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Impact of HDAC inhibitors on macrophage polarization to enhance innate immunity against infections

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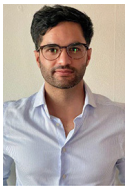
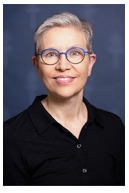
Innate immunity plays an important role in host defense against pathogenic infections. It involves macrophage polarization into either the pro-inflammatory M1 or the anti-inflammatory M2 phenotype, influencing immune stimulation or suppression, respectively. Epigenetic changes during immune reactions contribute to long-term innate immunity imprinting on macrophage polarization. It is becoming increasingly evident that epigenetic modulators, such as histone deacetylase (HDAC) inhibitors (HDACi), enable the enhancement of innate immunity by tailoring macrophage polarization in response to immune stressors. In this review, we summarize current literature on the impact of HDACi and other epigenetic modulators on the functioning of macrophages during diseases that have a strong immune component, such as infections. Depending on the disease context and the chosen therapeutic intervention, HDAC1, HDAC2, HDAC3, HDAC6, or HDAC8 are particularly important in influencing macrophage polarization towards either M1 or M2 phenotypes. We anticipate that therapeutic strategies based on HDAC epigenetic mechanisms will provide a unique approach to boost immunity against disease challenges, including resistant infections.



Mohammad Faizan Bhat obtained his Bachelor's degree in Pharmaceutical Sciences from the University of Kashmir in 2014. He then pursued a Master's degree in Pharmaceutical Chemistry at the Indian Institute of Technology, Banaras Hindu University (BHU), graduating in 2016. In October 2018, Faizan began his doctoral studies in the Department of Chemical and Pharmaceutical Biology at the University of Groningen, The Netherlands, under the supervision of Prof. Dr Gerrit J. Poelarends. His PhD research focused on chemoenzymatic and photobiocatalytic synthesis strategies. Currently, Faizan works as a postdoctoral researcher, specializing in drug design and the medicinal chemistry of immunity-enhancing drugs and working with Dr Frank J. Dekker.



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Frank J. Dekker studied at Utrecht University where he obtained his PhD (2004) while working with Rob M.J. Liskamp. He did his postdoctoral research with Herbert Waldmann at the Max-Planck Institute of Molecular Physiology in Dortmund, from 2004 until 2007. Subsequently, he moved to the University of Groningen to start his own research on the development of chemistry-based methods for the inhibition and detection of enzyme activity in the epigenetic regulation of inflammatory responses. He is currently an associate professor and director of the master's program in medical pharmaceutical sciences at the University of Groningen.

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Introduction

Inflammatory diseases, including those with infectious components, pose substantial challenges to global health.[p1] In response, there is a growing interest in therapeutic strategies aimed at modulating pro- or anti-inflammatory responses. One emerging approach involves the enhancement of innate immunity, which leverages the body's own defense mechanisms to combat diseases, particularly those with infectious etiologies.[p2] Central to this process are macrophages, which are versatile immune cells situated at the forefront of the immune defense that are responsible for detecting and eliminating invading pathogens. Their remarkable ability to transit between distinct functional states, a process known as polarization, is key in coordinating immune responses.[p3]

Macrophage polarization entails the differentiation of these cells into distinct phenotypes, characterized primarily as pro-inflammatory M1 or anti-inflammatory M2, each exerting unique immunomodulatory effects.[p3] This process is intricately regulated by a myriad of factors, including genetic and environmental influences. An increasing amount of research highlights the role of epigenetics in tailoring the mechanisms that govern macrophage polarization.[p4] These epigenetic modifications, characterized by reversible alterations in chromatin structure and gene expression patterns, are increasingly acknowledged as fundamental regulators of immune cell function, notably in macrophages.[p5] The major classes or enzymes that have been studied for their role in macrophage polarization include histone acetyltransferases (HATs), deacetylases (HDACs), methyltransferases (HMTs), and demethylases (HDMTs).[p6] HDACs have gained significant attention for their ability to control chromatin accessibility and gene transcription dynamically.[p7]

HDACs regulate the acetylation status of histone proteins, thereby influencing the degree of compaction of chromatin and the accessibility of DNA to the transcriptional machinery.[p8] Through their action, HDACs play a crucial role in controlling gene expression programs that are involved in various cellular processes, including inflammation and immunity. The role of HDACs in the epigenetic regulation of macrophage functional polarization presents a promising avenue for exploring alternative therapeutic strategies to strengthen innate immunity, especially innate immunity against highly resistant infections.[p9]

HDACs are broadly classified into three major classes, based on sequence homology, cellular localization, and enzymatic activity. Class I HDACs, including HDAC1, 2, 3, and 8, predominantly reside in the nucleus and modulate histone acetylation to regulate transcription. Class II HDACs, such as HDAC4, 5, 6, 7, 9, and 10, exhibit diverse subcellular localization and regulate various cellular processes beyond histone modification, including cell cycle progression and stress responses. Sirtuins, which are classified as Class III HDACs, require NAD^+ as a cofactor and are involved in metabolic regulation, aging, and stress responses.[p10] Various isoforms of HDACs have been implicated in epigenetically regulating distinct facets of macrophage polarization, thereby eliciting diverse responses depending on the stimulus, whether it be pathogen infection or tissue damage following the suppression of the initial stimulus. This modulation of polarization states by HDACs offers the inherent advantage of imprinting immune responses, thereby providing an effect that is more sustained than that resulting from the modulation of conventional transient signaling pathways.[p9],[p11]

In this review, we describe the intricate interplay between HDACs and macrophage polarization, and its role in regulating innate immunity. We focus on the diverse roles of HDACs in regulating macrophage function, highlighting their differential effects on innate immune responses. Furthermore, we explore the therapeutic implications of the role of HDAC inhibitors (HDACi) in modulating macrophage-mediated immune responses, offering insights into potential strategies for combating infectious diseases by modulating relatively untapped epigenetic mechanisms.

Epigenetic control of innate immunity

The immune system's primary defense mechanism relies on triggering highly precise gene expression programs that are tailored to specific signals, cell-lineages, and timings. This precise gene expression leads to alterations in signal transduction pathways and cellular metabolism across various immune cell types. The transcription factors that are activated, bind to inducible genes responsible for encoding cytokines, transcription factors, effector proteins, and metabolic regulators. These effectors play essential roles in progressing pathogen clearance, supporting adaptive immunity, eliminating cellular debris, and promoting tissue repair. Importantly, specific epigenetic factors, such as exposure to pathogens and environmental changes, further enhance the responsiveness of innate immune cells beyond their initial differentiation. Pathogenic infections can initiate the secretion of microbial ligands and metabolites that prime innate immunity, leading to a more robust secondary response upon subsequent exposure. This amplification of innate immune cell responsiveness serves as a crucial component in the epigenetic regulation of immune responses, particularly against pathogens.[p2],[p12],[p13]

In the face of these innate immunity regulatory mechanisms, pathogens have developed diverse epigenetic strategies to ensure their survival and replication. These strategies include direct manipulation of host proteins and chromatin by pathogen-specific gene products that dampen sensing by pattern recognition receptors (PRRs), modulating signaling pathways, and altering the expression of activators and repressors within the innate immune system.[p14] Remarkably, host organisms possess mechanisms to counteract the epigenetic alterations that are induced by pathogens, thereby preserving effective antipathogenic immunity.[p2] One of the major factors that regulates this interplay between host and pathogen epigenetics is macrophage functional polarization. It is becoming increasingly clear that epigenetic factors, in the form of DNA or histone modifications and the activities of noncoding RNAs, are critical for generating both the cell lineages and the context-specific gene expression necessary for an appropriate immune response, including the functional polarization of macrophages in 'adaptive innate immunity'.[p15],[p16]

