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Impact of HDAC inhibitors on dendritic cell functions

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Histone deacetylase inhibitors are presently used in the routine clinic treatment against cancers. Recent data have established that some of these treatments have potent anti-inflammatory or immunomodulatory effects at noncytotoxic doses that might be of benefit in immuno-inflammatory disorders or post-transplantation. At least some of these effects result from the ability of histone deacetylase inhibitors to modulate the immune system. Dendritic cells are professional antigen presenting cells that play a major role in this immune system. Data summarized in this review brings some novel information on the impact of histone deacetylase inhibitors on dendritic cell functions, which may have broader implications for immunotherapeutic strategies.

Dendritic cells (DCs) are the most potent antigen-presenting cells (APC) in vitro and in vivo. They play a key role in the initiation of immune responses and are considered to be promising targets for immunotherapy [1]. DCs induce effective immunity against pathogens and "altered self" like tumor, while maintaining tolerance to self-antigens (Ags) [2]. In mice, constitutive ablation of DCs breaks self-tolerance, producing spontaneous autoimmunity [3].

Based on many studies observed in mouse models, DCs have been classified into two major classes: conventional DCs and plasmacytoid DCs [4,5]. Although conventional DCs are the most studied cells, the functions of plasmacytoid DCs are more specific. Plasmacytoid DCs are a rare population of circulating cells that have the unique ability to rapidly produce large quantities of type I interferon in response to viral infections [6]. Under steady-state conditions they are present in the bloodstream and secondary lymphoid organs, but are normally absent from most peripheral tissues [7,8]. From the peripheral blood, plasmacytoid DCs are recruited to inflammation sites, where they accumulate and play a significant immunomodulatory role in many animal viral models and disease settings [9,10]. Conventional DCs, on other hand, are localized in all peripheral tissues in proximity to the epithelium of body surfaces, where there is risk of invasion by pathogens. These DCs can capture pathogen-derived material from the periphery via blood or afferent lymphatics to draining lymph nodes, where they activate T cells. In the thymus, DCs have an important role in maintaining self-tolerance by negatively selecting autoreactive T cells and positively selecting regulatory T cells (Tregs) [11]. These DCs are also localized in secondary lymphoid tissues (spleen and lymph nodes). Some lymphoid-resident DCs subsets have been described in mice, including $CD8\alpha^+$ DCs responsible for cross-presentation of exogenous Ags on major histocompatibility complex class I, and $CD8\alpha^-$ DCs specialized in $CD4^+$ T-cell activation [12]. Another DCs subpopulation that has been described relates to inflammatory DCs, which seem to derive from monocytes during inflammation. This differentiation of monocytes into DCs does not happen under noninflammatory, steady-state conditions [13,14]. Human monocyte-derived DCs generated in vitro in response to granulocyte-macrophage colony-stimulating factor and interleukin (IL)-4 are similar to these inflammatory DCs [14].

DCs control immunity

Under steady-state conditions, in peripheral tissues, DCs are in an immature state, constantly capturing Ags but

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lacking the ability to efficiently process and present these Ags to T cells. In contrast, when there are signals associated with infection and tissue damage, DCs mature into powerful APCs and migrate to secondary lymphoid organs. There they activate naïve and memory T cells and enhance effector T-cell responses. Mature DCs supply naïve T cells with major histocompatibility complex/peptide complexes (signal 1) and costimulatory molecules (signal 2) that synergistically promote development of Ag-specific T cells. Moreover, DCs provide signal 3: soluble or membrane molecules that are responsible for polarization of distinct T-cell subset (i.e., Th1, Th2, Th17, Treg...). The various T-cell subsets control different components of cellular and humoral immunity [15]. A typical example of signal 3 is IL-12. It is secreted by DCs in response to some microbes and effectively leads to development of Th1 cells [16,17]. Pathogen- and tissue-derived molecules can change the nature of signal 3. Thus, DCs operate as a connection between innate and adaptive immunity by transmitting essential information on the nature of damage and infection in the periphery to naïve T cells in the lymph nodes.

