Inhibiting epigenetic enzymes to improve atherogenic macrophage functions

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ARTICLE INFO

ABSTRACT

Macrophages determine the outcome of atherosclerosis by propagating inflammatory responses, foam cell formation and eventually necrotic core development. Yet, the pathways that regulate their atherogenic functions remain ill-defined. It is now apparent that chromatin remodeling chromatin modifying enzymes (CME) governs immune responses but it remains unclear to what extent they control atherogenic macrophage functions.

We hypothesized that epigenetic mechanisms regulate atherogenic macrophage functions, thereby determining the outcome of atherosclerosis. Therefore, we designed a quantitative semi-high-throughput screening platform and studied whether the inhibition of CME can be applied to improve atherogenic macrophage activities.

We found that broad spectrum inhibition of histone deacetylases (HDACs) and histone methyltransferases (HMT) has both pro- and anti-inflammatory effects. The inhibition of HDACs increased histone acetylation and gene expression of the cholesterol efflux regulators *ATP-binding cassette transporters* ABCA1 and ABCG1, but left foam cell formation unaffected. HDAC inhibition altered macrophage metabolism towards enhanced glycolysis and oxidative phosphorylation and resulted in protection against apoptosis. Finally, we applied inhibitors against specific HDACs and found that HDAC3 inhibition phenocopies the atheroprotective effects of pan-HDAC inhibitors.

Based on our data, we propose the inhibition of HDACs, and in particular HDAC3, in macrophages as a novel potential target to treat atherosclerosis.

1. Introduction

Current atherosclerosis medication efficiently lowers plasma cholesterol levels, but reduces the risk for cardiovascular disease only partially. Therefore, alternative treatment strategies are needed [1]. During atherosclerosis initiation, accumulation of *low-density lipoproteins* (LDL) and their modifications (e.g. oxidation to oxLDL) in the arterial wall activate endothelial cells. Attracted monocytes adhere to and migrate into the vessel wall, where they differentiate into macrophages and can become lipid-loaded foam cells. Upon atherosclerosis progression, additional immune cells propagate chronic inflammation and proliferating smooth muscle cells enclose the lesion with a fibrous cap. Apoptotic foam cells can be eliminated by neighboring macrophages through efferocytosis or can form a necrotic core. Additionally, macrophage-derived metalloproteases can induce thinning of the fibrous cap and when so-called 'vulnerable' plaques rupture this can cause myocardial infarction and stroke [2].

While macrophages clearly play a central role in different stages of atherogenesis, surprisingly little is known about the molecular mechanisms that regulate their phenotype within the plaque [3]. Macrophages display high heterogeneity and in response to the microenvironment adopt different polarization states [4]. Classically activated (M1) macrophages, activated by Toll-like-receptor (TLR) triggers and Th1 cytokines, are pro-inflammatory and are therefore regarded as pro-atherogenic. Interleukin-4 (IL-4)/IL-13-induced alternatively activated (M2) macrophages are considered

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anti-atherogenic by counteracting inflammation and by efferocytosis. However, M2 macrophages can also aggravate atherogenesis through oxLDL-uptake [1–3]. Oxidized phospholipids, produced in atherosclerotic plaques, induce Mox macrophages that are characterized by an anti-oxidant response [5]. A strategy that promotes anti-inflammatory M2 features and/or inhibits pro-inflammatory, pro-atherogenic macrophage actions could be envisaged as an atheroprotective treatment [3].

Histone modifications by CME govern multiple aspects of inflammation and immunity [6]. Histone acetylation on lysine (K) residues by histone acetyltransferases (HATs) is associated with transcriptional activation and is counteracted by HDACs. Histone methylation is regulated by histone methyltransferases (HMTs) and histone demethylases (HDMs). Di- or tri-methylation of histone H3K4, H3K36 and H3K79 is associated with activation of transcription, whereas H3K9me2/3 and H3K27me3 constitute repressive marks. In macrophages, the histone demethylase Jmjd3 regulates responses to various stimuli, and HDAC3 promotes inflammatory M1 activation while inhibiting M2 [7]. Pharmaceutical companies are currently pursuing CME-inhibitors as a therapy against inflammatory diseases and we consider such approaches to be valuable for atherosclerosis treatment as well.

To study which CME might be favorable to inhibit in macrophages during atherogenesis, we designed a screening that addresses all major atherogenic macrophage features. Based on our data, we propose macrophage-specific inhibition of HDAC3 as a potential therapeutic target in atherosclerosis.

