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FORUM REVIEW ARTICLE

Epigenetic Regulation of Monocyte and Macrophage Function

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Abstract

Significance: Monocytes and macrophages are key players in tissue homeostasis and immune responses. Epigenetic processes tightly regulate cellular functioning in health and disease. ***Recent Advances:*** Recent technical developments have allowed detailed characterizations of the transcriptional circuitry underlying monocyte and macrophage regulation. Upon differentiation and activation, enhancers are selected by lineage-determining and signal-dependent transcription factors. Enhancers are shown to be very dynamic and activation of these enhancers underlies the differences in gene transcription between monocytes and macrophages and their subtypes. ***Critical Issues:*** It has been shown that epigenetic enzymes regulate the functioning of these cells and targeting of epigenetic enzymes has been proven to be a valuable tool to dampen inflammatory responses. We give a comprehensive overview of recent developments and understanding of the epigenetic pathways that control monocyte and macrophage function and of the epigenetic enzymes involved in monocyte and macrophage differentiation and activation. ***Future Directions:*** The key challenges in the upcoming years will be to study epigenetic changes in human disease and to better understand how epigenetic pathways control the inflammatory repertoire in disease. *Antioxid. Redox Signal*. 25, 758–774.

Keywords: epigenetics, monocytes, macrophages, transcription factors

Introduction

MONOCYTES AND MACROPHAGES are central in tissue homeostasis and immune responses. Recently emerging data show that most tissue macrophages are seeded during embryonic development and maintained by locally residing and proliferating stem cell pools (42, 48). These tissue macrophages serve trophic functions, maintain tissue homeostasis, and mediate resolution of inflammation. Monocytes provide a macrophage precursor pool that is recruited upon inflammatory challenges to mediate host defense against pathogens, foreign antigens, or tissue damage. The total population of monocytes and macrophages comprises a maintenance and defense pool that is involved in many human diseases, including infections, cancer, obesity and diabetes, cardiovascular diseases, and chronic inflammatory diseases such as rheumatoid arthritis and Crohn's disease.

Monocytes are generated in the bone marrow from granulocyte–monocyte progenitor (GMP) cells and several subsequent more dedicated precursors (42). Their development depends on colony-stimulating factor 1 (CSF1, M-CSF) as mice lacking CSF1 or its receptor (CD115, M-CSFR) lack most of their monocytes and a significant portion of their macrophages (18, 24, 127). Monocytes occur in different subsets and can be distinguished in the mouse by being either $Ly6C^{hi}CCR2^{+}$ or $Ly6C^{lo}CX_3CR1^{hi}$ and in humans as $CD14^{+}$ or $CD14^{lo}CD16^{+}$. $Ly6C^{hi}$ monocytes are generally termed inflammatory monocytes and are particularly attracted in response to inflammatory stimuli (142), whereas the $Ly6C^{lo}$ monocytes are often referred to as patrolling monocytes and serve in maintaining endothelial function (5). Both subsets are generated in the bone marrow, and in the blood, the half-life of $Ly6C^{hi}$ monocytes is relatively short (approximately 20 h) and they can differentiate into the $ly6C^{lo}$ subset, which can circulate for days (137). In humans, similar subsets as in the mouse are found, but their functional implications in health and disease are less well defined and it remains to be seen whether similar distinctions can be made for their role in inflammatory responses.

Macrophages are very plastic cells and, in response to their microenvironment, adapt their phenotype and transcriptional program depending on the stimuli they encounter (46). This results in a wide variety of macrophage activation states, in which the two initially described macrophage phenotypes of classically activated macrophages (CAMs) and alternatively activated macrophages (AAMs) are the two most distinct and

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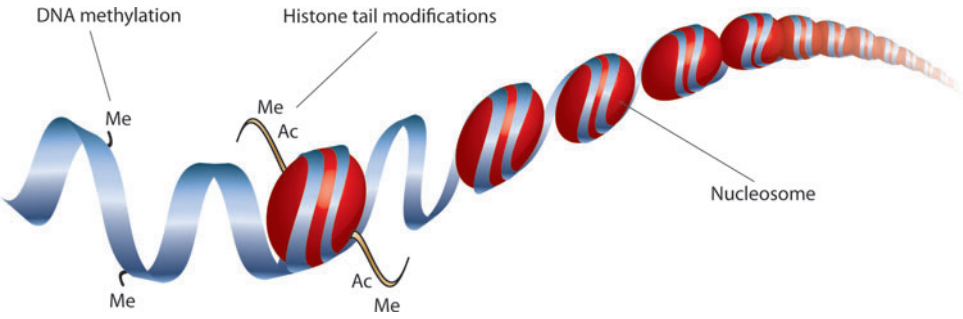