DCs control tolerance

Studies performed in the last few years have confirmed the hypothesis that immature DCs can induce tolerance, while mature DCs can induce immunity [18]. For example, in 2003, Probst et al. [19] generated a Cre/LoxP-based system that permitted inducible Ag presentation by DCs in vivo under steady-state or immune-activating conditions. In this study, Ags presented by resting immature DCs induced Ag-specific tolerance; whereas Ags presented by mature DCs promoted cytotoxic T lymphocytes expansion and protective effector functions. In contrast, it has become increasingly clear that the maturation state of DCs is not always linked with their activating or protective immune functions. For instance, mature DCs can efficiently expand naturally occurring Tregs [20–22]. Also, some microbial products cause the maturation of DCs by increasing major histocompatibility complex II and costimulatory molecule expression, but these DCs secrete anti-inflammatory IL-10 and provoke the expansion of IL-10–producing Tregs [23,24]. It is now known that mature DCs can demonstrate tolerogenic functions, and that their tolerogenicity can be promoted by signals that they receive during maturation. For example, immunosuppressive cytokines (IL-10, transforming growth factor–β) [25–27] or some pathogen-derived molecules [24] have all been shown to drive the differentiation of tolerogenic DCs.

Histone deacetylase inhibitors

Acetylation of histones represents one of several post-translational modifications. This epigenetic regulation of gene expression is controlled by the opposing activities of two enzymes, histone deacetylases (HDACs) and histone acetyltransferases. Histone acetylation by histone acetyltransferases is associated with activation of transcription through relaxed chromatin structure, whereas deacetylation by HDACs induces a more condensed or inactive chromatin state, leading to gene repression. Emerging data demonstrate that HDACs also modify the activity of diverse types of nonhistone cellular proteins [28]. It is therefore possible that acetylation state of nonhistone proteins induced by histone acetyltransferases and HDACs is an important in regulate function, stability, and interactions between proteins and proteins and DNA [29]. The imbalance of acetylation and deacetylation may be responsible for a wide range of human disorders, including oncogenesis and immune dysfunction [30].

HDACs include a family of 18 genes subdivided into the following distinct classes: class I (HDAC 1, 2, 3, and 8), class II (HDAC 5, 6, 7, 9, and 10), and class IV (HDAC 11) have sequence similarity and require Zn^{+} for enzymatic activity [31–35]. Class III is a structurally distinct NAD^{+}-dependent subfamily and belong to the Sirtuin family [32]. The classical HDAC inhibitors (HDACIs), which act on the zinc-dependent HDACs (HDAC 1–11), include benzamides (MS275), short-chain fatty acids (sodium butyrate and valproic acid [VPA]), hydroxamic acids (trichostatin A [TSA] and suberoylanilide hydroxamic acid [SAHA]) and cyclic tetrapeptides like trapoxin and depsipeptide [31,34,35] (Table 1). Two of them, SAHA and ITF 2357, were approved by the US Food and Drug Administration for treatment of cutaneous T-cell lymphoma [36,37]. Other HDACIs, such as butyrate and VPA, have long been utilized clinically in nononcologic contexts. For example, butyrate continues to be used as a therapy for inflammatory bowel disease, although whether its benefits are due to inhibition of HDAC activity stays controversial [38]. Similarly, for a while, VPA has been used as an anticonvulsive activity. There are no data available to confirm whether prolonged

Table 1. Different classes of HDAC inhibitors

	HDACIs	HDAC specificity	Clinical trial	Tumors
Hydroxamic acids	trichostatin A	Class I, II		Leukemia, lymphoma, myeloma, various solid tumors
	SAHA	Class I, II	FDA approval	Cutaneous T-cell lymphoma
Short-chain fatty acids	Butyrate	Class I, IIa	Phase I, II	Leukemia, lymphoma, intestinal cancers
	VPA	Class I, lla	Phase I, II, III	Leukemia, various solid tumors, myelodysplasia
Benzamides	MS-275	Class I	Phase I, II	Solid tumors, leukemia, lymphoma

FDA = US Food and Drug Administration.

treatment of epileptic patients with VPA also protects against comorbid immunoinflammatory diseases through inhibition of HDACs [39].