2. Materials and methods

2.1. Cell culture and reagents

Femurs and tibia from C57Bl/6 mice were flushed with PBS and bone marrow cells were cultured 8 days in RPMI-1640 2 mM L-glutamine, 10% FCS, penicillin (100 U/ml), streptomycin (100 μg/ml) (Gibco) and 15% L929-conditioned medium. Unless otherwise indicated, the resulting bone marrow-derived macrophages (BMMs) were plated in 150 μl at 10^6/ml in a confluent monolayer in 96-well microplates (Greiner). Cells were left untreated or were pretreated 30 min with the following commonly used [8–11] pan-inhibitors (Sigma): 50 nM trichostatin A (TSA; HDAC inhibitor [HDACi]), 50 μM epigallocatechin-3-gallate (EGCG; HATi), 10 μM pargyline (HDMi), 0.5 mM 5′-methylthioadenosine (MTA, HMTi) or 0.5% DMSO as a control for HMTi. Inhibitor concentrations were selected based on previous studies [8–11] and a MTT toxicity assay (data not shown). The pan-HDAC inhibitor ITF2357 (250 nM) and small molecule inhibitors with distinct specificities against particular HDACs were synthesized at Italfarmaco (Milan, Italy) [12]. The HDAC8i retains high selectivity for HDAC8 up to 10 μM and all information about the specificity of the other inhibitors is provided in Supplementary Table 1. BMMs were activated with 10 ng/ml LPS or 2 μg/ml LTA (lipoteichoic acid, Sigma) for 6 or 24 h, or were stimulated 24 h with 10 U/ml interferon-γ (IFNγ, Peprotech) + 10 ng/ml LPS, 100 U/ml IL-4 (Peprotech) or 50 μg/ml oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (oxPAPC, Invivogen) to elicit M1, M2 or Mox macrophages, respectively.

2.2. Gene expression analysis

RNA was isolated with High Pure RNA Isolation kits (Roche), cDNA was synthesized from with iScript (Bio-rad) and real-time PCR was performed using Sybr Green Fast mix (Applied Biosystems) on a ViiA7 apparatus (Applied Biosystems). Gene expression was normalized to the mean expression of housekeeping genes *Rplp0* (Arbp) and *Ppia* (CypA). Primer sequences are available on request.

2.3. Foam cell formation

After overnight inhibitor pretreatment and DiI-oxLDL (Biotrend) treatment (3 h, 10 μg/ml), oxLDL-uptake was measured by flow cytometry (BD Canto II). For lipid staining, BMMs were pretreated with the inhibitors for 30 min, stimulated with 50 μg/ml oxLDL (BTI) and stained with LipidTOX Red (Invitrogen) according to the manufacturer's protocol. The median fluorescence intensities (MFI) were calculated with FlowJo. Alternatively, cells were lysed in water with 1% Triton and free and total cholesterol was determined using an established enzymatic fluorometric assay [13]. Protein concentrations in the cell lysates were measured with a Pierce BCA assay and used to normalize cholesterol concentrations.

2.4. Apoptosis

After 30 min inhibitor pretreatment, BMMs were left untreated or were stimulated overnight with 20 μg/ml 7-ketocholesterol (7KC; Sigma), 50 μg/ml oxLDL or 10 μg/ml 25-hydroxycholesterol (25OHC; Sigma) and stained with propidium iodide (PI)/Annexin-V-Alexa-Fluor647 (Invitrogen) according the manufacturer's protocol. The percentage living (Annexin-V⁻/PI⁻), early apoptotic (Annexin-V⁺/PI⁻), late apoptotic (Annexin-V⁺/PI⁺) and necrotic (Annexin-V⁻/PI⁺) macrophages was measured in a FACS Canto II.

2.5. Metabolic assay

BMMs were plated in XF-96 cell culture plates and either left untreated or pretreated with the CME-inhibitors, followed by M1 or M2 polarization. Metabolic fluxes were analyzed in an XF-96 Flux Analyzer (Seahorse Bioscience) three times with 5 min intervals per the manufacturer's protocol.

2.6. Statistical analysis

Data represent the mean ± SEM of one representative experiment. Data were evaluated with GraphPad Prism4 using one-way ANOVA and values represent the mean ± SEM of 3 replicates; $^*P < 05$; $^{**}P < 01$; $^{***}P < 001$.

3. Results

3.1. Study design: a phenotypic in vitro screening platform to identify compounds that improve atherogenic macrophage functions

To identify CME-inhibitors that affect atherogenic macrophage activities, we designed a phenotypic screening that addresses major atherogenic macrophage features, including adhesion to endothelial cells, activation and inflammatory cytokine production, foam cell formation, apoptosis and efferocytosis. All assays were designed in a quantitative 96-well-plate-based semi-high-throughput format, providing a new tool to screen large numbers of modulators for their involvement in atherogenic macrophage functions in a fast manner and requiring minimal amounts of materials. First we performed a cell adhesion assay and found that macrophage/endothelial cell-adhesion was not affected by pre-treatment of BMMs with CME-inhibitors (Fig. S1).

