phages after LPS and IL-4 stimulation, respectively, were identical in WT and Arg1Δ macrophages. Therefore, the authors conclude that "arginase-1-independent polyamine production stimulates the expression of alternatively activated macrophage markers". This point puts into question the use of Arg1 as a marker of alternatively activated macrophages. The authors imply that there is a mechanism whereby polyamine synthesis occurs through a metabolic pathway that does not involve arginase activity, but this theoretical pathway remains to be elucidated. Regardless of which pathway(s) may be used by macrophages for polyamine biosynthesis, the data in this manuscript demonstrate that endogenous polyamines play a major function in regulation of macrophage activation.

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KEY WORDS:
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Editorial: **HDAC inhibition begets more MDSCs**

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SEE CORRESPONDING ARTICLE ON PAGE 701

Emerging data demonstrate that HDACi, initially developed as anticancer agents, are also potent anti-inflammatory agents at non-cytotoxic doses [1, 2]. The mechanisms and promise of HDACi-mediated immune modulation are increasingly understood [1]. In this issue, Rosborough and colleagues [3] report a novel role for HDACi in regulating immune responses (**Fig. 1**). They demonstrate that HDACi enhance the generation and expansion of MDSCs, a key subset of regulatory APCs [4].

HDACs remove acetyl groups from ε-*N*-acetyl lysine amino acids on histone tails and regulate chromatin structure and dynamics [5–7]. Emerging data also show that in addition to regulating acetylation of lysines within

Abbreviations: BM=bone marrow, GVHD=graft-versus-host disease, HDACi=histone deacetylase inhibitors, HSC=hematopoietic stem cell, MDSC=myeloid-derived suppressor cell, SAHA=suberoylinalide hydroxamic acid, Treg=regulatory T cell, TSA=trichostatin-A

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SUMMARY:
- **HDAC inhibition regulates innate responses and DC functions**
- **Inhibition of HDAC enzymes in GMCSF treated BM progenitors impairs DC but promotes MDSC differentiation**
- **The MDSCs generated by HDAC inhibition demonstrate the expected in vitro suppressive functions that are dependent on iNOS and HO-1**
- **HDAC inhibition augments MDSCs numbers in vivo**

IMPLICATIONS:
- **Insight into HDAC inhibition mediated immune regulation**
- **Provide a platform for ex vivo expansion of MDSCs that may be exploited therapeutically**
- **The stability, phenotype and functional relevance of the in vivo expansion of MDSCs remain to be analyzed**
- **The specific HDACs and HATs involved in MDSC differentiation need to be determined**
- **The critical epigenetic and the non-histone proteins that must be acetylated need to be understood**

Figure 1. Summary and implications.

histones, HDAC enzymes regulate the acetylation of several nonhistone proteins [8–11]. They are classified into four main classes: class I HDACs include HDAC1, -2, -3, and -8; class II HDACs are HDAC4, -5, -7, and -9 (class IIa) and HDAC6 and -10 (class IIb); class III HDACs are homologs of yeast silent information regulator 2 proteins; and class IV HDAC is HDAC11 [11]. Class I HDAC enzymes are expressed in most cells and are largely, but not exclusively, restricted to the nucleus. By contrast, class II HDAC enzymes demonstrate a more restricted, tissue-specific expression and shuttle between the nucleus and cytoplasm [7, 12]. Most HDACi inhibit class I and II enzymes with varying efficiency [11]. The catalytic activities of the class I and II HDACs differ [7]. Class I HDAC enzymes exhibit strong deacetylase activity, whereas most class II HDACs are enzymatically less active and act primarily as scaffolding proteins within large multimolecular complexes.

HDACi belong to different classes of drugs with distinct chemical structures and abilities to inhibit HDAC enzymes [11, 13–15]. The two HDACi, used by Rosborough et al. [3]—TSA and SAHA—are nonselective, pan-HDACi that inhibit class I and II [11, 16]. HDACi can also modulate the acetylation of HDAC proteins themselves, causing alterations in their stability or activity [7]. They have been shown to regulate the function of various immune cells and modulate in vivo disease states in experimental models [1, 17]. Specifically, their impact on Tregs and T cell responses is being appreciated increasingly [13, 18]. Recent data have demonstrated the ability of these agents to inhibit the production of multiple proinflammatory cytokines from various APCs [1]. HDAC inhibition has been shown to enhance IDO expression in DCs, promote conversion of inflammatory macrophages into a tolerogenic phenotype, decrease TLR signaling, reduce costimulatory molecule expression, and increase IL-10 expression [19–22]. But, the mechanisms of action of HDACi on different APC subsets and their generation and function are not understood completely.