Anticancer effects of HDAC inhibitors

At high concentrations, HDACIs exhibit antitumor properties and are considered cytotoxic. Approximately 80 clinical trials with HDACIs are currently ongoing and testing more than a dozen drugs in various solid and hematologic malignancies [40]. The anticancer potential of HDACIs arises from their capacity to influence several cellular processes that are usually deregulated in tumor cells. In general, inhibition of the cell cycle, activation of differentiation, and induction of apoptosis are the important antitumor activities of HDACIs (Fig. 1). HDACIs alter the differentiation of leukemia circulating cells [41], of breast cancer cell lines [42], of prostate cancer [43], and also of renal cell carcinoma [44]. Furthermore, the ability of HDACIs to repress angiogenesis and deactivate the host immune system may play a significant role in their therapeutic response [31]. Recent preclinical studies demonstrated a direct link between initiation of tumor cell apoptosis and therapeutic efficacy [45–48]. The capacity of HDACIs to induce death of cancer cells but not normal cells is an important point and suggests that HDACIs may be a more promising agent compared with conventional drugs. HDACIs can target apoptosis through the mitochondrial pathway [49], particularly through accumulation of reactive oxygen species and caspase activation in transformed but not normal cells [50]. HDACIs can also cause an increase in the level of thioredoxin, a major reducing protein for many targets in normal cells but not in transformed cells. In the case of tumor cells, they induce the binding of thioredoxin to thioredoxin binding protein (TBP2), its inactivation, and the induction of cell death [51]. This selective induction of tumor cell death can also be explained by the selective increase of tumor necrosis factor–related apoptosis-inducing ligand and its receptor (DR5) expression induced by HDACIs [52]. In addition, combining HDACIs with other proapoptotic agents can result in synergistic apoptosis and higher antitumor activities [31,53].

HDACIs modulate function of DCs

Although a large variety of HDACIs have been studied and developed for cancer therapy, emerging data demonstrate that HDACIs at lower and noncytotoxic concentrations possess potent anti-inflammatory and immunoregulatory effects [54,55]. In addition, multiple laboratories have shown that HDACIs can suppress several inflammatory and immune-mediated diseases, such as lupus, sepsis,

