) Activation of dendritic cells: translating innate into adaptive immunity. *Curr. Opin. Immunol.* **16,** 21–25.
9. Lanzavecchia, A., Sallusto, F. (2001) Regulation of T cell immunity by dendritic cells. *Cell* **106,** 263–266.
10. Mantovani, A., Allavena, P., Sozzani, S., Vecchi, A., Locati, M., Sica, A. (2004) Chemokines in the recruitment and shaping of the leukocyte infiltrate of tumors. *Semin. Cancer Biol.* **14,** 155–160.
11. Vulcano, M., Albanesi, C., Stoppacciaro, A., Bagnati, R., D'Amico, G., Struyf, S., Transidico, P., Bonecchi, R., Del Prete, A., Allavena, P., Ruco, L. P., Chiabrando, C., Girolomoni, G., Mantovani, A., Sozzani, S. (2001) Dendritic cells as a major source of macrophage-derived chemokine/CCL22 in vitro and in vivo. *Eur. J. Immunol.* **31,** 812–822.
12. Trinchieri, G. (2003) Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat. Rev. Immunol.* **3,** 133–146.
13. Oppmann, B., Lesley, R., Blom, B., Timans, J. C., Xu, Y., Hunte, B., Vega, F., Yu, N., Wang, J., Singh, K., et al. (2000) Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* **13,** 715–725.
14. Kastelein, R. A., Hunter, C. A., Cua, D. J. (2007) Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu. Rev. Immunol.* **25,** 221–242.
15. Veldhoen, M., Hocking, R. J., Atkins, C. J., Locksley, R. M., Stockinger, B. (2006) TGFβ in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* **24,** 179–189.
16. Mangan, P. R., Harrington, L. E., O'Quinn, D. B., Helms, W. S., Bullard, D. C., Elson, C. O., Hatton, R. D., Wahl, S. M., Schoeb, T. R., Weaver, C. T. (2006) Transforming growth factor-β induces development of the T(H)17 lineage. *Nature* **441,** 231–234.
17. Aggarwal, S., Ghilardi, N., Xie, M. H., de Sauvage, F. J., Gurney, A. L. (2003) Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J. Biol. Chem.* **278,** 1910–1914.
18. Acosta-Rodriguez, E. V., Napolitani, G., Lanzavecchia, A., Sallusto, F. (2007) Interleukins 1β and 6 but not transforming growth factor-β are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat. Immunol.* **8,** 942–949.
19. Wilson, N. J., Boniface, K., Chan, J. R., McKenzie, B. S., Blumenschein, W. M., Mattson, J. D., Basham, B., Smith, K., Chen, T., Morel, F., Lecron, J. C., Kastelein, R. A., Cua, D. J., McClanahan, T. K., Bowman, E. P., de Waal Malefyt, R. (2007) Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat. Immunol.* **8,** 950–957.
20. Acosta-Rodriguez, E. V., Rivino, L., Geginat, J., Jarrossay, D., Gattorno, M., Lanzavecchia, A., Sallusto, F., Napolitani, G. (2007) Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat. Immunol.* **8,** 639–646.
21. Happel, K. I., Dubin, P. J., Zheng, M., Ghilardi, N., Lockhart, C., Quinton, L. J., Odden, A. R., Shellito, J. E., Bagby, G. J., Nelson, S., Kolls, J. K. (2005) Divergent roles of IL-23 and IL-12 in host defense against *Klebsiella pneumoniae*. *J. Exp. Med.* **202,** 761–769.
22. Huang, W., Na, L., Fidel, P. L., Schwarzenberger, P. (2004) Requirement of interleukin-17A for systemic anti-*Candida albicans* host defense in mice. *J. Infect. Dis.* **190,** 624–631.
23. Murphy, C. A., Langrish, C. L., Chen, Y., Blumenschein, W., McClanahan, T., Kastelein, R. A., Sedgwick, J. D., Cua, D. J. (2003) Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. *J. Exp. Med.* **198,** 1951–1957.
24. Langrish, C. L., Chen, Y., Blumenschein, W. M., Mattson, J., Basham, B., Sedgwick, J. D., McClanahan, T., Kastelein, R. A., Cua, D. J. (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* **201,** 233–240.