Effects of HDACs on macrophage polarization and innate immunity

Most tissues contain innate immune cells in the form of macrophages. Under normal physiological conditions, macrophages produce trophic factors, clear debris, and prevent excessive inflammation in response to environmental stresses.[p3] Factors such as tissue injury or infections activate the host defense function of macrophages, primarily by inducing the production of cytokines and chemokines. In turn, these molecular mediators play important roles in processes that are vital for tissue repair and microbial clearance.[p17] The switch in functional state, from 'pro-inflammatory or killer' to 'anti-inflammatory or repair', broadly differentiates the two major types of macrophages.[p18],[p19],[p20],[p21] M1 (killer) macrophages show the classical activation phenotype and are typically induced by factors such as interferon (IFN)-γ and microbial products, including Toll-like receptor (TLR) ligands. Conversely, M2 (repair) macrophages show the alternative activation phenotype and are predominantly induced by T helper (Th)2 cytokines, such as interleukin (IL)-4 and IL-13.[p22]

In addition to exploring the effects of physiological conditions or stress stimuli on macrophage polarization,[p23],[p24],[p25],[p26] recent research has unveiled the role of epigenetic mechanisms in regulating macrophage polarization.[p27] The signaling pathways and transcription factors that are crucial for macrophage polarization have the capacity to trigger epigenetic changes, as evidenced by shifts in chromatin state.[p28],[p29],[p30] The epigenetic configuration that is established during macrophage development both directs and constrains the influence of signaling pathways and transcription factors, thereby shaping the pattern of gene expression and the resulting functional outcomes.[p31]

Post-translational modifications (methylation, acetylation, and phosphorylation) predominantly mediate epigenetic mechanisms by acting on histones and other chromatin-associated proteins that regulate DNA binding.[p32],[p33],[p34],[p35],[p36] Dynamic regulation of epigenetic chromatin-associated marks in response to environmental cues is evident. Although these marks are subject to dynamic changes, they are generally relatively stable when compared to rapidly fluctuating post-translational modifications of conventional upstream signaling proteins. Conventional post-translational modifications of upstream signaling proteins are frequently transient, whereas epigenetic modifications provide a more enduring cellular response, potentially persisting for extended periods, spanning hours, days, or even longer time frames.[p23],[p24],[p25],[p26],[p27],[p37],[p38]

Effect of HDACs on macrophage polarization

Histone modifications by post-translational changes remain the main focus of investigations related to the regulation of macrophage polarization.[p32],[p36],[p39] Accordingly, histone modifications are broadly classified into two main categories: positive marks, which encourage gene activity; and negative marks, which dampen it. The specific combination of these marks determines the velocity at which a gene responds to signals from the cell's surroundings. These marks collectively influence both the basal transcriptional activity of the gene and its responsiveness to extracellular stimuli.[p4],[p32],[p36],[p39]

Histone-modifying proteins and chromatin remodelers are recognized as key regulators in macrophage activation and polarization. Despite their significance, the detailed mechanisms that govern these processes remain to be elucidated fully.[p40],[p41] Four primary classes of enzymes have been relatively well explored in the context of epigenetic regulation of macrophage polarization: HATs, HDACs, HMTs, and HDMTs.[p6] HDACs, also known as lysine deacetylases (KDACs), constitute a diverse family of proteins that are primarily responsible for the removal of acetyl groups from histone lysine residues, effectively modulating chromatin accessibility and gene transcription.[p7] More specifically, HDACs catalyze the deacetylation of ε-N-acetyl lysine residues (dynamically counter regulated by the action of HATs), exerting profound effects on the epigenetic landscape within the cell.[p8]

HDACs play a crucial role in governing both innate and adaptive immunity. Class I HDACs are key players in innate immunity, where they regulate the production of inflammatory cytokines by controlling the expression of TLR- and IFN-regulated genes. This function underscores their significance in shaping immune responses at the molecular level.[p9] By contrast, Class II HDACs are important in regulating adaptive immunity, mainly affecting T cell activities. They regulate essential functions such as antigen presentation and the activation and maturation of B and T lymphocytes. Consequently, they play an essential role in shaping the adaptive immune response that is crucial for maintaining immune balance and for effective defense against pathogens.[p42]

Despite the multifaceted roles attributed to HDACs, class I HDACs are chiefly labeled as negative modulators of TLR signaling. Notably, HDAC1 functions as a critical negative feedback regulator, dampening inflammatory responses by attenuating the promoter activity of TLR-induced genes such as cyclooxygenase 2 (Cox-2),[p43] interleukin 12 subunit p40 (IL-12p40),[p44] and IFN-β.[p45] Interestingly, HDAC3 plays a role in reducing the expression of genes that are dependent on NF-κB in mouse macrophages. This occurs through a partnership with promyeloid leukemia zinc-finger protein (PLZF), which helps to stabilize the transcriptional inhibitory complex involving HDAC3 on gene promoters.[p46]

TLR signals are essential for activating NF-κB signals, which play a crucial role in regulating inflammatory responses. Within this framework, classical HDACs, including HDACs 1, 2, and 3, exert a negative regulatory influence on NF-κB. HDAC1 recruits HDAC2 by directly interacting with p65, leading to the repression of target genes, whereas HDAC3 indirectly modulates NF-κB signaling by deacetylating the p65 subunit, facilitating its interaction with the inhibitory subunit NF-κB inhibitor-α (IκBα).[p10]

By contrast, the regulation of Class II HDACs suggests that only a particular isoform, HDAC7-u, is upregulated in inflammatory macrophages. This upregulation coincides with the heightened expression of a subset of pro-inflammatory genes that are induced by TLRs, a process mediated by the hypoxia inducible factor alpha (HIF-1α)-dependent mechanism.[p47] Moreover, the suppression of HDAC7 inhibits the transcriptional reprogram-

ming necessary for acquisition of the key inflammatory phenotype.[p48] A pro-inflammatory role of HDAC5 has been observed in both human and murine macrophages. By contrast, HDAC6 has been seen to produce mixed outcomes: it promoted Interferon Regulatory Factor (IRF)-dependent INFβ expression in human fibroblasts, but resulted in no discernible difference in TLR-induced production of inflammatory mediators in mice.[p49]

Finally, Class III HDACs, also known as Sirtuins, are gaining increasing attention because they show distinct structural differences when compared to classical HDACs. Their roles in longevity and contradictory effects in cancer development and progression have sparked significant interest.[p50] In relation to macrophage polarization, the direct impact of Sirtuins remains relatively understudied, with only a handful of investigations shedding light on their specific roles. Nevertheless, research has highlighted the involvement of SIRT1 in promoting macrophage activation towards the M1 phenotype in murine models.[p51],[p52] In addition, studies suggest that SIRT2 regulates inflammation related to sepsis in obese or septic mice through the deacetylation of p65.[p53] Moreover, emerging evidence indicates that SIRT1 exacerbates inflammation in response to lipopolysaccharide (LPS), whereas both SIRT1 and SIRT6 contribute to tissue inflammation and insulin resistance.[p6]

HDAC-assisted regulation of innate immunity

Beyond their direct involvement in functional macrophage polarization, HDACs have been implicated in various facets of innate immunity regulation, including myeloid cell development and differentiation.[p9] Notably, HDAC5 has emerged as a key player in controlling myeloid cell fate, evidenced by its upregulation during the differentiation of human monocytes into macrophages.[p54] Conversely, HDAC3 has been identified as a negative regulator of myeloid cell differentiation, underscoring the nuanced roles of specific HDAC isoforms in immune cell fate determination.[p55] Specifically, HDACs exert influence on the TLR and IFN signaling pathways, thereby intricately regulating the production of essential inflammatory mediators, including chemokines, cytokines, and matrix metalloproteinases (MMPs).