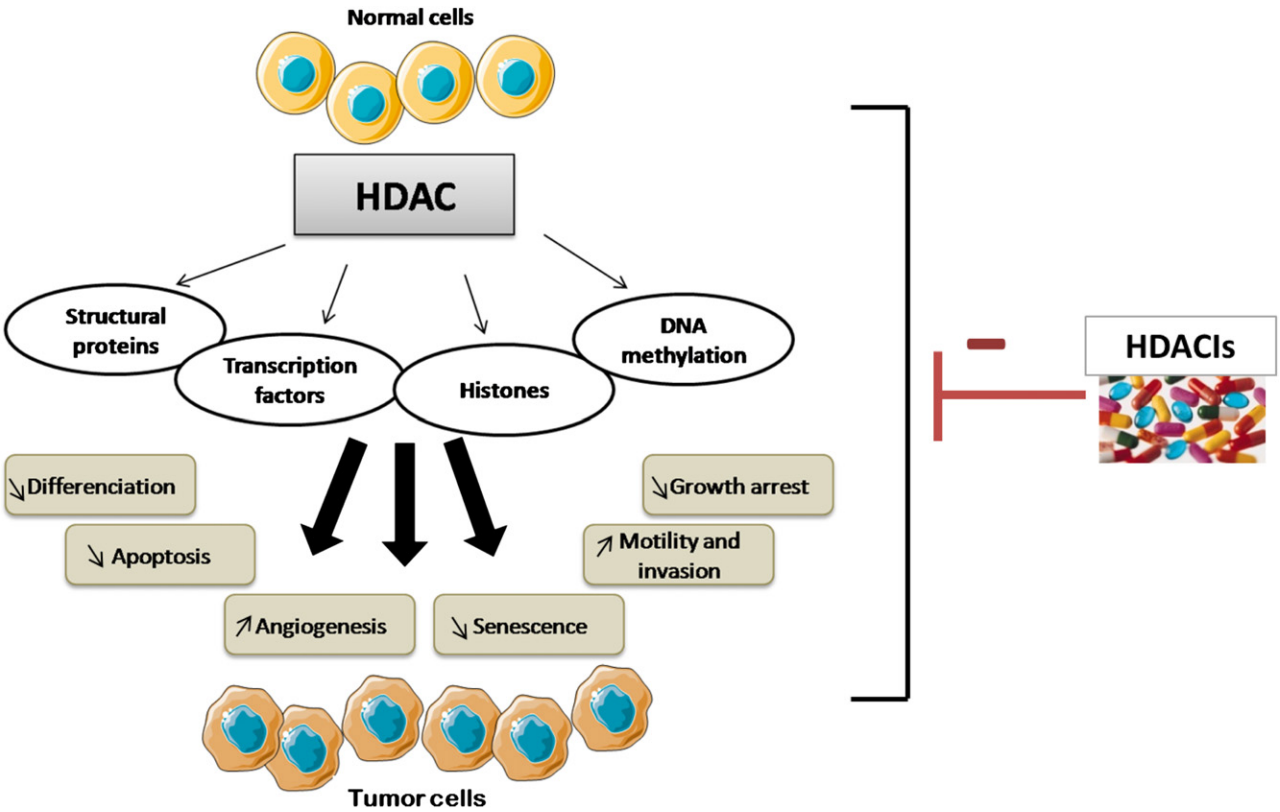

Figure 1. Schematic representation of anticancer activities of HDACIs. The antitumoral potential of HDACIs relies on their ability to influence many cellular processes that are deregulated in tumor cells. In general, inhibition of cell cycle, activation of differentiation and apoptosis are important antitumor activities of HDACIs.

inflammatory bowel disease, rheumatoid arthritis, autoimmune diabetes, allograft tolerance, and graft-vs-host disease, in preclinical models [55–59]. HDACIs have direct and indirect impacts on a variety of immune cell subsets. By reducing the secretion of inflammatory cytokines, they play an important role in the negative regulation of APCs. HDACIs also increase the number and function of naturally occurring Tregs, exert various effects on natural killer (NK) cell function, and inhibit the activity of genes involved in immune functions of macrophages [60]. Because of their central role in orchestrating innate and adaptive immunity, many groups have investigated the effect of HDACIs on DCs. Most results suggest that HDACIs affect biologic activities of DCs at different levels.

HDACIs repress expression of costimulatory molecules on DCs

CD40 is a well-characterized costimulatory molecule, with its ligand CD40L present on the surface of T cells. It is upregulated on activated DCs and functions as a trigger for the expression of two other important costimulatory molecules, CD80 and CD86 [61]. CD40–CD40L interaction is also essential for IL-12 secretion, which polarizes T-cell responses to a T-helper 1 (Th1 type) [62]. CD80 and CD86 are expressed on DCs and bind to CD28 on T cells. Costimulation of CD28 with CD80 and CD86 induces T-lymphocyte proliferation and cytokine secretion.

CD83 is a maturation marker on DCs [63]. Weakly expressed on immature DCs, it is strongly upregulated during DCs maturation together with CD80 and CD86. Previous studies in mice have shown that this molecule positively regulates $CD4^+$ T development as well as major histocompatibility complex class II Ag expression [64].

The effect of several HDACIs on these costimulation molecules was investigated in numerous studies. In 2007, for example Nencioni et al. showed that two HDACIs, MS-275 and VPA, affect the expression of costimulation and adhesion molecules on human monocyte-derived DCs [65]. They observed an important reduction of CD40, CD80, and CD83 expression, whereas the expression of CD86 was minimally affected. Similar observations were made by our group using VPA [66]. Exposure to LBH589, another HDACIs, also affected the costimulatory molecule expression on immature and mature DCs by decreasing CD83 and CD40 while increasing CD86 expression.