25. Annunziato, F., Cosmi, L., Santarlasci, V., Maggi, L., Liotta, F., Mazzinghi, B., Parente, E., Fili, L., Ferri, S., Frosali, F., Giudici, F., Romagnani, P., Parronchi, P., Tonelli, F., Maggi, E., Romagnani, S. (2007) Phenotypic and functional features of human Th17 cells. *J. Exp. Med.* **204,** 1849–1861.
26. Yen, D., Cheung, J., Scheerens, H., Poulet, F., McClanahan, T., McKenzie, B., Kleinschek, M. A., Owyang, A., Mattson, J., Blumenschein, W., Murphy, E., Sathe, M., Cua, D. J., Kastelein, R. A., Rennick, D. (2006) IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. *J. Clin. Invest.* **116,** 1310–1316.
27. Duerr, R. H., Taylor, K. D., Brant, S. R., Rioux, J. D., Silverberg, M. S., Daly, M. J., Steinhart, A. H., Abraham, C., Regueiro, M., Griffiths, A., et al. (2006) A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* **314,** 1461–1463.
28. Li, Y., Chu, N., Hu, A., Gran, B., Rostami, A., Zhang, G. X. (2007) Increased IL-23p19 expression in multiple sclerosis lesions and its induction in microglia. *Brain* **130,** 490–501.
29. Kim, H. R., Cho, M. L., Kim, K. W., Juhn, J. Y., Hwang, S. Y., Yoon, C. H., Park, S. H., Lee, S. H., Kim, H. Y. (2007) Up-regulation of IL-23p19 expression in rheumatoid arthritis synovial fibroblasts by IL-17 through PI3-kinase-, NF-κB- and p38 MAPK-dependent signaling pathways. *Rheumatology (Oxford)* **46,** 57–64.
30. Lee, E., Trepicchio, W. L., Oestreicher, J. L., Pittman, D., Wang, F., Chamian, F., Dhodapkar, M., Krueger, J. G. (2004) Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. *J. Exp. Med.* **199,** 125–130.
31. Fuss, I. J., Becker, C., Yang, Z., Groden, C., Hornung, R. L., Heller, F., Neurath, M. F., Strober, W., Mannon, P. J. (2006) Both IL-12p70 and IL-23 are synthesized during active Crohn's disease and are down-regulated by treatment with anti-IL-12 p40 monoclonal antibody. *Inflamm. Bowel Dis.* **12,** 9–15.
32. Mannon, P. J., Fuss, I. J., Mayer, L., Elson, C. O., Sandborn, W. J., Present, D., Dolin, B., Goodman, N., Groden, C., Hornung, R. L., Quezado, M., Yang, Z., Neurath, M. F., Salfeld, J., Veldman, G. M., Schwertschlag, U., Strober, W. (2004) Anti-interleukin-12 antibody for active Crohn's disease. *N. Engl. J. Med.* **351,** 2069–2079.
33. Langowski, J. L., Zhang, X., Wu, L., Mattson, J. D., Chen, T., Smith, K., Basham, B., McClanahan, T., Kastelein, R. A., Oft, M. (2006) IL-23 promotes tumor incidence and growth. *Nature* **442,** 461–465.
34. De Ruijter, A. J., van Gennip, A. H., Caron, H. N., Kemp, S., van Kuilenburg, A. B. (2003) Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem. J.* **370,** 737–749.
35. De Schepper, S., Bruwiere, H., Verhulst, T., Steller, U., Andries, L., Wouters, W., Janicot, M., Arts, J., Van Heusden, J. (2003) Inhibition of histone deacetylases by chlamydocin induces apoptosis and proteasome-mediated degradation of survivin. *J. Pharmacol. Exp. Ther.* **304,** 881–888.
36. Marks, P. A., Breslow, R. (2007) Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. *Nat. Biotechnol.* **25,** 84–90.
37. McLaughlin, F., Finn, P., La Thangue, N. B. (2003) The cell cycle, chromatin and cancer: mechanism-based therapeutics come of age. *Drug Discov. Today* **8,** 793–802.
38. Platta, C. S., Greenblatt, D. Y., Kunnimalaiyaan, M., Chen, H. (2007) The HDAC inhibitor trichostatin A inhibits growth of small cell lung cancer cells. *J. Surg. Res.* **142,** 219–226.