The contradictory effects of HDACi on the expression of TLR target genes underscore the dual regulatory role of HDACs in TLR signaling pathways, acting as both positive and negative regulators.[p49],[p56] Despite extensive mapping of the expression profiles of key TLR target genes,[p9] the precise mechanisms that underlie the HDAC-mediated regulation of these genes remain incompletely understood. Emerging evidence suggests that HDACs may promote the expression of TLR target genes through the deacetylation of signaling molecules involved in TLR signaling pathways. However, investigations identifying specific HDAC isoforms that are responsible for this effect are lacking.[p57]

HDACs regulate transcription factor activity in TLR responses, impacting the production of inflammatory mediators such as type I interferons by the IRF family of transcription factors. Certain members of the IRF family, such as IRF7, undergo lysine acetylation that compromises their DNA-binding ability.[p58] The direct mechanism of the HDAC isoforms responsible for this effect is not yet known. Nevertheless, HDAC6 has been shown to exhibit virus-inducible, IRF-dependent IFN-β expression in human fibroblasts.[p45] In macrophages, HIF-1α plays a crucial role in inflammation triggered by TLR-4. Its stability and/or transcriptional activity during hypoxia depend on HDAC activity. The HDAC isoforms HDAC1, HDAC3, HDAC4, HDAC6 and HDAC7 have been documented to have a direct or indirect effect on such pro-inflammatory pathways.[p59],[p60],[p61]

Moreover, HDACi diminish the recruitment of NF-κB p65 to inflammatory promoters mediated by TLRs. Nevertheless, the prevailing evidence suggests that, contrary to expectations, HDACs predominantly act as negative regulators of TLR-mediated NF-κB activation.[p62],[p63] In this context, Class I HDACs, which predominantly facilitate transcriptional repression via histone modification, are most likely to be responsible for exerting these negative regulatory effects on TLR responses. Conversely, Class IIb enzymes (HDAC6, HDAC4) hinder TLR-4 responses by suppressing signaling pathways that are crucial for targeting TLR responses.[p9] Class I HDACs also play a significant role in IFN responses, contributing positively to signaling pathways. Acetylation of the IFN-α/β receptor 2 subunit enhances IFN signaling by promoting the recruitment and acetylation of the interferon-stimulated gene factor (ISGF)-3 complex. Moreover, cytoplasmic HDAC activity, particularly the activity of HDAC1, 2, and/or 3, is crucial for resetting Signal Transducer and Activator of Transcription protein 1 (STAT1) and for driving STAT1-dependent gene expression upon ligand binding, ultimately influencing effective antiviral responses and host defense mechanisms.[p9] HDACs also play crucial roles in regulating innate immune responses, impacting both myeloid cell fate and key signaling pathways such as the TLR and IFN pathways. Finally, Sirtuins, a class of NAD^+-dependent deacetylases (Class III HDACs), have been implicated in the regulation of macrophage lifespan. Notably, Sirtuins have been shown to extend the lifespan of platelets, which potentially possess immune functions.[p64] Furthermore, pharmacological inhibition of Sirtuins has been observed to activate the NF-κB pathway and to reduce the *in vitro* production of lipopolysaccharide (LPS)-induced cytokines in J774 macrophages.[p65]

HDACi and their role in macrophage polarization

HDACi generally act by relaxing chromatin by allowing increased histone acetylation, enhancing the transcription of various genes, including those involved in immune responses. HDACi have a well-established position in cancer treatment, but their impact on macrophage polarization and innate immunity is complex and multifaceted. HDACi can both impair and enhance the immune response, depending on the disease context and the specific HDAC targets involved.[p9] These implications could be exploited in the development of treatments for diseases in which macrophage behavior plays a crucial role, such as in chronic inflammation and infections.[p66]

A major class of HDACi function by chelating the zinc ion that is essential for HDAC enzymatic activity, thereby disrupting the function of the enzyme. These inhibitors are categorized into various classes on the basis of their chemical structure and specificity, with classes including hydroxamic acids, benzamides, cyc-

lic peptides and aliphatic acids, each with unique biological properties and therapeutic potentials.[p67] Five HDACi have been FDA-approved, primarily for the treatment of cancer,[p68] but with a recent approval for the treatment of muscular dystrophy (Figure 1).[p69] In addition, numerous HDACi are currently in clinical trials,[p70] showing promise for the expansion of their therapeutic applications beyond oncology to include autoimmune and inflammatory diseases.

The ability of HDACi to modulate macrophage polarization has significant implications for diseases that are characterized by dysregulated immune responses, which have been extensively reviewed.[p66],[p70],[p71],[p72],[p73] Table 1 presents examples from the past five years, detailing various classes of HDACi and their role in immune modulation, particularly through the strategic polarization of macrophages.

The study by Li *et al.*[p74] found that the HDAC8 inhibitor PCI-34051 attenuates allergic asthma by suppressing the expression of galectin-3, thereby reducing the polarization of macrophages towards the M2 phenotype, which is associated with inflammatory responses. In this context, HDAC inhibition impacts macrophage polarization by modifying the expression of proteins such as galectin-3 that drive M2 polarization, which is crucial for the allergic inflammation seen in asthma.[p74] Benjaskulluecha *et al.*[p75] employed epigenetics compound library screening and found that the HDACi vorinostat, ricolinostat, and nexturastat reversed the suppressive effects of LPS-induced tolerance, effectively enhancing the production of inflammatory cytokines such as TNFα. The study by Ghiboub *et al.*[p76] showed that HDAC3 inhibition does not affect macrophage polarization in human cells but modulates pro-inflammatory cytokine production, thereby affecting the induction of tolerance in inflammatory macrophages. Specifically, HDAC3 mediates macrophage reactivity, which is important for managing exacerbated immune responses and limiting tissue damage in inflammatory conditions.

Hoeksema *et al.*[p77] found that *Trichuris suis* soluble products (TsSPs) modulate monocyte-to-macrophage differentiation by promoting an anti-inflammatory M2 macrophage phenotype through epigenetic remodeling involving histone deacetylation. This remodeling can be reversed by the HDACi givinostat, suggesting that HDACs play a key role in maintaining the expression of pro-inflammatory genes, such as TNF and IL-6, that are typically seen in M1 macrophages.[p77] Noonepalle *et al.*[p78] reported that selective inhibition of HDAC6 with the drug SP-2-225 can enhance the immune response induced by radiotherapy, leading to decreased tumor growth. This occurs