3.2. HDAC and HMT inhibitors dampen inflammatory macrophage responses in M1 and regulate M2 and Mox macrophage polarization

To test the involvement of epigenetic enzymes in macrophage activation, we pretreated BMMs with the different CME-inhibitors and challenged them with LPS for 6 or 24 h. Blocking HDACs and

HMTs profoundly impaired LPS-induced production of most inflammatory cytokines (except for *tumor necrosis factor* [TNF] at 24 h) and nitric oxide (NO, Fig. S2A and B). HAT and HDM inhibition had only minor effects on the secretion of pro-inflammatory cytokines. Similar results were observed for LTA-induced TLR2/6-responses (Fig. S2C).

In accordance with their inhibitory effect on TLR-responses, HDACi also reduced IFNγ + LPS-induced *Tnf*, *Il6* and *Nos2* expression in M1 macrophages (Fig. 1A) and the resulting secretion of IL-6 and NO (Fig. S2D). On the other hand, HDAC inhibition profoundly enhanced the expression of the M1 transcription factor *Stat1* (Fig. 1A). While the HATi and HDMi did not affect IFNγ + LPS-induced activation, the HMTi impaired *Tnf* expression and resulted in a drop in IL-6 and TNF secretion (Fig. 1A and Fig. S2D). In contrast, HMT inhibition enhanced IFNγ + LPS-induced *Il1b* expression (Fig. 1A).

Overall, these data show that HDAC and HMT inhibition partly impair inflammatory macrophage responses, but at the same time promote the expression of other M1 marker genes. In addition to inflammatory activation, macrophages can be alternatively activated by IL-4 or can be polarized towards Mox macrophages by oxPAPC [5] but the effect of CME-inhibition on these modes of macrophage activation remains unexplored. Here, we found that HDAC inhibition impaired the IL-4-induced expression of the M2 markers [4] *Arg1*, *Retnla* and *Chi3l3* (Fig. 1B). Conversely, the gene encoding the M2 marker E-cadherin (*Cdh1*) [15] was more efficiently induced by IL-4 after HDAC inhibition. Additionally, the HDACi and HMTi potentiated the expression of some Mox-associated genes [5] (Fig. 1C). Together these observations show that both HDACs and HMTs regulate M1, M2 and Mox polarization.

3.3. HDAC inhibition enhances histone acetylation and expression of Abca1 and Abcg1, but does not affect foam cell formation

Since foam cell formation is a crucial hallmark of atherosclerosis, we set out to analyze the effect of the CME-inhibitors on oxLDL-uptake and the resulting foam cell formation. Hereto, we first pretreated BMMs with the CME-inhibitors before adding fluorescent DiI-oxLDL. All compounds reduced DiI-oxLDL uptake after 3 h incubation (Fig. 2A). Yet, only the HATi EGCG effectively reduced foam cell formation after 24 h (Fig. 2B). While EGCG is an established HATi and could affect foam cell formation by regulating the expression of scavenger receptors and efflux proteins, EGCG also influences micellar cholesterol solubility [16] and could in this way also alter foam cell formation. In an attempt to explain the observed effects on oxLDL uptake, we measured the expression of *Cd36* and *Msr1* as oxLDL uptake mediators and *Abca1* and *Abcg1* as cholesterol efflux regulators. While the expression of *Cd36* and *Msr1* was not altered by any compound tested, *Abca1* and *Abcg1* expression were increased upon HDAC inhibition (Fig. 2C) and these levels correlated with increased histone acetylation at both promoters (data not shown). Yet, increased *Abca1* and *Abcg1* expression and histone acetylation did not affect foam cell formation. EGCG did not modulate the expression of oxLDL uptake receptors or efflux proteins and thus probably exerts its effect on foam cell formation through the modulation of cholesterol solubility.