39. Golay, J., Cuppini, L., Leoni, F., Micò, C., Barbui, V., Domenghini, M., Lombardi, L., Neri, A., Barbui, A. M., Salvi, A., Pozzi, P., Porro, G., Pagani, P., Fossati, G., Mascagni, P., Introna, M., Rambaldi, A. (2007) The histone deacetylase inhibitor ITF2357 has anti-leukemic activity in vitro and in vivo and inhibits IL-6 and VEGF production by stromal cells. *Leukemia* **21,** 1892–1900.
40. Reilly, C. M., Mishra, N., Miller, J. M., Joshi, D., Ruiz, P., Richon, V. M., Marks, P. A., Gilkeson, G. S. (2004) Modulation of renal disease in MRL/lpr mice by suberoylanilide hydroxamic acid. *J. Immunol.* **173,** 4171–4178.
41. Chung, Y. L., Lee, M. Y., Wang, A. J., Yao, L. F. (2003) A therapeutic strategy uses histone deacetylase inhibitors to modulate the expression of genes involved in the pathogenesis of rheumatoid arthritis. *Mol. Ther.* **8,** 707–717.
42. Leoni, F., Zaliani, A., Bertolini, G., Porro, G., Pagani, P., Pozzi, P., Dona, G., Fossati, G., Sozzani, S., Azam, T., Bufler, P., Fantuzzi, G., Goncharov, I., Kim, S. H., Pomerantz, B. J., Reznikov, L. L., Siegmund, B., Dinarello, C. A., Maqscagni, P. (2002) The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines. *Proc. Natl. Acad. Sci. USA* **99,** 2995–3000.
43. Luhrs, H., Gerke, T., Muller, J. G., Melcher, R., Schauber, J., Boxberge, F., Scheppach, W., Menzel, T. (2002) Butyrate inhibits NF-κB activation in lamina propria macrophages of patients with ulcerative colitis. *Scand. J. Gastroenterol.* **37,** 458–466.
44. Vernia, P., Annese, V., Bresci, G., d'Albasio, G., D'Inca, R., Giaccari, S., Ingrosso, M., Mansi, C., Riegler, G., Valpiani, D., Caprilli, R. (2003) Topical butyrate improves efficacy of 5-ASA in refractory distal ulcerative colitis: results of a multicentre trial. *Eur. J. Clin. Invest.* **33,** 244–248.
45. Moreira, J. M., Scheipers, P., Sorensen, P. (2003) The histone deacetylase inhibitor trichostatin A modulates CD4+ T cell responses. *BMC Cancer* **3,** 30.
46. Saemann, M. D., Parolini, O., Bohmig, G. A., Kelemen, P., Krieger, P. M., Neumuller, J., Knarr, K., Kammlander, W., Horl, W. H., Diakos, C., Stuhlmeier, K., Zlabinger, G. J. (2002) Bacterial metabolite interference with maturation of human monocyte-derived dendritic cells. *J. Leukoc. Biol.* **71,** 238–246.

47. Diakos, C., Prieschl, E. E., Saemann, M., Novotny, V., Bohmig, G., Csonga, R., Baumruker, T., Zlabinger, G. J. (2002) Novel mode of interference with nuclear factor of activated T-cells regulation in T-cells by the bacterial metabolite *n*-butyrate. *J. Biol. Chem.* **277,** 24243–24251.
48. Brogdon, J. L., Xu, Y., Szabo, S. J., An, S., Buxton, F., Cohen, D., Huang, Q. (2007) Histone deacetylase activities are required for innate immune cell control of Th1 but not Th2 effector cell function. *Blood* **109,** 1123–1130.
49. Chen, L. F., Greene, W. C. (2004) Shaping the nuclear action of NF-κB. *Nat. Rev. Mol. Cell Biol.* **5,** 392–401.
50. Bernstein, B. E., Tong, J. K., Schreiber, S. L. (2000) Genomewide studies of histone deacetylase function in yeast. *Proc. Natl. Acad. Sci. USA* **97,** 13708–13713.
51. Kurdistani, S. K., Tavazoie, S., Grunstein, M. (2004) Mapping global histone acetylation patterns to gene expression. *Cell* **117,** 721–733.
52. De Ruijter, A. J., Meinsma, R. J., Bosma, P., Kemp, S., Caron, H. N., van Kuilenburg, A. B. (2005) Gene expression profiling in response to the histone deacetylase inhibitor BL1521 in neuroblastoma. *Exp. Cell Res.* **309,** 451–467.