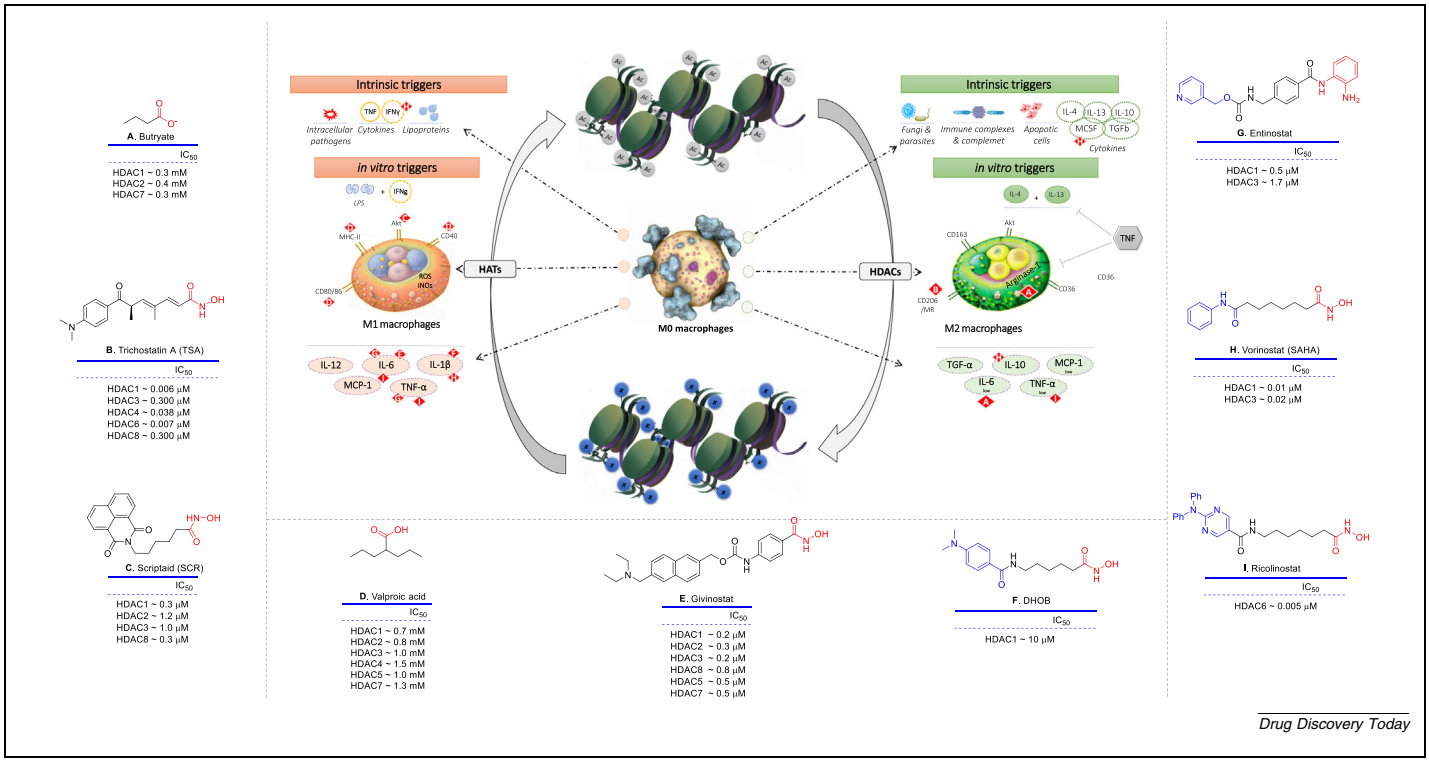

FIGURE 1

Role of histone deacetylases (HDACs) on macrophage polarization. The figure illustrates the key factors involved in macrophage polarization, with specific emphasis on HDAC inhibitors (HDACi) investigated for their impact on macrophage polarization, through either direct or indirect pathways. **A.** butyrate, **B.** trichostatin A, **C.** scriplaid, **D.** valproic acid, **E.** givinostat, **F.** 4-(dimethylamino)-N-[6-(hydroxyamino)-6-oxohexyl]-benzamide (DHOB), **G.** entinostat, **H.** vorinostat, and **I.** ricolinostat. Components of the HDACi are color-coded, with the Zinc binding group (ZBG), linker and cap denoted in red, black and blue, respectively. Note, that the IC_{50} values of certain compounds, including valproate and butyric acid, are high, which may suggest potential off-target effects. In general, IC_{50} values should be interpreted, cautiously as assay results often do not fully represent the physiological effects in a physiological system. Abbreviations: CD, Cluster of differentiation; HAT, Histone acetyltransferase; IFN, Interferon; IL, Interleukin; iNOS, Inducible nitric oxide synthase; LPS, Lipopolysaccharides; MCP, Methyl-accepting chemotaxis protein; MCSF, Macrophage colony-stimulating factor; MHC, Major histocompatibility complex; MR, Mineralocorticoid receptor; ROS, Reactive oxygen species; TGF, Transforming growth factor; TNF, Tumor necrosis factor.

TABLE 1

Recent advances in the epigenetic targeting of macrophage polarization with histone deacetylase (HDAC) inhibitors

Reported inhibition of HDAC isoenzyme	Drug	Disease indication	Effect on macrophage polarization	Signaling pathway and effect	Signaling molecules	Ref.
HDAC2	Class 1 inhibitor CAY10398 (SAHA, VPA also tested)	Lung cancer	Shift from M2-like (protumor) to M1-like (antitumor) macrophages	HDAC2i regulated the M2-like TAM phenotype through acetylation of histone H3 and transcription factor SP1	M1 (CCR7, IL1B, IL8, IL12B) and M2 (ALOX15, CCL18, IL1RA, MRC1) markers	(p80)
HDAC1, 2, 3	MS-275 (entinostat)	Acute lung injury	Regulation of proinflammatory and anti-inflammatory cytokine balance	Involvement of TLR4, NF-κB, and MyD88-dependent and -independent pathways, impacting cytokine transcription and secretion	IL-10, IL-12b, TNFα, CXCL2, IL-6, MIF	(p81)
HDAC3	RGFP966	Ischemic brain damage	Suppressed production of inflammasome-associated cytokines, leads to neuroprotection	Inhibition of the AIM2 inflammasome, modulation of STAT1 acetylation and phosphorylation	AIM2, ASC, IL-1β, IL-18, STAT1	(p87)
HDAC6	CKD-506	Inflammatory bowel disease, murine colitis	Promotes anti-inflammatory profile	Inhibits NF-κB signaling, leading to reduced inflammation	IL-6, IL-8, TNF-α	(p123)
HDAC6	CAY10603	Acute lung injury	Anti-inflammatory M2 polarization	NFκB and inflammasome inhibition; blocks inflammatory signaling	NFκB, IκB, IL-1β, Caspase-1, MMP9 ↓TNF-α, IL-1β, IL-6	(p124)
HDAC6	SP-2-225	Cancer	Promotes M1 polarization	Preventing the usual shift from pro-inflammatory M1 macrophages to pro-tumor M2 macrophages following radiotherapy	Il-10, Socs3 (STAT3 signaling), Il-1b	(p78)
HDAC8	PCI-34051	Asthma	Reducing M2 polarization	Suppressed HDAC8-Gal-3 interaction	HDAC8, Gal-3, IL-4	(p74)
HDAC1, 2	4-(Dimethylamino)-N-[6-(hydroxyamino)-6-oxohexyl]-benzamide (DHOB)	Tuberculosis	Promotes M1 polarization	Increased IL-1β production through NLRP3 inflammasome activation	NLRP3, Caspase-1, IL-1b, ASC oligomerization	(p82)
HDAC1, 2, 3 HDAC6	MS-275 (entinostat) Ricolinostat, nexturastat	General mechanisms of innate immune memory, which may have implications for inflammatory diseases and infection response	Promotes M1 polarization	Reverses LPS-induced tolerance, enhances inflammatory response	↑ TNFα, IL-6	(p75)
HDAC3	ITF3100	Inflammatory diseases	Reduced cytokine secretion in M1 macrophages	Suppresses LPS-induced cytokine production in monocytes and M1 macrophages	↓TNFα, p40 and IL-6 cytokine secretion	(p76)
HDAC6 HDAC11	Nexturastat A FT895	Cancer	Suppressing M2 macrophages Promoting M2 macrophages	Opposite effects of HDAC6 and HDAC11 inhibition on macrophage phenotype and function	↑ IFN-γ, Tnf-α, Il-1β, Cxcl10 ↑ IL10, IL13, Egf, Pdgf	(p125)
HDAC1, 2, 3	Tefinostat	Neuroinflammatory disorders	Promotes pro-regenerative macrophage phenotype	Upregulates peroxisomal genes involved in β-oxidation	Modulation of very long-chain fatty acid (VLCFA) metabolism	(p79)
HDAC1, 2, 3, 6	CG-745	Pancreatic, colorectal, non-small cell lung cancer	Suppresses M2 macrophage polarization	Enhances anti-cancer immunity by promoting T cell activation and M1 macrophage polarization	IL-2, IFN-γ	(p83)

TABLE 1 (*CONTINUED*)