Similarly, the HDACIs apicidin, SAHA, ITF2357, and TSA were reported to significantly attenuate the expression of costimulatory molecules on mouse DCs both in vivo and in vitro [67–69].

HDACIs reduce cytokines secretion from DCs

After activation, DCs produce a large variety of chemokines and cytokines that contribute to T-cell priming (e.g., IL-1β, IL-6, IL-15, and tumor necrosis factor–α [TNFα]) as well as T-cell polarization (e.g., IL-12, IL-18, IL-7).

Nencioni et al. tested the effect of the HDACIs MS-275 on cytokine secretion by DCs and found a decrease in secretion of TNFα, IL-6, and IL-12. It is noteworthy that MS-275 also decreased the secretion of the anti-inflammatory cytokine IL-10 in response to poly(I-C).

The impact of butyrate was also analyzed on human DCs [70]. This study demonstrated that treated DCs showed lower production of IL-12p40 and IL-6 in response to lipopolysaccharides. The HDACI LBH589 also significantly repressed the production of IL-6, IL-12p70, IL-23, TNFα, as well as IL-10, by TLR3- and TLR4-activated DCs [71]. Moreover, in murine DCs, the HDACIs suberoylanilide hydroxamic acid, TSA, and VPA were reported to block secretion of this proinflammatory cytokines TNFα, IL-1β, IL-6, and IL-12 [56,69,72,73].

Given the importance of DC-derived cytokines for the stimulation of lymphocyte responses, inhibition of cytokine production by HDACIs is likely to contribute to impairment of DCs immunostimulatory capacity.

HDACIs impact indoleamine 2,3-dioxygenase expression in DCs

Indoleamine 2,3-dioxygenase (IDO) is an immunomodulatory enzyme produced by some alternatively activated macrophages and other immunoregulatory cells. This enzyme is responsible for the catabolism of tryptophan, an amino acid that is essential for T-cell activation [74]. In 2008, Reddy demonstrated that SAHA treatment of murine DCs increased IDO expression at the messenger RNA and protein levels by acetylation of histone 4 in the promoter region of IDO [68]. In this study, three complementary approaches were used to investigate the importance of IDO induction in HDACI-treated DCs. First, they silenced the messenger RNA expression of IDO in SAHA-treated DCs by using IDO-specific small interfering RNA. This approach significantly reversed the suppression of the proinflammatory cytokine TNFα. Similarly, in lipopolysaccharide-stimulated DCs from $IDO^{-/-}$ animals or those treated with 1-MT, they demonstrated the loss of suppression of proinflammatory cytokine secretion. Finally, direct injection of HDACIs early after allogeneic bone marrow transplantation to chimeric animals whose bone marrow–derived cells lacked IDO failed to protect from graft-vs-host disease, demonstrating in vivo a functional role of IDO. Together, these data suggest that HDACIs regulate several DC functions through the induction of IDO.

This same team showed in 2009 that the acetylation of the nonhistone protein signal transducers and activators of transcription (STAT) 3 was also necessary for induction of IDO by HDACIs [75] (the essential role of STAT3 in suppressing immune responses [76,77] and in the negative regulation of DCs [78] has already been demonstrated). This study showed that HDAC inhibition has a critical role in increasing acetylation and activation of STAT3,

which regulates DCs, in part, by promoting the transcription of IDO.

Effect of HDACIs on polarization of naïve T cells

Mature DCs acquire the ability to send signals that are required for the polarization of the adaptive immune response. DC immune signals include cell–cell contact and the production of cytokines determining the differentiation of naïve T lymphocytes into diverse types of mature effector cells (e.g., Th1, Th2, Th17, and Treg). An imbalance of Th1, Th2, Th17, and Treg responses is critical in the pathogenesis of autoimmune diseases [79–81].