3.4. Apoptosis of macrophages and foam cells is reduced by HDAC inhibition

Since macrophage apoptosis and impaired efferocytosis impair plaque stability, we tested whether CME-inhibitors could be
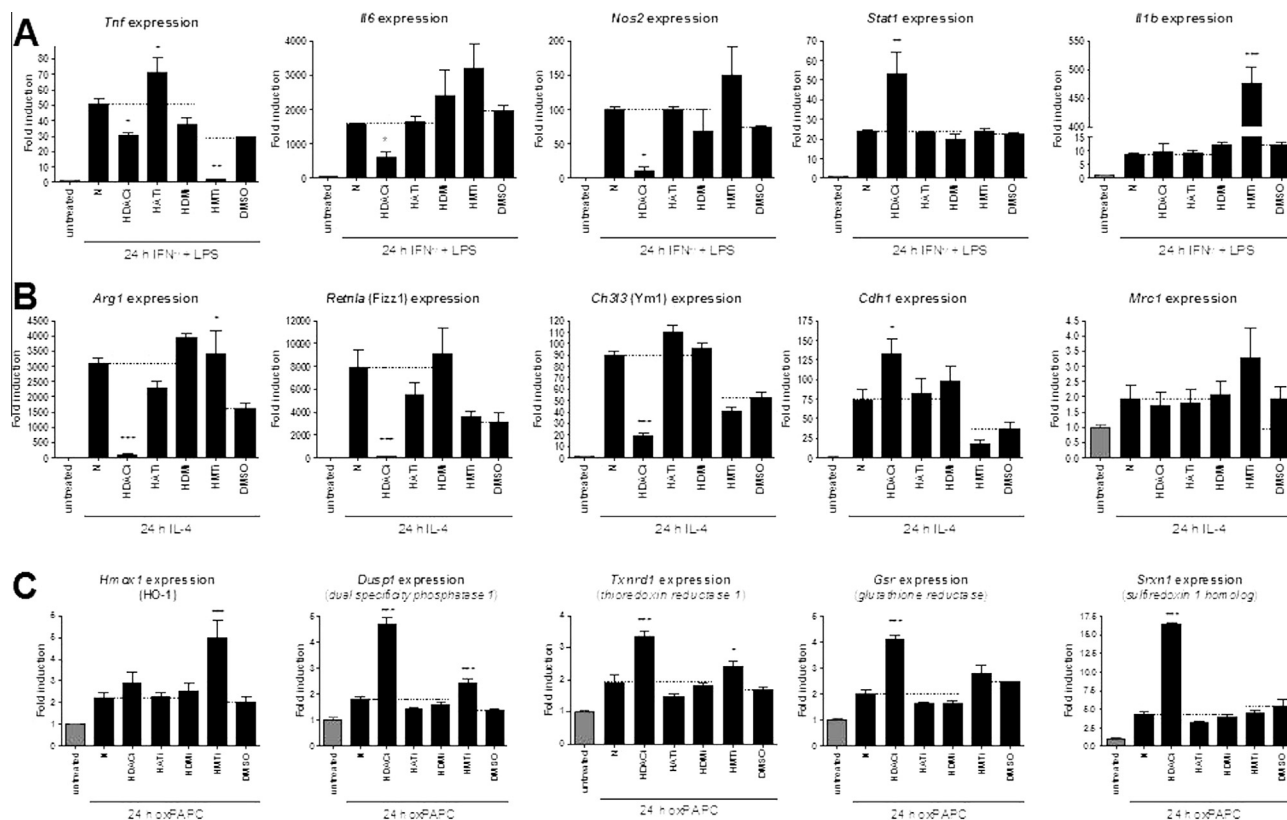
Fig. 1. HDAC and HMT inhibition partly impair M1 macrophage activation, and affect M2 and Mox polarization. BMMs were pretreated with CME-inhibitors or were left untreated for 30 min, followed by 24 h (A) LPS + IFNγ (M1), (B) IL-4 (M2) or (C) oxPAPC (Mox) stimulation. Fold inductions were determined relative to the expression in untreated cells. HDACi-, HATi- and HDMi-pretreated conditions were compared to a control without pretreatment (N) and DMSO-pretreated cells served as control for HMTi (dotted lines).
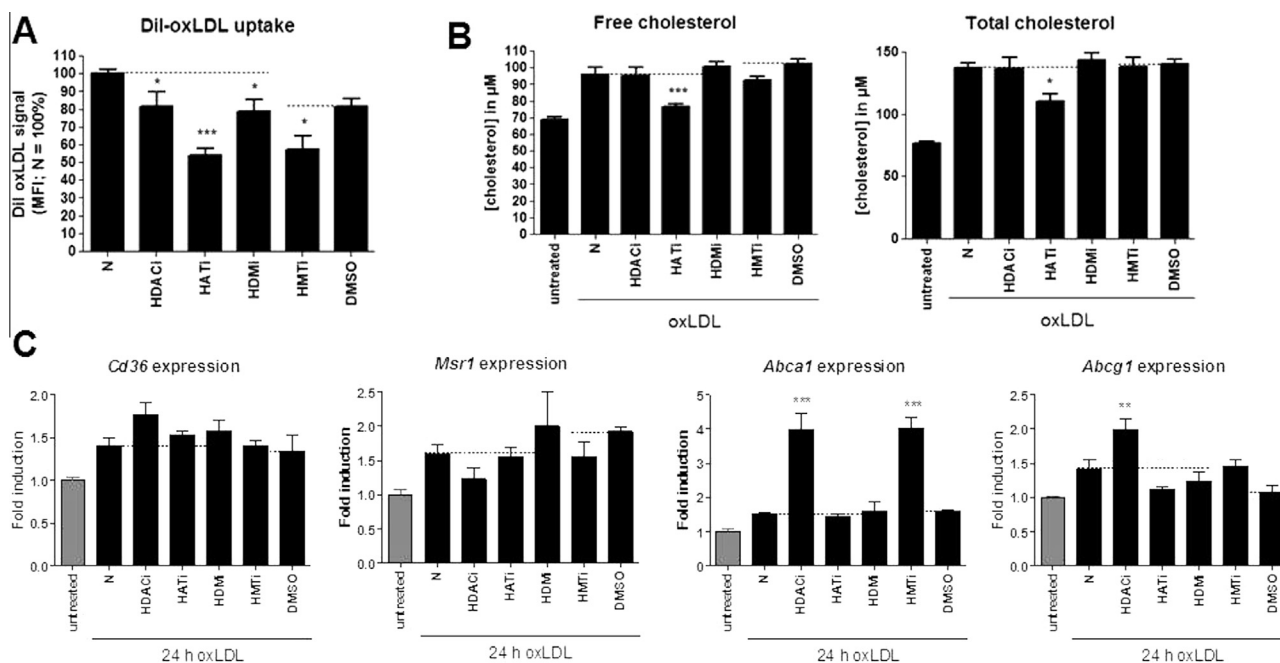
Fig. 2. HDAC inhibition reduces foam cell formation, promotes *Abca1* and *Abcg1* expression and increases histone acetylation in the promoter regions of both cholesterol efflux regulators. (A) BMMs were pretreated overnight with different CME-inhibitors or were left untreated, followed by 3 h incubation with DiI-labeled oxLDL. (B) To analyze longer-term foam cell formation, pretreated (30 min) BMMs were stimulated for 24 h with oxLDL followed by the measurement of free and total cholesterol. (C) Fore gene expression analysis, BMMs were pretreated for 30 min with different CME-inhibitors or were left untreated, followed by 24 h oxLDL stimulation. Fold inductions were determined relative to the expression in untreated cells. HDACi-, HATi- and HDMi-pretreated conditions were compared to a control without pretreatment (N) and DMSO-pretreated cells served as control for HMTi (dotted lines).