FIG. 1. The epigenetic level of gene regulation. DNA is wrapped around histones and the combined loop of DNA and histone proteins is called a nucleosome. Epigenetic modifications can occur directly at the DNA (methylation) or at the histone tails. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

extreme subsets (44, 119). The CAMs are known to be associated with chronic inflammatory diseases and type 2 diabetes (70). In contrast, AAMs are important in the pathogenesis of cancer, in parasite infections, allergy, wound repair, and lung fibrosis (116). Where CAMs produce high amounts of inflammatory mediators, AAMs are known to produce high levels of the anti-inflammatory cytokine interleukin 10 (IL-10) and profibrotic transforming growth factor β (TGFβ). Although the oversimplified CAM-AAM model provides a keen reductionist tool to describe cellular phenotypes, transcriptomic profiling of human and mouse activated macrophages shows a more complex spectrum of macrophage activation states (46, 74, 86, 131), greatly expanding this traditional model. Several excellent recent reviews have been written about monocytes, macrophages, their subsets, and development and we refer to these articles for further reading (25, 42, 48, 56, 82, 141).

Recent technical developments have allowed detailed characterizations of the molecular circuitry underlying monocyte and macrophage subtype regulation. Fine-tuned epigenetic processes that tightly regulate cellular differentiation and their responses under different challenges control the great plasticity of these cells. In this review, we will discuss the latest developments and understanding of the epigenetic pathways that control monocyte and macrophage function in health and disease.

Epigenetic Processes

Epigenetic processes control the use of DNA, without altering its sequence itself. Epigenetic alterations are often inheritable and are affected by environmental factors, are reversible, and therefore amendable for therapeutic interventions. Several levels of epigenetic processes exist: regulation by DNA methylation, by histone modifications, and by noncoding RNAs. Noncoding RNAs, such as miRNAs, can induce gene silencing and thereby contribute greatly to the gene expression programs. Several excellent recent reviews exist on this topic (63, 118) and we consider it outside the scope of the current article, in which we will focus on DNA methylation and histone modifications as processes regulating monocyte and macrophage function. DNA methylation and histone modifications, including acetylation and methylation (Fig. 1), alter the chromatin structure, which in turn determines the accessibility of DNA for binding of transcription factors, thereby affecting gene expression. The combination of DNA methylation patterns and specific histone modifications controls the epigenetic state of the chromatin ranging in the extremes from heterochromatin, that is, densely packed chromatin, where DNA is less accessible, resulting in gene silencing to euchromatin, which is the open conformation of the chromatin allowing transcription factor binding and gene expression.

DNA Methylation

DNA methylation, together with post-translational modifications of histone tails, is one of the most common mechanisms causing changes in DNA accessibility. DNA methylation occurs at cytosines that are adjacent to guanines in the DNA (CpG) and is associated with gene silencing. This cytosine methylation can block binding of transcription factors and transcriptional activators, which leads to decreased transcription factor accessibility or a less open chromatin structure (2). Methyl marks are placed on the DNA by DNA methyltransferases (DNMTs) and can be passively or actively removed by the ten-eleven translocation (TET) family of proteins (110).

Histone Methylation

Histone methylation can be associated with either gene induction or repression, depending on the position of methylation and the number of methyl groups (*i.e.*, mono-, di-, or trimethylation). While di- or trimethylation of histone H3 at lysine-4 and -79 is associated with gene activation, H3K9me2/3 and H3K27me3 are repressive histone marks (87), as described in Figure 2. Active promoters are marked by trimethylation of lysine 4 on histone H3 (H3K4), whereas enhancers, which are detailed below, are marked by mono- or

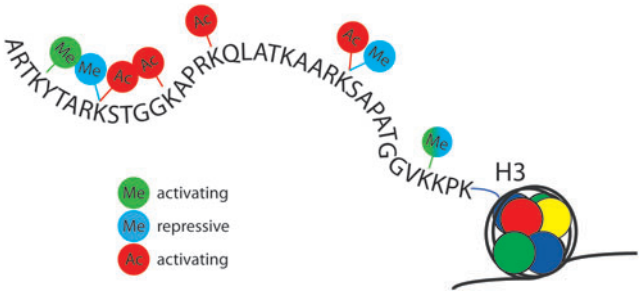

FIG. 2. Histone tail modifications. Histone modifications can occur on several lysines of the histone tail. Shown here is the tail of histone 3. Acetyl modifications are associated with gene transcription, while methyl marks can be activating or repressive, depending on the lysine that is targeted. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

dimethylation of H3K4 (51). The methylation status of histone lysines is determined by the activity of histone methyltransferase (HMT) and the opposing histone demethylase (HDM) activity.