53. Natoli, G., Saccani, S., Bosisio, D., Marazzi, I. (2005) Interactions of NF-κB with chromatin: the art of being at the right place at the right time. *Nat. Immunol.* **6,** 439–445.
54. Allavena, P., Badolato, R., Facchetti, F., Vermi, W., Paganin, C., Luini, W., Giliani, S., Mazza, C., Bolzern, U., Chiesa, I., Notarangelo, L., Mantovani, A., Sozzani, S. (2001) Monocytes from Wiskott-Aldrich patients differentiate in functional mature dendritic cells with a defect in CD83 expression. *Eur. J. Immunol.* **31,** 3413–3421.
55. Reis e Sousa, C., Germain, R. N. (1999) Analysis of adjuvant function by direct visualization of antigen presentation in vivo: endotoxin promotes accumulation of antigen-bearing dendritic cells in the T cell areas of lymphoid tissue. *J. Immunol.* **162,** 6552–6561.
56. Reis e Sousa, C., Hieny, S., Scharton-Kersten, T., Jankovic, D., Charest, H., Germain, R. N., Sher, A. (1997) In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. *J. Exp. Med.* **186,** 1819–1829.
57. Bode, K. A., Schroder, K., Hume, D. A., Ravasi, T., Heeg, K., Sweet, M. J., Dalpke, A. H. (2007) Histone deacetylase inhibitors decrease Toll-like receptor-mediated activation of proinflammatory gene expression by impairing transcription factor recruitment. *Immunology* **122,** 596–606.
58. Rahman, M. M., Kukita, A., Kukita, T., Shobuike, T., Nakamura, T., Kohashi, O. (2003) Two histone deacetylase inhibitors, trichostatin A and sodium butyrate, suppress differentiation into osteoclasts but not into macrophages. *Blood* **101,** 3451–3459.
59. Mahlknecht, U., Will, J., Varin, A., Hoelzer, D., Herbein, G. (2004) Histone deacetylase 3, a class I histone deacetylase, suppresses MAPK11-mediated activating transcription factor-2 activation and represses TNF gene expression. *J. Immunol.* **173,** 3979–3990.
60. Klampfer, L., Huang, J., Swaby, L. A., Augenlicht, L. (2004) Requirement of histone deacetylase activity for signaling by STAT1. *J. Biol. Chem.* **279,** 30358–30368.
61. Nencioni, A., Beck, J., Werth, D., Grunebach, F., Patrone, F., Ballestrero, A., Brossart, P. (2007) Histone deacetylase inhibitors affect dendritic cell differentiation and immunogenicity. *Clin. Cancer Res.* **13,** 3933–3941.
62. Iwata, K., Tomita, K., Sano, H., Fujii, Y., Yamasaki, A., Shimizu, E. (2002) Trichostatin A, a histone deacetylase inhibitor, down-regulates interleukin-12 transcription in SV-40-transformed lung epithelial cells. *Cell. Immunol.* **218,** 26–33.
63. Suuronen, T., Huuskonen, J., Pihlaja, R., Kyrylenko, S., Salminen, A. (2003) Regulation of microglial inflammatory response by histone deacetylase inhibitors. *J. Neurochem.* **87,** 407–416.
64. Huang, N., Katz, J. P., Martin, D. R., Wu, G. D. (1997) Inhibition of IL-8 gene expression in Caco-2 cells by compounds which induce histone hyperacetylation. *Cytokine* **9,** 27–36.
65. Lu, J., Sun, H., Wang, X., Liu, C., Xu, X., Li, F., Huang, B. (2005) Interleukin-12 p40 promoter activity is regulated by the reversible acetylation mediated by HDAC1 and p300. *Cytokine* **31,** 46–51.
66. Sozzani, S. (2005) Dendritic cell trafficking: more than just chemokines. *Cytokine Growth Factor Rev.* **16,** 581–592.
67. Sato, W., Aranami, T., Yamamura, T. (2007) Cutting edge: human Th17 cells are identified as bearing CCR2+CCR5– phenotype. *J. Immunol.* **178,** 7525–7529.
68. Marks, P. A. (2007) Discovery and development of SAHA as an anticancer agent. *Oncogene* **26,** 1351–1356.
69. Duvic, M., Talpur, R., Ni, X., Zhang, C., Hazarika, P., Kelly, C., Chiao, J. H., Reilly, J. F., Ricker, J. L., Richon, V. M., Frankel, S. R. (2007) Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). *Blood* **109,** 31–39.