applied to decrease apoptosis and to improve efferocytosis. BMMs were pretreated with the different CME-inhibitors and were next left untreated or were stimulated with 7KC, oxLDL or 25OHC. HDACi and HDMi treatment reduced basal macrophage apoptosis (Fig. 3A). Inhibition of HDACs reduced apoptosis induced by the mild stressors 7KC and oxLDL, but could not overcome the strong 25OHC-induced necrosis. In contrast, HMT inhibition rather increased oxLDL- and 25OHC-induced apoptosis. Efferocytosis was not affected by the distinct CME-inhibitors (Fig. S3). These experiments indicate that HDAC inhibition could be applied to prevent stress-induced macrophage apoptosis in an atherosclerosis setting.

3.5. HDAC inhibition alters cellular metabolism upon inflammatory macrophage activation

Recent data demonstrate that inflammatory macrophages display a glycolytic metabolism while M2 macrophages show high mitochondrial oxidative phosphorylation (OXPHOS) [17]. Inhibition of glycolysis or OXPHOS hamper M1 or M2 activation, respectively [18,19]. To assess whether inhibition of CME affects cellular metabolism, we performed a metabolic flux analysis and determined the oxygen consumption rate (OCR) as a measure of OXPHOS, and extra-cellular acidification rate (ECAR) as a measure of glycolysis. While the HATi, HDMi and HMTi had no noteworthy metabolic effects (data not shown), HDAC inhibition resulted in remarkable alterations in cellular metabolism in M1 macrophages. We found that inflammatory macrophage activation by IFNγ + LPS induces glycolysis and impairs OXPHOS (Fig. 3B and C). Conversely, IL-4-induced M2 polarization enhanced OXPHOS (Fig. 3C). The HDACi had no significant effect on ECAR and OCR in naïve and M2 macrophages. Upon M1 polarization however, the HDACi strongly induced ECAR (Fig. 3C) and prevented the IFNγ + LPS-induced drop in OCR which is normally observed during classical macrophage

activation (Fig. 3D). Hence, while inflammatory macrophage activation increased the ECAR/OCR ratio as described earlier [17], HDACi-pretreated M1 macrophages display a shift towards both high ECAR and OCR (Fig. 3D).