Histone Acetylation

Acetylation on lysine residues by histone acetyltransferases (HATs) leads to the addition of negative charges to the positive lysines and thereby reduces the interaction between DNA and histones. This leads to a more open chromatin state, increasing transcriptional accessibility (109). The activity of HATs can be counteracted by repressor complexes with histone deacetylase (HDAC) activity removing lysine acetylation.

Other Histone Modifications

Besides histone methylation and acetylation, other marks such as histone phosphorylation (105), ubiquitination (15), and citrullination (23) can also be placed on histones, with diverse effects on chromatin structure and gene regulation.

Enhancer Formation Driving Differentiation and Cell Identity

The regulation of gene transcription programs requires the interaction between gene promoters and regulatory enhancer elements. Promoter regions are located proximal to gene transcription start sites and enhancer regions are found more distally (87). Besides their location in the genome, promoters and enhancers differ on more aspects, where gene transcription, if often driven by only a single promoter, can be regulated by multiple enhancers. Moreover, in contrast to promoters, the activity of enhancers has been proven to be very cell-type specific (104). Enhancers are gene regulatory regions that are marked by specific histone modifications, mainly H3K4me1, and can be classified into poised and active enhancers based on the absence or presence of histone acetylation, respectively (94), as visualized in Figure 3. Interestingly, genomic studies have shown that active enhancers overlap with RNA Pol II loading, which generates active bidirectional transcripts called enhancer RNAs (eRNAs) (27, 64, 85, 111). Although the exact role of enhancer transcription remains unknown, it is considered a hallmark of functionally active enhancers (50).

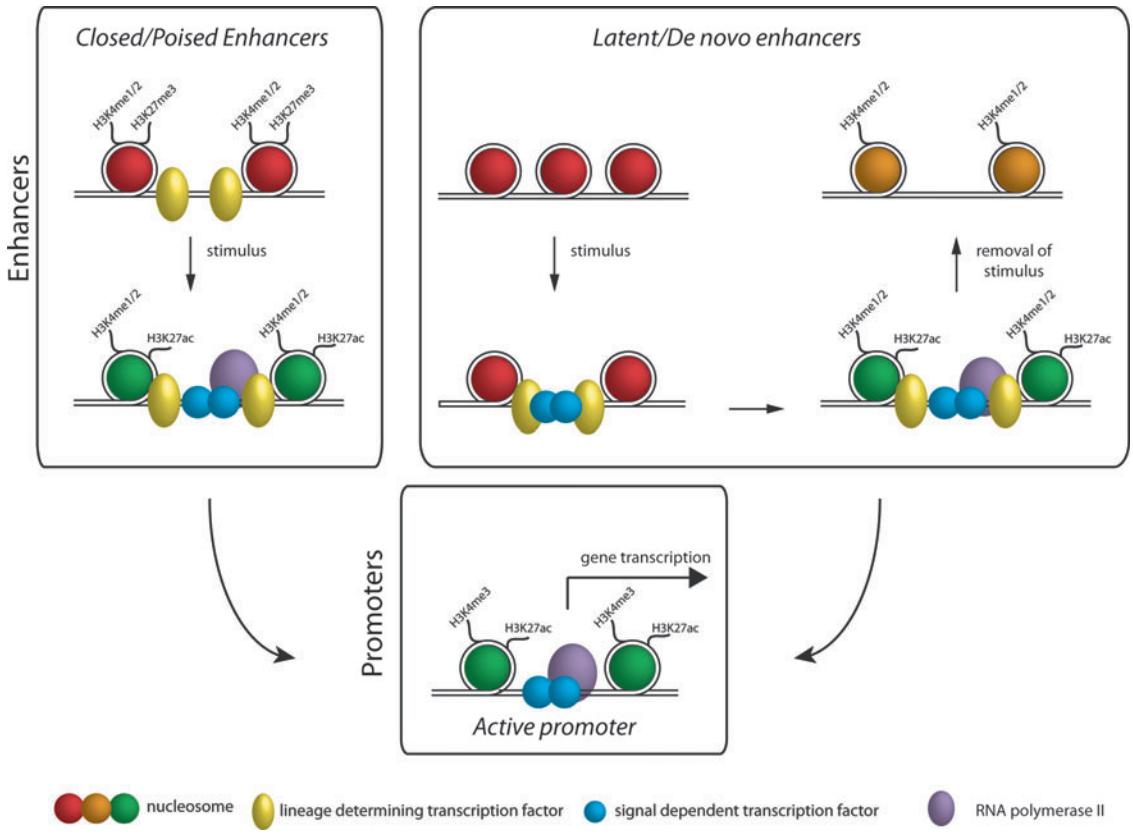

FIG. 3. Chromatin features of different types of enhancers and active promoters. Enhancers are characterized by H3K4me1/me2 marks; closed or poised enhancers carry, besides the H3K4 mark, also the repressive H3K27me3 mark. Latent or *de novo* enhancers do not contain any histone modifications. Upon stimulation, SDTF binding to poised enhancers results in loss of H3K27me3 and induction of H3K27 acetylation and subsequently RNA Pol II binding and transcription. *De novo* enhancers gain active modifications upon stimulation through collaborative efforts of SDTFs with LDTFs. Active enhancers drive promoter activity and gene transcription. LDTF, lineage-determining factor; SDTF, signal-dependent transcription factor. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