In vivo use of HDACIs has been shown to induce $CD4^+$ T-cell anergy by a mechanism that still needs to be delineated [82]. It has also been reported that HDACIs cause a decrease in the secretion of cytokines that prime T cells, potentially by induction of cyclin-dependent kinase inhibitor p21 and suppression of nuclear factor-κB [82,83]. Brogdon et al. [70] have shown that the HDACI LAQ824 is a potent inhibitor of IL-12p40, a common subunit for IL-12 and IL-23, in both DCs and macrophages and is necessary for the induction and perpetuation of Th1 responses. In 2008, Bosisio et al. also demonstrated that TSA or SAHA reduces the Th1- as well as the Th17-inducing potential of DC in vitro by decreasing the production of IL-12/interferon–β and IL-6/IL-23 [84]. Moreover, in mice, another HDACI apicidin appears to suppress Th1 polarization of murine bone marrow–derived DCs [67]. These findings represent relevant mechanisms through which HDACIs, at nonapoptotic doses, apply their immunomodulatory properties.

HDACIs increase Tregs

During the last few years, roles for $Foxp3^+$ Tregs in maintaining immune homeostasis have been identified and these cells have emerged as a main target for therapeutic manipulation to control autoimmunity and transplant rejection [85]. In recent studies, HDACIs have been shown to increase the number and suppressive functions of Tregs both in vitro and in vivo [65,68,86], leading to the notion that HDACI use might provide a pharmacological means to exploit the actions of these cells.

Most HDACIs have been studied recently for this effects on $Foxp3^+$ Treg function in vitro [87,88]. HDACIs of the hydroxamic acid group, such as TSA, SAHA, and M344, enhanced suppression by Tregs in vitro when used at