Reported inhibition of HDAC isoenzyme	Drug	Disease indication	Effect on macrophage polarization	Signaling pathway and effect	Signaling molecules	Ref.
Class I	Tacedinaline (CI-994)	MYC-driven medulloblastoma	Enhances macrophage phagocytosis	Activation of NF-kB pathway leading to tumor inflammation	NF-kB, TGM2, inflammatory cytokines	(p84)
HDAC1, 2, 3	TTA03-107	Autoimmune arthritis	Dampens M1 differentiation and cytokine production	Inhibits differentiation and activation of macrophages and Th17 cells	↓IL-1β, TNF-α, IL-17A	(p85)
HDAC1, 2, 3	MS-275 (entinostat), RGFP966	Hypertension	Decreased macrophage infiltration	Reduction in vascular remodeling and vasoconstriction via the NO pathway	TNF-α, IL-1β, MCP-1, NO levels	(p126)
Class I & II	Trichostatin A (TSA)	Polymicrobial sepsis	Promotes M2 macrophage polarization, reduces M1 polarization	Enhancement of autophagy reduces inflammation	Autophagy-related proteins such as LC3 and p62, mTOR	(p86)
HDAC1, 2, 3, 4	TSA, SAHA (vorinostat)	Potential inflammatory diseases, cancer	Shifts towards anti-inflammatory profile	TLR-4 signaling pathways, NF-κB inhibition, alteration in gene expression	IKKε, IL-1β, iNOS, TNFα, COX-2	(p127)
–	Benzimidazole–hydroxamate hybrids (9k, 9l) improved vs. SAHA	Cancer	Promotes M2-type polarization with antitumor activity	Inhibits Treg cell recruitment, enhances T cell activation	Not specified	(p128)
General HDAC inhibition	SAHA (vorinostat)	Tuberculosis	Promotes pro-inflammatory profile	Enhances glycolysis, increases IL-1β production	IL-1β, IL-10, IFN-γ, GM-CSF	(p129)
HDAC1, 2, 3, 8, 10	Trichostatin A	Wound healing	Promotes M2-like macrophage polarization	Influences macrophage plasticity through modulation of iNOS protein levels	iNOS, CD11b, Ly6C	(p130)

through modulation of macrophage polarization, preventing the usual shift from pro-inflammatory M1 macrophages to pro-tumor M2 macrophages following radiotherapy, thus enhancing the overall antitumor immune response.[p78]

Villoria-González *et al.*[p79] showed that HDACi, specifically tefinostat, enhance the degradation of very long-chain fatty acids and shift human macrophages towards a pro-regenerative phenotype, which could be beneficial in treating neuroinflammatory disorders. Zheng *et al.*[p80] demonstrated that the inhibition of HDAC2 in tumor-associated macrophages shifts their polarization from a protumor (M2-like) to an antitumor (M1-like) state, a shift mediated by changes in histone and transcription factor acetylation that ultimately impacts lung cancer progression and immune response. Stanfield *et al.*[p81] showed that inhibition of class I HDACs leads to increased transcription of anti-inflammatory cytokines and a reduction in proinflammatory responses, suggesting a potential therapeutic target in acute lung injury.

NLRP-3 (Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3), a key component of the immune system and a modulator of inflammatory response, has an effect on macrophage polarization. Moreira *et al.*[p82] found that 4-(dimethylamino)-N-[6-(hydroxyamino)-6-oxo hexyl]-benzamide (DHOB) (Figure 1), an HDAC1 inhibitor that also affects HDAC2, enhances IL-1β production through increased expression and activation of NLRP3, which promotes macrophage polarization toward a more inflammatory phenotype in tuberculosis infection.[p82] CG-745, a Class I and IIb (HDAC6) inhibitor, enhances the anti-cancer effects of anti-PD-1 therapy by modulating the immune microenvironment, particularly by suppressing M2 macrophage polarization and promoting M1 polarization.[p83] This supports a more effective anti-tumor immune response.[p83] Tacedinaline (CI-994), a Class I HDACi, targets MYC-driven medulloblastoma by enhancing macrophage-mediated phagocytosis and tumor inflammation through the NF-kB and tissue transglutaminase 2 (TGM2) pathways, which increase the secretion of inflammatory cytokines and boost anti-tumor immune responses.[p84] The novel HDAC1-selective inhibitor TTA03-107 attenuates autoimmune arthritis by suppressing inflammatory cytokine production and inhibiting the polarization of macrophages towards an inflammatory M1 phenotype.[p85] Trichostatin A (TSA), modulates macrophage polarization towards the M2 phenotype by enhancing autophagy, leading to reduced inflammation and improved survival in a sepsis model. TSA shifts macrophage activity from pro-inflammatory M1 to anti-inflammatory M2, a change that is crucial for resolving inflammation and promoting recovery in sepsis.[p86]

CG-745 enhances the anti-cancer effects of anti-PD-1 therapy by modulating the immune microenvironment, particularly by

suppressing M2 macrophage polarization and promoting M1 polarization, which supports a more effective anti-tumor immune response.[p83] The study by Zhang *et al*.[p87] shows that the HDAC3 inhibitor RGFP966 reduces ischemic brain damage by modulating the AIM2 inflammasome and altering STAT1 acetylation, leading to neuroprotection and improved outcomes post-stroke. Remarkably, Zhao *et al*. designed a HDAC3-directed PROTAC that shows anti-inflammatory potential by effectively blocking the polarization of M0-like macrophages into M1-like macrophages.[p88] HDACi such as suberoylanilide hydroxamic acid (SAHA) and valproic acid (VPA) upregulate inflammatory-related genes, attracting macrophages to hemangiosarcoma cells and thus impacting immune responses.[p89] Taken together, HDACi play a crucial role in macrophage polarization and thus govern the epigenetic regulation of inflammatory and immune responses.

In combination with modulation of the vitamin D pathway, HDACi have been linked to enhanced *Mtb*-killing activity of macrophages. Specifically, phenylbutytrate (PBA), which has been shown to induce *CAMP*/LL-37 gene expression in cell lines, has been suggested to have a role in the treatment of *Mtb* infection.[p90] Moreover, an *in vitro* study using a combination of PBA and vitamin D3 showed the differentiation of dendritic cells into stretched CD14+/CD1a-DC, and promoted both autophagy and an overall enhanced production of reactive oxygen species (ROS) and cathelicidin.[p91]

The effect of HDACi on bacterial infections, both *in vivo* and *in vitro*, is influenced by compound selectivity and treatment timing. The phagocytosis of *Escherichia coli* by human macrophages was seen to be impaired when primed with SAHA or TSA. However, simultaneous co-administration of SAHA and TSA enhanced mitochondrial ROS generation, promoting the clearance of intracellular bacteria. Notably, MS-275, a class I HDACi, did not improve bacterial killing by macrophages, whereas the HDAC6-specific inhibitor tubastatin A (TubA) did increase the killing of bacteria, indicating a significant role for HDAC6 in this process. Similarly, following sepsis induced by cecal ligation and puncture (CLP), HDAC6 inhibition restored the populations of innate immune cells, including macrophages, in the bone marrow. Moreover, selective inhibition of HDAC8 with PCI-34051 partially counteracted the suppression of IL-1β production by Anthrax lethal toxin (LeTx) during *Bacillus anthracis* infection, thereby restoring some immune cell functions. These findings imply that the non-selective inhibition of HDACs may hinder the innate immune response, whereas the targeted inhibition of specific HDAC isoforms could promote the eradication of certain microbial pathogens.[p92]

In addition to their impact on macrophage-mediated bacterial clearance, HDACi have been shown to influence the production of antimicrobial peptides (AMPs). These peptides play a crucial role in both innate and adaptive immune responses against pathogens. Specifically, cationic antimicrobial peptides (CAMPs), which are generally less than 10 kDa in size, serve as key microbicidal effector molecules that enhance the body's mechanisms for defense against invading microorganisms. Epithelial cells, as the initial barrier against pathogenic microorganisms, produce large quantities of CAMPs. HDACi have been identified as potent inducers of two principal classes of mammalian CAMPs, cathelicidins and defensins, in colonic and airway epithelial cells. Various HDACi compounds, including entinostat, TSA, butyrate, and phenylbutyrate, have been associated with enhanced CAMP production, highlighting their potential as modulators of epithelial immune responses. Several mechanistic studies have elucidated the pathways through which HDACi increase CAMP production. In the colonic epithelial cell line Caco-2, TSA has been demonstrated to upregulate human β-defensin 2 (hBD2). This upregulation occurs through a mechanism that involves the preferential phosphorylation of histone H3S10 at the hBD2 promoter, mediated by the phosphorylation of the IKKα/β complex, alongside the acetylation of the NF-κB p65 subunit.