The selection and activity of enhancers are tightly regulated by two types of transcription factors: lineage-determining factors (LDTFs) and signal-dependent transcription factors (SDTFs). LDTFs are the pioneer factors in defining epigenetic and transcriptomic states of macrophages (53). LDTFs are critical transcription factors for a specific cell type and initiate selection of regulatory enhancers (43, 45, 46), as described in Figure 3. Hereby, the LDTFs determine cellular identity. Upon an encounter with an environmental trigger, SDTFs will switch on the transcriptional program necessary for an adequate stimuli-specific response by activating enhancers and driving promoter activity.

Over the past few years, it has become increasingly clear that the enhancer repertoire determines cell identity as the transcriptome needs to change depending on the function of the different cell types. Heinz *et al.* studied the transcriptomic differences between B cells and macrophages. They showed that although the master LDTF for both cell types is PU.1, genome-wide binding of PU.1 greatly differs between the two cell types. Moreover, in macrophages, PU.1 colocalizes with other macrophage-specific LDTFs, AP-1/JunB, and C/EBP, while in B cells, other factors such as Oct and E2A are more prominent (52). It was shown as a proof of principle that when PU.1 was depleted in primary macrophages, this resulted in decreased activating H3K4 methylation at many macrophage enhancers (40). Using natural genetic variation in mice as an *in vivo* mutagenesis model, it was found that binding of PU.1 and C/EBP was indeed necessary for enhancer formation, induction of activating histone modifications, and binding of SDTFs when stimulated (53).

In a more recent extensive study by Lara-Astiaso *et al.*, chromatin modifications during hematopoiesis in mice were studied (73). They performed chromatin immunoprecipitation (ChIP) sequencing experiments for four chromatin modifications and performed ATAC sequencing, a method to study chromatin openness, across 16 stages of hematopoietic differentiation. They identified over 48,000 enhancer elements, H3K4me1-positive regions located distally from promoters, and studied their dynamics in different subsets. During differentiation, monocytes gain about 5000 enhancers and lose 3000 enhancers compared with the hematopoietic stem cell (HSC) precursors, while macrophages gain 6000 enhancers and lose a similar number when formed from monocytes. Erythrocytes upon formation gain a similar number of enhancers, but lose about 20,000 enhancers, indicating an enormous shutdown of the transcriptional program, which fits with low transcriptional activity in these cells. These data clearly show high flexibility and plasticity in the enhancer repertoire in different hematopoietic cells, demonstrating the importance of these enhancer elements in cellular function. Monocytes and macrophages show a large overlap in the gained enhancers and already gain 40% to 50% of the *de novo* myeloid enhancers in the first step of myeloid commitment when differentiated into a common myeloid progenitor (CMP). Motif analysis of the enhancers overlapping with ATAC-seq peaks identified PU.1, C/EBPα, and C/EBPβ as most prominent LDTFs in GMPs. Furthermore, for macrophages, the Junb motif is enriched, while monocytes have a high enrichment of Atf3 (73), indicating the importance of these transcription factors in cellular commitment, see Figure 4. Overall, these data show a highly dynamic chromatin during hematopoiesis, regulated by specific LDTFs, controlling the transcriptional program of different hematopoietic cells.

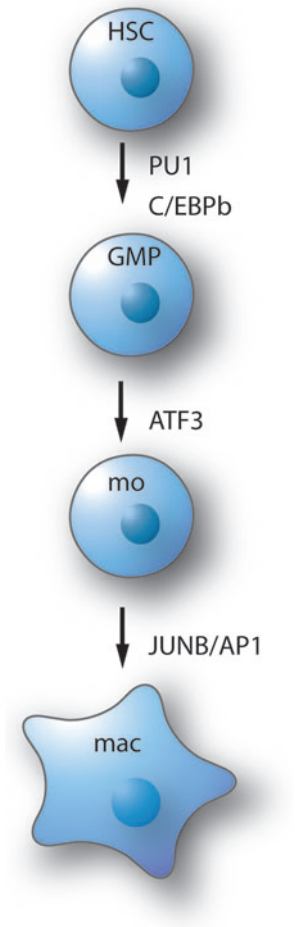

FIG. 4. LDTFs in monocyte and macrophage differentiation. LDTFs drive differentiation toward a specialized cell type. PU.1 and C/EBPβ are the driving LDTFs in HSC to GMP differentiation. In monocyte differentiation from GMPs, ATF3 is the crucial LDTF. Besides LDTFs, PU.1 and C/EBPβ, JunB/AP-1 is a critical determinant for macrophage differentiation. GMP, granulocyte-macrophage progenitor; HSC, hematopoietic stem cell. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