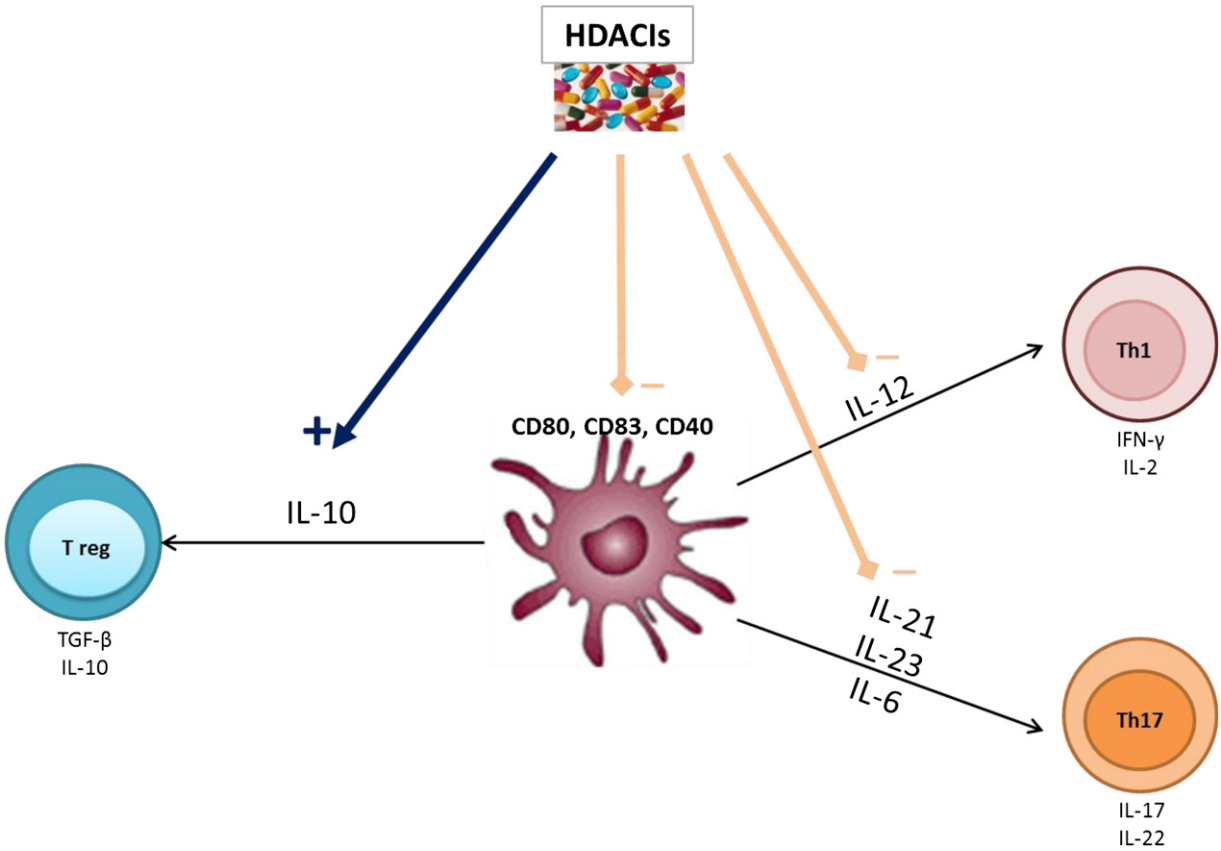

Figure 2. Regulation of immune cells by HDACIs. Several studies have demonstrated that HDAC inhibitors have numerous effects on immune cells. They increase the number and suppressive functions of Tregs both in vitro and in vivo. They also can reduce expression of costimulatory molecules and secretion of inflammatory cytokines by DCs, inducing the suppression of the development of Th1 and Th17 responses. IFN = interferon; TGF = transforming growth factor.

nanomolar concentrations, and the short-chain fatty acids, phenylbutyrate and VPA, improved Treg function when used at micromolar and millimolar levels, respectively. TSA, VPA, and sodium butyrate also increased Foxp3 messenger RNA expression and promoted peripheral conversion upon adoptive transfer of T cells into immunodeficient mice [86]. The effects of TSA and SAHA compared with the class I HDACI MS275 in colitis models showed variations in modulation of Treg function and lack of HDACI efficacy in Treg-depleted mice [89]. TSA was shown to reduce the differentiation of Foxp3$^+$ Tregs into Th17 cells [90]. HDACI therapy increased Treg function and decreased inflammatory responses in arthritis [91,92] and renal transplant rejection [93].

Impact of HDACi on NK cells

It is now well established that NK cells can play a major role in antitumor immunity, triggering cytotoxicity and interferon-γ secretion. NK cells can sense target tumor cells through activating receptors, such as NKG2D, DNAM-1, 2B4, and the NCRs NKp46, NKp44, and NKp30 [94,95] or upon sensing proinflammatory stimuli [96]. Different studies showed that (at least in vitro) HDACIs can sensitize tumor cells to NCR-, DNAM-1-, and NKG2D-dependent cytotoxicity by promoting upregulation of specific ligands on tumor cells [97,98]. However, in vivo, in the context of clinical trials, it appeared that HDACIs can severely impair NK cell activation, receptor expression, and effector functions, suggesting that they may deteriorate NK cell immune surveillance [99], a fact that may promote relapse in treated patients.

Conclusions and perspectives

In recent years, HDACIs have been mainly developed as anticancer agents. However, there is emerging evidence that HDACIs could have therapeutic potential for many nonmalignant diseases, as they possess potent anti-inflammatory and immunoregulatory effects. In this review, we discussed the impact of various structurally distinct HDACIs on DCs. It is increasingly established that HDACIs have direct and indirect effects on these cells, and they have a crucial role in the negative regulation of APCs, as well as the reduction of secretion of inflammatory cytokines, they also suppress development of Th1 and Th17 cells and increase the number and function of naturally occurring Tregs (Fig. 2). Th1 cells have the ability to favor cytotoxic T lymphocytes responses, which are crucial for an effective antitumor effect. HDACI-treated DCs were shown to be incapable of inducing Th1 responses. Thus, this deleterious effect of HDACIs on immunostimulatory responses might be an obstacle for an optimal cancer treatment. However, the anti-inflammatory and immunosuppressive properties of HDACIs at low concentrations could be useful in other clinical settings, such as chronic inflammatory diseases or graft-vs-host disease occurring after allogeneic stem cell transplantation [100]. Immunomodulatory effects of HDACIs have been established in preclinical autoimmunity models [101] and future objectives are to translate these findings into clinical applications.

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Conflict of interest disclosure

No financial interest/relationships with financial interest relating to the topic of this article have been declared.

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