Recent research utilizing a luciferase-based reporter system in the HT-29 colonic epithelial cell line revealed that entinostat enhances the upregulation of LL-37 by modulating the transcription factors STAT3 and HIF-1α.[p93] In addition, an *in vivo* model of bacillary dysentery induced by *Shigella flexneri* has highlighted the critical role of short-chain fatty acids (SCFAs) in CAMP induction. Oral administration of butyrate in infected rabbits led to a significant reduction in both colon inflammation and bacterial load, correlating with increased expression of cathelicidin CAP-18 (the rabbit analog of LL-37). These promising findings prompted a clinical trial to evaluate butyrate as an adjunct to antibiotics for treating shigellosis (ClinicalTrials.gov: NCT00800930). Future research should focus on exploring the specific roles of different HDACi in CAMP induction and on the development of more effective inhibitors to enhance AMP production, potentially leading to new antimicrobial therapies against resistant infections.[p92]

In conclusion, the current literature suggests that several HDAC isoforms and their respective inhibitors play significant roles in modulating macrophage polarization towards either the pro-inflammatory M1 or the anti-inflammatory M2 phenotype. Specifically, Class I HDACs (HDAC1, 2, 3, and 8) and HDAC6 (Class IIb) are primarily involved in promoting M1 macrophage polarization. By contrast, selective inhibition of HDAC1, 6, and 8 has been associated with the induction of M2 polarization. It is important to acknowledge, however, that the selectivity observed in *in vitro* or animal models may not always reflect the outcome in clinical settings. For instance, compounds such as butyrate, which exhibit low HDAC inhibition values, may exert off-target effects, indicating that their mechanisms of action could extend beyond the inhibition of HDAC alone. Further research is needed to clarify these complexities and to provide a better understanding of the clinical implications of HDAC inhibition in macrophage polarization, especially in relation to disease context.

It is important to address several key considerations when translating findings from animal and pre-clinical studies to human physiology. First, there are differences in HDAC expression and function across species, which can lead to discrepancies between findings observed in preclinical models and clinical outcomes. In addition, variations in the pharmacokinetics and pharmacodynamics of different inhibitors, which play a crucial role in drug absorption, distribution, metabolism, and excretion, can significantly impact dosing regimens and the therapeutic window. Moreover, immune responses observed in animal models may not always be replicated in humans because of differences in immune system structure, tumor microenvironment,

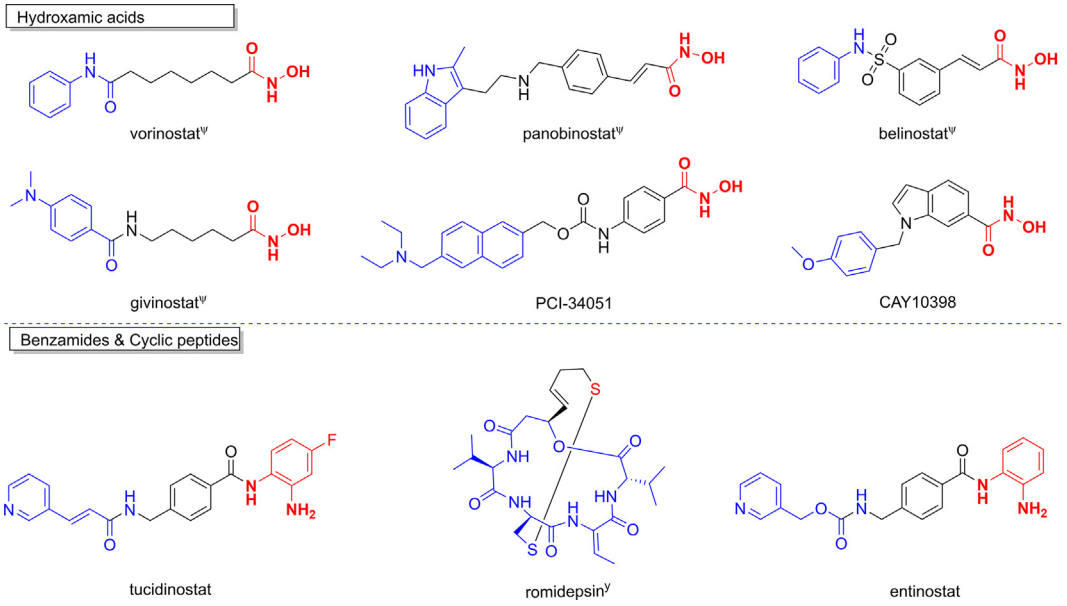

FIGURE 2

Major classes of histone deacetylase (HDAC) inhibitors. $^{\Psi}$FDA approved HDAC inhibitors. The zinc-binding group (ZBG), linker and cap are denoted in red, black and blue colors, respectively.

and other species-specific physiological factors. Despite these challenges, several effective HDACi have successfully progressed to clinical use (Figure 2), demonstrating their potential for continued advancement in drug development.[p94]

Role of HDACs in epigenetic defense against infections

Microbial infections cause a systemic innate immune response. Microbial nucleic acids and pathogen-associated molecular patterns (PAMPS) are recognized by several receptors, such as the endocytic RRs and cytoplasmic molecules such as retinoic acid-inducible gene-I (RIG-I), C-type lectin receptors, melanoma-dif ferentiation-associated gene 5 (MDA5) and others.[p95] This sensing depends on receptor abundance in different types of immune cells, such as dendritic cells, macrophages, T cells, B cells, and natural killer cells, among others, and leads to the transcription of type I and III interferon genes and to the production of NF-kB-induced cytokines. Recent evidence suggests that the innate immune response is also affected by infection-induced epigenetic changes. These changes are also cell-type specific and will affect the future response to subsequent infection. In addition, the epigenetic state of inducible genes will contribute to the overall immune response upon infection.

Epigenetic defense against viral infections

Recent studies of epigenetic reprogramming that results in the death of viral pathogens or that limits infection encourage the therapeutic targeting of epigenetic processes as an important future strategy to treat infections and to eradicate latent infections. Two different strategies are suggested as therapies for latent HIV infection, namely the 'shock and kill' strategy and the 'block and lock' strategy. The shock and kill strategy starts with the use of HDACi, HMT or DNA methyl transferase (DNMT) inhibitors to revert the transcriptional silencing of latent viruses, producing active viral forms that are more prone to antiviral treatments and/or host-directed therapies. Recently, treatment with DNMT inhibitors and HDACi was shown to reactivate latent forms of HIV-1.[p96] An opposite approach is to cause super-latency to prevent reactivation efficiently. This approach can be promoted by blocking the HIV-1-specific protein Trans-Activator of Transcription (Tat), which recruits transcriptional factors, thereby locking the HIV-1 virus in a super-latency state. As Tat is specific to HIV-1, blocking it does not interfere with defense against other virus infections.

These two strategies can also be used against other viruses, such as Human cytomegalovirus (HCMV), which poses a threat to immunocompromised individuals. Although the US Food and Drug Administration (FDA) has approved antiviral drugs that target the DNA replication step for HCMV, genes that are involved in the early stages of infection are not inhibited and the drugs do not target the latent forms of the virus. As the HCMV lytic and latent cycles are regulated by epigenetic mechanisms, antiviral drugs could be effectively used together with epigenetic modifiers to activate latent HCMV and then eradicate the activated viruses.[p97],[p98] HDACi could also induce the transient expression of viral antigens, which would be recognized and reacted to by cytotoxic T lymphocytes. By contrast, the block and lock strategy can be based on histone demethylase inhibitors (HDMi). Prior research on HCMV suggests that the inhibition of HDMs reduces HCMV lytic infection and reactivation, and controls diseases that are associated with viral infection.[p99] HDMs could effectively halt the infection in its nascent stages and prevent any potential reactivation. Such capabilities are not only crucial for averting graft rejection but also for impeding the ini-

tiation of oncogenic signaling pathways, underscoring their role in preventive healthcare.