In addition, in human monocytes, it was shown that PU.1 and C/EBPα synergistically mediate enhancer creation in THP-1 cells (62). More recently, human monocytes and macrophages have been epigenetically characterized by the BLUEPRINT consortium (www.blueprint-epigenome.eu) (1). ChIP-seq experiments were performed for the histone marks, H3K4me3 (promoters), H3K4me1 (enhancers), and H3K27ac (active promoters and enhancers). Comparing monocytes with macrophages for differences in histone acetylation, which was the most dynamic mark, showed that it was altered at 2547 promoters, while 4036 enhancers showed a differential histone acetylation pattern (106). These data again demonstrate that major epigenetic changes occur during macrophage differentiation and that enhancers are important regulatory regions in defining the epigenetic landscape necessary for macrophage differentiation.

Enhancers and Macrophage Microenvironment

It was recently shown that specific stimuli in *in vitro* differentiated macrophages induce a completely different enhancer repertoire leading to signal-dependent differences in gene expression (94). In this article, regions in the genome were identified that initially lack histone marks and are not bound by transcription factors, but gain histone modifications and transcription factor binding upon macrophage activation by the stimuli. These enhancers are called *de novo* or latent enhancers (64, 94). Upon stimulation, SDTFs collaborate with LDTFs to activate these *de novo* enhancers, resulting in gene expression of genes that were epigenetically switched off before the trigger (94), as described in Figure 3. In the case of an inflammatory signal *via* Toll-like receptor (TLR) 4, approximately 3000 *de novo* enhancer-like regions are induced. The regions that gained H3K4 methylation upon TLR4 activation exhibited highly significant enrichment for motifs recognized by SDTFs, nuclear factor κB (NF-κB), interferon regulatory factors (IRFs), and Signal Transducer and Activator of Transcription factors (STATs), and LDTFs, C/EBP, AP-1, and PU.1 (52), also indicating a collaboration between SDTFs and LDTFs for the selection of *de novo* enhancers.

Next to typical enhancers, it was also observed that some DNA regions exist where enhancers are clustered together near key genes that drive cell identity. These regions are called superenhancers (55, 126). Although the functional significance of superenhancers is still under debate (99), it was observed that genome-wide association study (GWAS) variants are more enriched in superenhancers compared with regular or short enhancers. This may imply that particularly mutations in superenhancers affect susceptibility for disease (55, 96), indicating that superenhancers play pivotal roles in health and disease. In macrophages, it was shown that during activation through TLR4 stimulation, superenhancers for inflammatory gene expression become active. In addition, it was shown that these superenhancers are more conserved between mouse and human macrophages than typical enhancers. Moreover, superenhancers are associated with enhanced eRNA transcription, indicating increased enhancer activity. However, genes repressed by TLR4 signaling are also associated with superenhancer domains and accompanied by massive repression of eRNA transcription (50).

Two recent studies investigated the epigenetic landscape of macrophages derived from different tissues (46, 74). Gosselin *et al.* compared mouse microglia, residential large and small peritoneal macrophages, thioglycollate-elicited peritoneal macrophages, and bone marrow-derived macrophages with each other (46). In another study, Lavin *et al.* isolated macrophages from the brain, liver, spleen, lung, peritoneum, ileum, and colon and, moreover, isolated monocytes from mice for extensive epigenetic analysis (74). Cellular function and transcriptomic pathways differ a lot between these different cell types. While in the peritoneal macrophages, retinoid acid receptor and GATA 6 signaling was prominent (92), in microglia, TGFβ-SMAD and MEF2 signaling was over-represented compared with other cell types (46, 74). In spite of major transcriptomic differences, promoters showed hardly any variety in the activity between different macrophage subpopulations (46). In contrast, drastic differences in enhancers among macrophage subtypes were observed, again indicating that these enhancer regions are the drivers of differences in cellular function and that the tissue microenvironment influences macrophage phenotype by differentially activating different enhancer subsets (14, 46, 74). These data show that macrophages are highly plastic and that epigenetic mechanisms contribute to the diversity of tissue macrophages.