### 3.6. Macrophage HDAC3 inhibition results in an athero-protective macrophage phenotype

While broad spectrum HDAC inhibitors clearly have beneficial athero-protective effects, they also have contraindications that prevent their use in atherosclerosis therapy [20] and inhibiting specific HDACs might be more ideal. To investigate which particular HDAC enzymes should be targeted, we applied small molecule inhibitors which preferentially inhibit specific HDACs (Supplementary Table 1). Compounds HDAC3/6i(a) and (b) that preferentially inhibit HDAC3 and HDAC6 phenocopied the inhibitory effects of the pan-HDACi TSA and ITF2357 on IL-6 and NO secretion by M1 (Fig. 4A and B). The HDAC1/2i, HDAC6i and HDAC8i had no effect on IL-6 and NO secretion. Moreover, HDAC1/2 and HDAC3/6 inhibition, but not HDAC6 or HDAC8 inhibition, blunted oxLDL- and 7KC-induced apoptosis (Fig. 4C and D). Importantly, none of the tested specific HDACi had an adverse effect on foam cell formation (Fig. S4). Overall, HDAC3 inhibition appears to be sufficient to obtain the athero-protective effects of pan-HDAC inhibition.

4. Discussion

In this paper, we applied a new platform and demonstrate that chromatin modifying enzymes regulate atherogenic macrophage activities and polarization. Since broad-spectrum HMT-inhibition and especially HDAC inhibition have beneficial anti-atherogenic effects, targeting specific HDACs and HMTs in macrophages in atherosclerosis will be of particular interest in future studies.
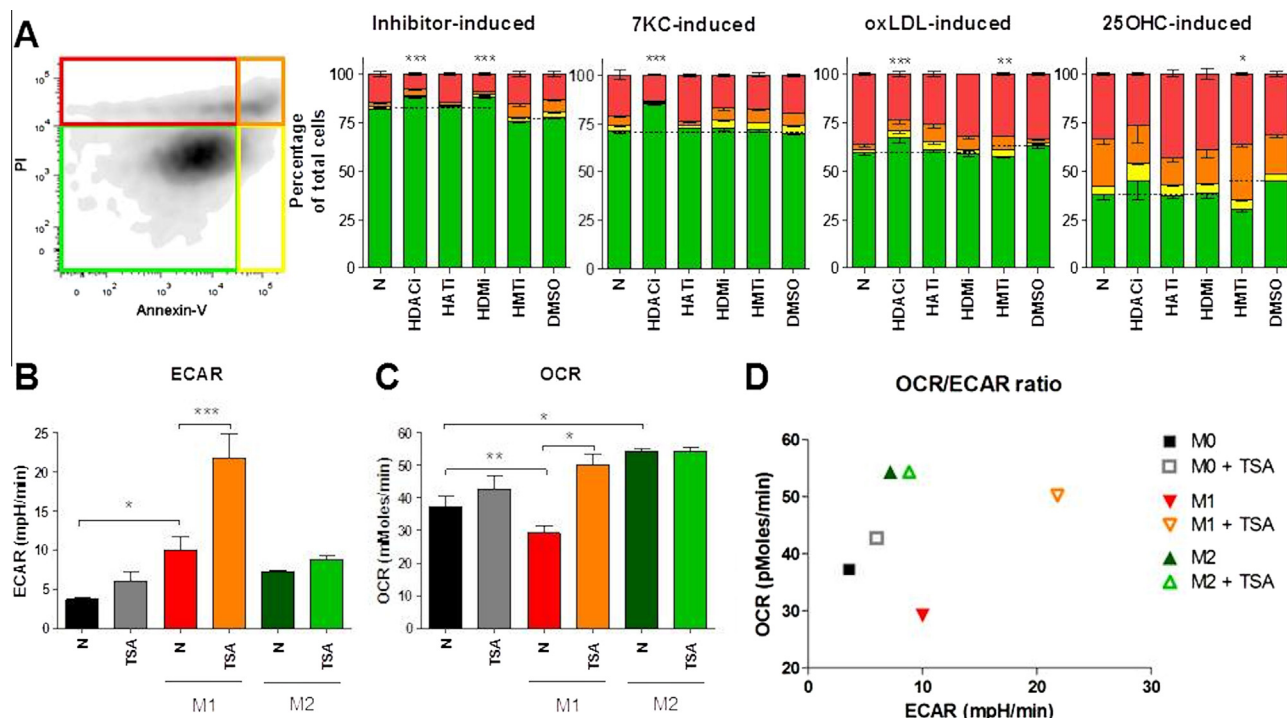
Fig. 3. HDACi reduces macrophage apoptosis and affects the cellular metabolism in M1 macrophages. (A) BMMs were pretreated 30 min with CME-inhibitors or were left untreated (N), followed by 24 h naïve culture (inhibitor-induced), or 7KC, oxLDL, or 25OHC stimulation. Annexin-V/PI-staining was performed, followed by flow cytometry. For metabolic characterization, BMMs were pretreated or not with TSA and differentiated towards M1 or M2, or remained untreated (M0). (B) ECAR (glycolysis) and (C) OCR (OXPHOS) were measured and (D) corresponding OCR/ECAR ratios were plotted.