MDSCs are now being appreciated increasingly as key APC subsets that are responsible for regulating immune responses. They potently suppress T effector cell responses while enhancing Tregs [23]. They are a heterogeneous population of immature myeloid cells that consists of myeloid progenitors and precursors [4]. MDSCs are identified in murine studies as cells that are positive for CD11b and Gr-1. Based on expression of Ly-6C or Ly-6G, they can be characterized further as monocytic MDSCs ($CD11b^+$ Ly-$6G^-$ Ly-$6C^{high}$) or granulocytic MDSCs ($CD11b^+$ Ly-$6G^+$ Ly-$6C^{low}$) [23]. The mechanisms of suppression of T cell responses by MDSCs include a high level of arginase activity, NO, ROS production, induction of Tregs, secretion of TGF-β, depletion of cysteine, and up-regulation of PGE_2. The distinct subsets of MDSCs use differential pathways for regulating immune responses [4, 22, 23]. For example, granulocytic MDSCs are reported to be dependent on ROS, whereas monocytic MDSCs are more dependent on arginase and NO for regulating T cell responses [4].

The powerful immune-suppressive features of MDSC make them attractive candidates for use in cell therapy to reduce unwanted and exuberant immune responses, such as in autoimmunity, graft rejection, and GVHD [23, 24]. To facilitate such studies, it would be essential to generate, ex vivo, relatively large and stable immune-suppressive MDSCs. Murine studies have demonstrated that G-CSF expands MDSCs in vitro and in vivo [23]. Rosborough et al. [3] analyzed the impact of HDAC inhibition on the generation and function of MDSCs. They demonstrate that HDAC inhibition of BM GM-CSF-treated cultures with TSA or SAHA expanded the HSC and progenitor compartments, skewed myeloid differentiation, impaired the development of DCs, and enhanced the generation of MDSCs. Although addition of rIL-4 to the cultures expanded the progenitor cells and demonstrated skewed myeloid differentiation, it did not enhance the generation of MDSCs. Upon further characterization, they found that these MDSCs expressed $CD11b^+$ $F4/80^{int}$ and Ly-$6C^{high}$, suggesting generation of a monocytic MDSC phenotype. These cells demonstrated equivalent suppression of allogeneic T cell responses in vitro. However, in contrast to control MDSCs, those derived following HDAC inhibition showed reduced expression of arginase, iNOS, and HO-1. Furthermore, arginase was not required, whereas iNOS and HO-1 activity was required for the suppressive effects on allogeneic T cells. Importantly, HDAC inhibition also augmented in vivo expansion of MDSCs in BM and spleen by GM-CSF, al-

though the functional impact of this in vivo expansion was not addressed.

This study, like all interesting and seminal observations, while illuminating a role for HDAC inhibition in the generation of MDSCs, also raises several additional questions. Why did the addition of IL-4 prevent the increase in MDSC generation despite the increase in HSC and progenitors? Is the increase in HSCs and progenitors critical and relevant? Or is it merely an epiphenomenon? The developmental pathways that are critical for MDSC generation remain largely unknown, and how would those pathways affected by HDAC inhibition remain to be deciphered? What would be the impact on the phenotype of MDSCs, especially in vivo? Is that functionally relevant? An intriguing observation is that the differential mechanisms that might be used by the HDACi induced MDSCs. The role of arginase, iNOS, and HO-1, in addition to the other pathways, such as induction of Tregs, relevance of cysteine depletion, and PGE_2, remains to be understood as well [23, 25–27]. Furthermore, the key molecular mechanisms remain to be explored. In addition to histone deacetylation and epigenetic alterations, is acetylation of nonhistone proteins critical? Further identification of the specific HDAC enzyme, the specific histone acetyltransferase, and their putative targets in generating MDSCs will refine our understanding of the role of protein acetylation and MDSC biology.

This study expands the scope of HDAC inhibition-mediated immune regulation and provides a novel method for generating MDSCs with greater efficiency, both in vitro and in vivo. It provides texture to our current understanding of the role of HDAC inhibition in regulating immune responses. Importantly, in light of the known immune-regulatory effects of MDSCs, the observations by Rosborough and colleagues [3] may pave way for building a platform to robustly expand MDSCs ex vivo and thus, facilitate well-designed, adoptive cell-therapy trials to study the potential of MDSCs in regulating autoimmunity, allograft rejection, and GVHD.

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