Epigenetic Memory in Tolerance and Training

Although immune memory has classically been considered exclusively present in cells from the adaptive immune system, over the past years, it has become increasingly clear that innate immune cells also have a memory. For instance, insects that do not have an adaptive immune system, but only have an innate immune system, also show memory responses (103). Dependent on the dose and the type of a first trigger, a second stimulation can lead to diverse responses. Epigenetic memory can lead to both tolerance, with reduced responses and preventing further tissue damage, and training, resulting in a stronger and more effective immune response. After training with *Candida albicans* or β-glucans, monocytes respond with increased cytokine production upon a second inflammatory trigger. By contrast, prestimulation of monocytes or macrophages with high doses of lipopolysaccharide (LPS) can induce LPS tolerance (2). Low doses of LPS, however, can augment the response to the second LPS trigger, resulting in training instead of tolerance, indicating that effects on memory are dose dependent (59, 125).

Tolerance

LPS tolerance has traditionally been viewed as a hyporesponsive state of macrophages resulting from receptor desensitization. A comprehensive study on the effects of LPS tolerance on gene expression in macrophages, however, demonstrated that there are two classes of genes found in tolerance: tolerizable and nontolerizable genes (37). This would indicate that macrophages are not in a complete hyporesponsive state, but instead tightly regulate which genes are repressed at a second hit and which ones are not. These different types of regulations ensure that proinflammatory mediators that cause tissue damage are transiently inactivated, while other antimicrobial proteins that do not negatively affect tissue physiology remain inducible. At an epigenetic level, it was found that H3K4 trimethylation was induced in naive macrophages at promoters of both tolerizable and nontolerizable genes. However, following a second hit of LPS, this modification was rapidly and selectively lost at tolerizable promoters, but was maintained at nontolerizable promoters, as visualized in Figure 5. Treatment of macrophages with pargyline, an inhibitor of H3K4 demethylase LSD1, prevented *Il6* silencing in tolerant macrophages and maintained H3K4me3 levels at the *Il6* promoter (37), indicating that demethylase activity is essential for shutting down tolerizable genes. Besides LPS, there are various bacterial or viral products that can program monocytes or macrophages for either an enhanced or a decreased inflammatory state, a function mediated by epigenetic changes (59, 101), depending on both the type of trigger and the dose. We recently demonstrated that the antiviral cytokine interferon γ (IFNγ) has dual effects on the inflammatory

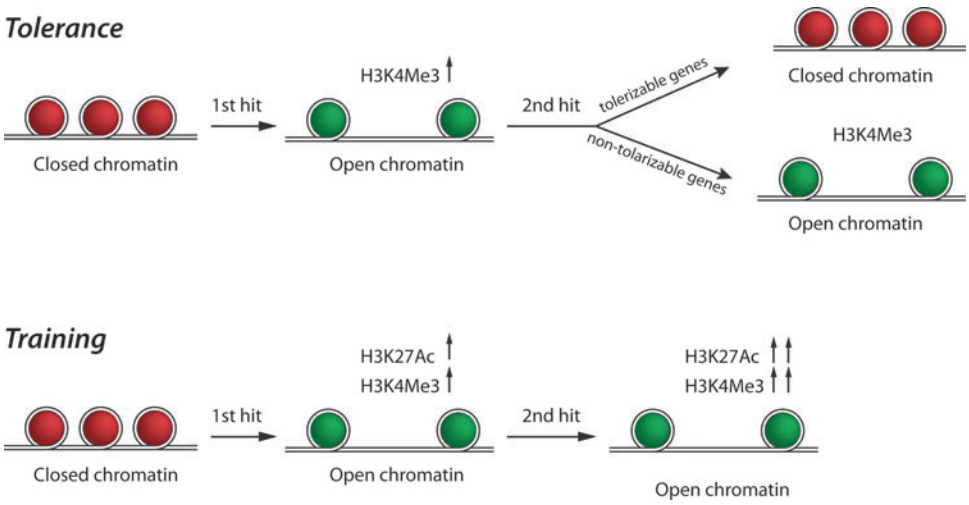

FIG. 5. Epigenetic regulation of tolerance and training. Monocytes and macrophages can be tolerized or trained for specific (inflammatory) stimuli, depending on the dose and the type of trigger. In tolerance, the first activates chromatin by increasing H3K4Me3. Upon a second hit, the tolerizable genes are silenced by removal of H3K4Me3 and gene expression is repressed, while nontolerizable genes are normally induced and keep H3K4Me3. In contrast, in training or trained immunity, a first hit opens up the chromatin leaving active histone marks; these marks are long-lasting. In case of a second hit, the chromatin is already primed leading to increased H3K27Ac and H3K4Me3 and enhanced gene expression. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

state of macrophages. Besides enhancing known inflammatory properties such as increased NO production (training), we surprisingly found that IFNγ priming represses a large subset of LPS-induced genes (tolerance). We found that repression by IFN-γ priming was dependent on STAT1 signaling and resulted in epigenetic remodeling on enhancer or promoter sites of repressed genes, resulting in decreased NF-κB p65 recruitment to these sites. The repressed genes were particularly involved in cellular movement and leukocyte recruitment and functionality, and the epigenetic and transcriptional changes induced by IFN-γ priming reduced neutrophil recruitment *in vitro* and *in vivo* (58). Such memory may also explain the increased susceptibility to bacterial infections of patients who have suffered from specific viral infections (91, 128). Overall, different bacterial and viral mediators can induce tolerance of specific gene programs, depending on the dose and type of the trigger.