SARS-CoV-2 results in innate immune hyperactivation, which causes many difficult symptoms in COVID-19 disease.[p100] Hyperactivation leads to high levels of pro-inflammatory cytokines, such as interleukins IL-2, IL-6 IFN-α, IFN-β and IFN-γ, to name a few. These cytokines may result in severe symptoms in some patients, for example causing acute respiratory syndrome and multi-organ dysfunction reminiscent of a sepsis-like disease.[p101] As they hyperactivate the immune system, HDACi offer a potential therapy against COVID-19 and several HDACi have shown anti-inflammatory properties. In addition, five clinical HDACi have been shown to downregulate the expression of the SARS-CoV-2 receptor ACE2 in the cell surface and to further prevent ACE-2-mediated entry of the virus.[p102] In addition to downregulating ACE-2 expression in endothelial cells, the HDAC2 inhibitor valproic acid was shown to reduce the expression of IL-6 significantly, suggesting that valproic acid could be used to prevent and treat COVID-19.[p103]

Epigenetic drug candidates are being studied in clinical trials to assess their ability to limit viral infections or malignancies caused by viral infections. In the case of HIV infection, several HDACi, such as panobinostat (NCT01680094), vorinostat (NCT01319383), romidepsin (NCT02092116) and valproid acid (NCT00289952) have been tested in combination with antiretroviral therapies. All of these treatments produced some increase in viral transcription, but none of them could induce total clearance of latent infection.[p104],[p105],[p106],[p107] In the case of virus-induced malignancies, the HDACi mocetinostat (MGCD0103) was effective in patients with relapsed Hodgkin lymphoma.[p108] In addition, HDACi were also shown to increase sensitivity to antivirals in Epstein–Barr virus-induced lymphomas.[p109]

Epigenetic defense against bacterial infections

In addition to viral infections, many bacterial pathogens also have the ability to interfere with the epigenetic regulation of host immune responses, enhancing their ability to colonize and infect. Such virulence-enhancing and immune-suppressing mechanisms involve histone acetylation mechanisms in host cells.[p92],[p110],[p111] Initial research with HDACi in the context of bacterial infections suggested negative rather than supportive effects. In the first studies, the HDACi VPA, SAHA, and TSA were observed to impair host defense mechanisms widely, with valproate treatment leading to decreased pathogen survival during bacterial (*Klebsiella pneumoniae*) and fungal (*Candida albicans*) infections in mice.[p63] However, the results also indicated that these inhibitors played a significant role in reducing toxic and septic shock in mice.[p63] Work by Mombelli *et al.*[p112] showed that VPA and TSA impaired phagocytosis of *E. coli* and *Staphylococcus aureus* using mouse bone marrow-derived mononuclear macrophages.

More recent studies have provided supporting evidence on the potential of HDACi as antibacterials, opening perspectives on the complexity of factors that influence immune response outcomes. First, timing of delivery may play a role in determining the efficacy of entinostat (a Class I HDACi): when administered simultaneously with *E. coli* and *Salmonella* exposure, an increase in bacterial clearance by macrophages was mediated by an elevated mitochondrial ROS response.[p105] Entinostat treatment was also shown to be efficient in an *in vivo* rabbit model, maintaining the integrity of the epithelial barrier and preventing infection and mortality related to *Vibrio cholerae*.[p113] These results suggest the potential use of entinostat in the prevention and treatment of gut infections.

Rösler *et al.*[p114] compared the effects of panobinostat (a pan-HDACi), entinostat (an inhibitor of HDAC1, 2 and 3) and RGFP966 (a HDAC3-specific inhibitor) in peripheral blood mononuclear cell (PBMCs) stimulated with *C. albicans* and *S. aureus*. In healthy donor cells, panobinostat disrupted the TNF-α and cytokine responses during *C. albicans* stimulation, especially when used at high doses, and this effect was also found for the TNF-α response under *S. aureus* stimulation. In cells isolated from STAT-1 GOF patients, however, the results were the opposite: entinostat and RGFP966 increased TNF-α and cytokine responses in response to *S. aureus*.[p114] These mixed results highlight the complexity of human genetic backgrounds and HDACi targets in selecting optimal antimicrobial approaches.

Mycobacteria can cause persistent intracellular infections, and thus represent one of the promising indications for HDACi-directed therapeutics, with tuberculosis in particular being a potential indication. A few studies have explored the role of different HDACi in this context. RGFP966 was observed to cause a decrease in TNF and IL-6 levels but was efficient in controlling the growth of *Mycobacterium tuberculosis*, *M. bovis* BCG and *M. avium* in human alveolar macrophages and monocyte-derived macrophages.[p115] This growth reduction was also observed as a direct effect in pure bacterial cultures, but only in a mycobacteria-specific manner. In a study by Moreira *et al.*,[p116] TSA caused a significant reduction in *M. tuberculosis* growth in both M1 and M2 macrophages, and also in macrophages that differentiated in presence of HDACi (including TMP-195 and TMP-269). This study also demonstrated an *in vivo* reduction in bacterial burden in zebrafish in response to TSA and TMP-195. Furthermore, as noted above, improved induction of macrophages by IL-1β during *M. tuberculosis* infection was obtained using the HDACi DHOB.[p82] Nevertheless, contradicting results exist, HDACi (TSA and SAHA) have been observed to lead to impaired bacterial killing by macrophages, decreased ROS production, and autophagy, resulting in reduced host survival in a mouse infection model.[p116]

Furthermore, a study focusing on the use of approaches based on HDAC inhibition in various inflammatory gut conditions showed context-dependency for experimental work and patient biopsies.[p117] In CaCo2/TC7 cells, the HDACi SAHA and sodium butyrate (SB) induced the antimicrobial peptides (AMPs) human beta defensin 2 (hBD2) and IL8 in response to *E. coli* infection. In patient colonic biopsy samples, however, these responses were absent, although HDACi MS-275 together with *E. coli* resulted in a significant increase in hBD2 expression.[p117]

In addition to specific infections, bacterial septicemia and sepsis could be potential targets for HDACi treatments. Trichostatin A increased macrophage polarization and autophagy, and improved the survival of mice with polymicrobial sepsis.[p86] Encouraging results have also been obtained for MS-275, tubas-

tatin A and TSA, all of which increased survival in CLP-induced mice.[p118] HDACi also attenuated lung, liver and heart injuries, and suppressed inflammatory mediators. In sepsis induced using LPS from *Salmonella typhosa*, rats treated with SAHA were protected from septic shock and had significantly improved survival.[p119] Similar results were obtained in another study, this time on mice, in which a positive effect of SAHA was obtained via inhibition of lactate dehydrogenase.[p120]

Overall, the studies highlight distinct HDAC isoforms that are associated with antiviral and antibacterial effects. For antiviral effects, Class I HDACs, particularly HDAC1, 2, and 3, play a crucial role in modulating viral latency and reactivation. In addition, although not directly implicated in antiviral mechanisms, HDAC6 contributes to antiviral activity through its significant role in modulating inflammation. Meanwhile, the anti-bacterial effects of HDACi highlight the roles of HDAC1, 3, and 6 in modulating immune responses through various mechanisms. HDAC3 plays a critical role in both anti-bacterial and anti-viral responses, highlighting the importance of investigating selective inhibitors in order to gain deeper insights into immune regulation. It is essential to recognize that the effectiveness of HDACi in antibacterial therapy is often both pathogen- and compound-specific. Therefore, in clinical settings, the use of HDACi requires careful optimization in order to achieve desired outcomes while avoiding potential immune-suppressive effects. By targeting host epigenetic mechanisms, HDACi offer a strategy to combat resistant microbial strains. However, such interventions must be designed with specificity to minimize off-target effects and to reduce toxicity, thereby ensuring the provision of safe and effective treatment options.