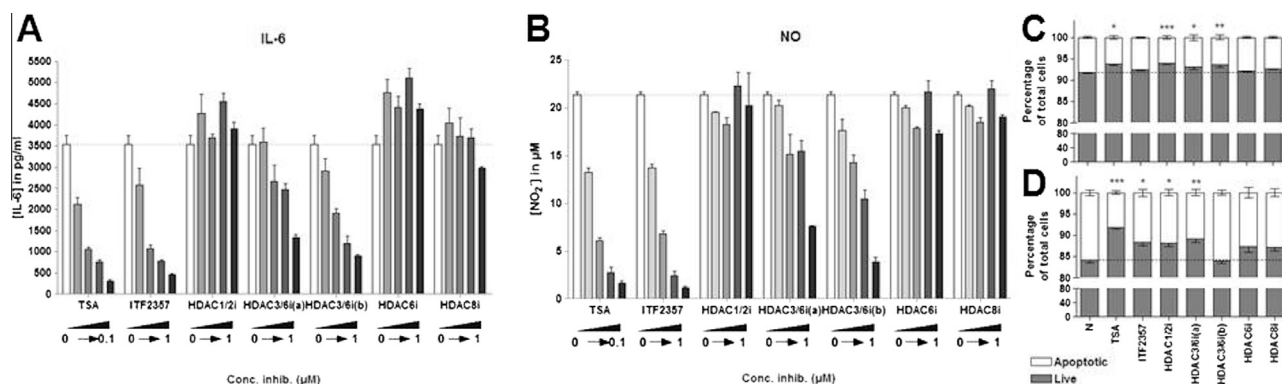
Fig. 4. Inhibition of HDAC3 is anti-inflammatory and anti-apoptotic. BMMs were pretreated with decreasing inhibitor concentrations (1/3 dilution series, starting at 1 μM for the Italfarmaco compounds and 0.1 μM for TSA), followed by LPS + IFNγ stimulation and (A) IL-6 and (B) NO measurement. For the apoptosis experiment, BMMs were pretreated with indicated inhibitors, followed by (C) 7KC or (D) oxLDL treatment and Annexin-V-staining.

4.1. Inhibition of HDACs and HMTs affects macrophage activation

Suppressing inflammatory responses is probably the best-described feature of HDAC inhibition in macrophages [8,21,22]. In agreement we observed reduced *Nos2*, *Il12b*, *Il6* and *Tnf* expression upon HDAC inhibition. While we observed that the HDACi increased the gene expression of *Stat1*, IFNγ-induced STAT1 signaling itself requires HDAC activity and is known to be suppressed upon HDAC inhibition [23].

We found that inhibition of HMT activity enhances LPS + IFNγ-induced *Il1b* expression. Moreover, *Il1b* expression was potentiated early during the LPS response and is not efficiently repressed later on (data not shown). These data, together with the observation that H3K9 methyltransferase G9a establishes an epigenetically silenced state during LPS tolerance [24], reflects the need for methyltransferase activity (probably G9a) to silence the pro-inflammatory gene

Il1b. Conversely, we found that the activity of other HMTs that deposit activating H3K4me2/3 or H3K36me2/3 histone marks is needed for TNF, IL-12, IL-6 and NO production. Members of the *mixed lineage leukemia* (MLL) family of HMT plausibly account for this, as they have been associated with inflammatory macrophage responses [25]. Thus, to effectively block inflammatory responses through HMT modulation the pro- and anti-inflammatory effects of the pan-HMT inhibitors need to be disconnected and specific compounds may be more relevant.