Trained Immunity

In contrast to tolerance, in training or trained immunity, priming of monocytes or macrophages by an initial trigger results in an enhanced response to a second challenge (88). An excellent example of training is by Bacille Calmette-Guérin (BCG) vaccination, a live attenuated vaccine against tuberculosis, which, besides tuberculosis, is also protective against a wide variety of other infections. It was observed that BCG vaccination in healthy volunteers increased the production of IFNγ, tumor necrosis factor (TNF), and IL-1β in response to unrelated pathogens. The enhanced function of circulating monocytes persisted for at least 3 months and was accompanied by increased H3K4 trimethylation on the *TNF* and *IL6* promoters (67). In addition, β-glucans (components of the cell wall of *C. albicans*) induce training by enhancing the production of proinflammatory cytokines through increased H3K4 trimethylation at these cytokine promoters (102, 106). More recently, it was demonstrated that training with β-glucans also results in a gain of H3K27 acetylation in both promoters and enhancers throughout the genome (106), see Figure 5. Further analysis of the β-glucan-induced transcriptome identified the increased expression of genes involved in glucose metabolism. β-Glucan-trained monocytes displayed high glucose consumption and high lactate production (13, 21), which fits with the strong association between metabolism and inflammation in macrophages (7, 38, 123). Very recently, it was found that the stress response transcription factor, ATF7, is crucial for innate memory. ATF7 suppressed innate immune genes in mouse macrophages by recruiting the histone H3K9 methyltransferase, G9a. Training with LPS or β-glucans resulted in phosphorylation of ATF7, which led to the release of ATF7 from the chromatin and a decrease in repressive histone H3K9me2 marks on inflammatory genes (138). Overall, this study reveals a novel part of the mechanism by which training increases proinflammatory gene expression.

Genetic Variation

Besides differences in transcriptional programs driven by environmental stimuli, functioning of the epigenetic landscape in monocytes and macrophages in humans may also be determined by individual genetic differences. Such differences, for instance, in LDTF or SDTF binding motifs, might be causing different epigenetic landscapes between humans. The impact of common genetic variants in the human population on macrophage function is largely unknown, but it has been the topic of several interesting recent studies. Until recently, research focused mainly on genetic variation in coding regions of the genome, while it is known that the majority of GWAS loci are actually in noncoding DNA regions (54). For example, in T cells, it was recently shown that 90% of causal autoimmune disease variants are noncoding and 60%