Conclusions and future prospects

In conclusion, the intricate interplay between HDACs, macrophage polarization, and innate immunity presents a promising avenue for understanding and potentially modulating immune responses against inflammatory diseases in general and infectious diseases in particular (Figure 3). Several HDACi have been investigated for their effects on macrophage polarization in relation to various diseases and infections. Leveraging this knowledge to direct macrophage polarization epigenetically towards a sustained immune response represents a promising strategy. HDACi that regulate macrophage polarization and enhance the host's defense against pathogens offer a promising alternative, particularly for combating highly resistant bacteria. Nevertheless, owing to the complexity of the responses that are elicited by HDACi and the associated signaling pathways, detailed mechanistic investigations are needed. Moreover, some HDACi (vorinostat, romidepsin, panobinostat, valproic acid, and TSA) that are known for their role in cancer therapy also exhibit immunosuppressive effects. This can be counterproductive in infectious diseases, where a robust immune response is crucial for clearing pathogens. At low doses, HDACi beneficially modulate gene expression and enhance immune responses, making them suitable for epigenetic reprogramming in viral infections.

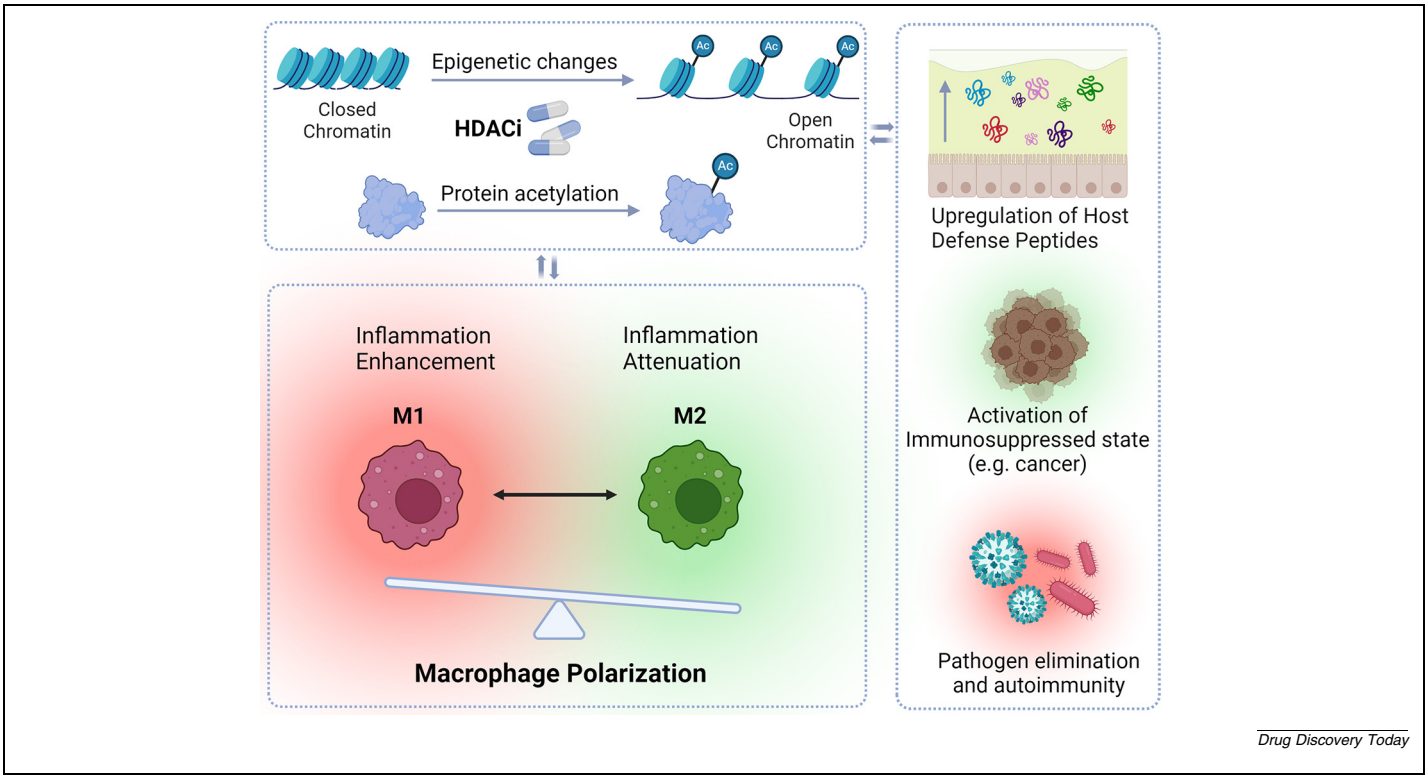

FIGURE 3

Summary of the modes of action by which small-molecule histone deacetylase inhibitors (HDACi) can cause increased anti-microbial defense. HDACi can shift the balance of M1 and M2 macrophage polarization, which affects antimicrobial defense. Furthermore, HDACi can affect the expression of antimicrobial peptides, activate immunosuppressed states or affect pathogen elimination.

At higher doses, they exhibit immunosuppressive effects, potentially undermining the body's ability to combat infections. Therefore, careful dose management studies and design of more potent/selective inhibitors are needed to exploit the real therapeutic potential of HDACi while avoiding adverse impacts on immune function.[p121] In addition, different studies on the effects of HDACi on T-cells, particularly CD4+ and CD8+ T-cells, have shown conflicting outcomes. In some models, HDACi enhanced T-cell proliferation and function, whereas in others, they induced apoptosis or impaired proliferation of T-cells. Such discrepancies complicate the interpretation of the overall impact of HDACi on immune function, highlighting the importance of considering the specific immune cells involved, the disease context, and the timing of drug administration.[p122] Moving forward, it will be essential for experts in the field to focus on validating the effects of HDACi in *in vivo* models in order to gain a clearer understanding of their impact.

We believe that future studies aimed at fully understanding and optimizing the HDACi approach should focus on innate immunity and the key factors that are involved in macrophage polarization. The role of different HDAC isoforms and the concentrations of specific HDACi significantly impact the immune enhancement outcome. Substantial focus should also be placed on identifying biomarkers for HDACi response, targeting genetic, proteomic, and metabolic markers to predict and monitor responses, and facilitating personalized HDACi therapies that improve both efficacy and safety. Last, the development of combination therapies in which HDACi are paired with immune-boosting agents, such as cytokines or checkpoint inhibitors, could strengthen the immune response against pathogens while minimizing suppression. Experiments that consider all of these factors while studying HDACi in the context of specific disorders or components of innate immunity are crucial. This comprehensive approach appears to be a promising way forward in optimizing the use of HDACi for immune modulation, especially against resistant infectious diseases.

Further research in this field holds promise for the development of more effective and targeted treatments against a wide range of pathogens. In the design of new drugs, however, the low selectivity of HDACi against different isoforms of HDACs is still a constraint. This effect is especially relevant in clinical settings, because the isoform selectivity seen in *in vitro* settings does not always reflect selectivity in physiological conditions. This challenge also creates a risk that HDACi will have adverse effects, which can vary from mild gastrointestinal disturbances such as nausea and diarrhea, to hematological toxicities such as thrombocytopenia and neutropenia, and cardiac issues such as QT interval prolongation. These toxicities reflect the broad impact of HDAC inhibition on various physiological processes, including hematopoiesis, immune function, and cardiac electrophysiology. Moreover, there are potential long-term risks associated with HDACi therapy, including the possibility of immunosuppression, cognitive impairment, and even secondary malignancies resulting from widespread epigenetic changes. Although HDACi offer considerable therapeutic promise, significant future efforts are necessary to fine-tune their efficacy and to minimize potential adverse effects, setting the stage for radical advancements in future drug development.

Declarations of interest

The authors declare that they have no conflicts of interest relevant to the work described in this article.

CRediT authorship contribution statement

Mohammad Faizan Bhat: Writing – review & editing, Writing – original draft, Conceptualization. **Sonja Srdanović:** Writing – review & editing, Project administration, Writing – original draft. **Lotta-Riina Sundberg:** Writing – review & editing, Writing – original draft. **Helga Kristín Einarsdóttir:** Writing – review & editing, Writing – original draft. **Varpu Marjomäki:** Writing – review & editing, Writing – original draft. **Frank J. Dekker:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Data availability

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