In contrast to the fast-growing amount of studies on the epigenetic regulation of inflammatory responses, the involvement of CME in other modes of macrophage polarization remains ill-defined. We therefore investigated the effect of CME-inhibitors on M2 polarization and found that some M2 marker genes [4,26] like *Arg1*, *Chi3l3* (Ym1) and *Retnla* (Fizz) rely on HDAC activity for their induction by IL-4 and that others (*Mrc1* and *Clec10a*) are

unaffected or induced (*Cdh1*) by HDAC inhibition. In agreement with our results, Serrat et al. recently showed that HDAC inhibition acetylates and inhibits the M2-regulating transcription factor C/EBPβ, thereby impairing the IL-4-induced expression of some M2 marker genes [27]. Thus, HDAC activity appears to be required to establish a complete M2 activation program. Since M2 macrophages are anti-inflammatory, a pan-HDACi might not be optimal to maximally dampen inflammatory responses within the plaque.

Although epigenetic enzymes have never been studied in Mox macrophages, our observation that a HDACi increase the expression of some Mox-associated marker genes is not entirely unexpected. Indeed, the Mox-regulating transcription factor Nrf2 is activated by HDAC inhibition in neuronal cells and microglia [28,29].

4.2. Inhibition of chromatin modifying enzymes does not improve foam cell formation

TSA was previously shown to increase CD36 expression and oxLDL-uptake in Raw264.7 macrophages [20]. In our setting, the expression of oxLDL-receptors CD36 and SRA was not affected by the different CME-inhibitors. This discrepancy could be explained by differences in experimental protocols. We used primary macrophages (instead of Raw264.7 cells) and investigated foam cell formation not only by assessing short-term oxLDL-uptake (like Choi did [20]), but also measured the longer-term accumulation of cholesterol after 24 h oxLDL-treatment. Actually, we observed an HDACi-induced increase of histone acetylation and gene expression of *Abca1* and *Abcg1*. In agreement with our data, two independent studies showed that HDACi enhances the ABC transporter expression and thereby stimulates cellular cholesterol efflux [30,31]. In contrast to previous observations in cell lines [20], HDAC inhibition appears to have no detrimental effects on foam cell formation in primary macrophages.

4.3. HDAC inhibition dampens apoptosis in macrophages

Excessive macrophages apoptosis, combined with impaired efferocytosis elicits plaque instability. We showed that HDAC inhibition beneficially reduced macrophage apoptosis. Possibly, our own metabolic flux data could at least partly explain why HDACi-pretreated cells display a survival benefit. Unlike normal M1 macrophages, M1 generated in the presence of TSA did not show a drop in OXPHOS and displayed efficient glycolysis. Since, an efficient cellular metabolism with increased OXPHOS could provide a bioenergetic survival advantage [17], it is tempting to speculate that this switch in cellular metabolism might at least partly explain the apoptosis-inhibiting effect of HDAC inhibition.

4.4. HDAC3 inhibition in macrophages is a highly attractive new target for atherosclerosis therapy

We demonstrated that pan-HDACi have beneficial anti-atherogenic effects as they partly inhibit M1 activation and blunt apoptosis, without augmenting foam cell formation in primary macrophages. Yet, HDAC inhibitors have contra-indications that prevent their usage in atherosclerosis therapy [20]. Anti- and pro-inflammatory effects of distinct inhibitors are separable over a concentration range, suggesting that individual HDACs have differential effects on macrophage activation [22]. For example, HDAC9 deletion in macrophages during atherosclerosis promotes cholesterol efflux and M2 polarization and impairs inflammatory responses [32]. Moreover, deletion of HDAC3 boosts M2 polarization and at the same time dampens pro-inflammatory activation of M1 macrophages [7], two features which are considered highly advantageous as atherosclerosis treatment. Here, we inhibited specific HDAC enzymes in macrophages and found that inhibiting HDAC3 elicits an athero-protective macrophage phenotype. These data support our very recent *in vivo* study in which we demonstrated that macrophage-specific deletion of HDAC3 stabilizes atherosclerotic lesions through enhanced collagen deposition and improved lipid handling [14].

Altogether, our data strongly suggest that inhibiting macrophage HDAC3 is a highly attractive new therapeutic target in cardiovascular disease. In addition, unraveling the involvement of particular HMTs (especially MLLs) in atherogenic macrophage functions should also support the development of specific compounds for anti-atherogenic remedy. Overall, our study provides a screening method to identify compounds that ameliorate atherogenic macrophage functions and using this platform, we illuminated the role of epigenetic enzymes in macrophage activation.

5. Conflict of interest

The authors have no actual or potential conflict of interests.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.11.029.

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