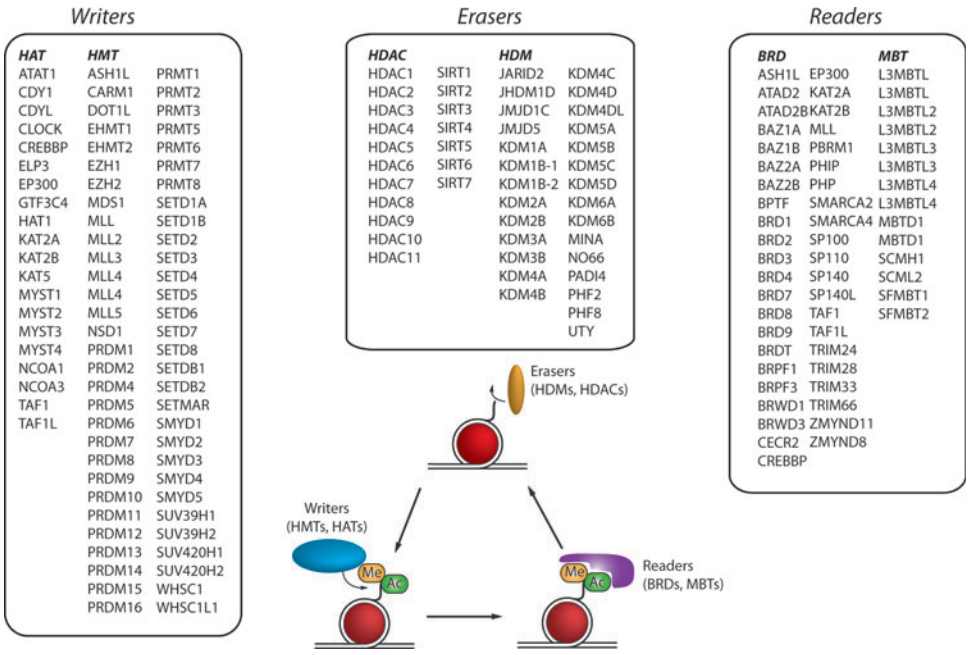

FIG. 6. Epigenetic enzymes regulating chromatin accessibility. Epigenetic marks on histone tails are placed by writers, such as HATs and HMTs. The marks are removed by erasers, such as HDACs and HDMs. Reading of the marks is performed by readers, including BRD and MBT domain proteins. For each family, an overview of the known enzymes is provided based on Arrowsmith *et al.* (3). BRD, bromodomain; HAT, histone acetyl transferase; HDAC, histone deacetylase; HDM, histone demethylase; HMT, histone methyltransferase; MBT, malignant brain tumor. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

of causal variants mapped to enhancer elements (34). This implies that phenotypic and disease-causing consequences of such variation are largely due to effects on regulation of gene expression through changes in enhancer function.

Both promoters and enhancers contain DNA motifs that are recognized by specific transcription factors. Promoter regions are mainly bound by general SDTFs, while enhancers are driven by LDTFs. Farh *et al.* (34) presented an excellent example of how genetic variation in an enhancer results in altered transcription factor binding and associates with disease. The Crohn's disease-associated variant, rs17293632 (C>T), is exactly located in an AP-1 consensus site. This motif-disrupting single-nucleotide polymorphism (SNP) is found in an intron of *SMAD3*, which encodes a transcription factor downstream of TGF-β with pleiotropic roles in immune homeostasis. The SNP disrupts a conserved AP-1 site, suggesting that rs17293632 may increase Crohn's disease risk by directly disrupting AP-1 regulation of the TGF-β–SMAD3 pathway (34).

Using natural genetic variation in mice, it was recently shown that the genetic differences in especially binding elements for LDTFs in enhancer regions are key determinants of inflammatory responses of macrophages and, for instance, strongly influence NF-κB responses (53).

Genetic differences between patients in noncoding DNA may form a new approach to identify patients who are most or least likely to respond favorably to treatment (49). As a proof of concept, it was recently shown that genetic variants in noncoding regions alter the effect of the antidiabetic drug, rosiglitazone (117). Differences in genetically determined binding of peroxisome proliferator-activated receptor γ (PPARγ) to regulatory enhancer sites account for mouse strain-specific transcriptional effects of the antidiabetic drug, arguing for personalized medicine related to nuclear receptor genomic occupancy. In addition, in humans, SNPs determining genomic binding of PPARγ are associated with changes in nearby genes and metabolic phenotypic differences (117), indicating that natural genetic variation determines individual disease risk and drug response.

Epigenetic Enzymes in Monocyte and Macrophage Function

Epigenetic regulators can be divided into three groups based on their function: writers, erasers, and readers. Epigenetic writers lay down epigenetic marks; these marks are removed by epigenetic erasers and recognized by epigenetic readers (32), as illustrated in Figure 6. These enzymes are important targets for intervention as they regulate gene expression by altering the epigenetic status of promoters and enhancers. In this study, we will give an overview of the different classes of epigenetic enzymes and, when applicable, their role in monocytes or macrophages.

Epigenetic Writers

DNA methyltransferases

As discussed previously, DNMTs methylate cytosines at CpG sites in the DNA, resulting in closed chromatin, thus silencing genes. In mammals, three active DNMTs have been identified, DNMT1, DNMT3A, and DNMT3B. In an obesity mouse model, it was found that DNMT3B is lower in AAMs than CAMs. Moreover, DNMT3B knockdown resulted in an AAM phenotype and suppressed macrophage inflammation. DNMT3B is involved in methylating and thereby silencing CpG sites at the promoter of *Pparg1*, a key regulator of the alternative macrophage phenotype (135). Inhibiting general DNA methylation by 5-aza-2-deoxycytidine suppresses inflammation and ameliorates atherosclerosis in mice. Treatment with this compound resulted in demethylated liver-x-receptor α (LXRα) and PPARγ1 promoters, which are both enriched with CpG sites, resulting in overexpression of LXRα and PPARγ. These two mediators are known to exert both anti-inflammatory and atheroprotective effects (17).

Histone methyltransferases

Three families of enzymes have been identified that catalyze the addition of methyl groups donated from S-adenosyl methionine to histones. The SET domain-containing proteins and DOT1-like proteins have been shown to methylate lysines, and members of the protein arginine N-methyltransferase family have been shown to methylate arginines (47). Depending on their target, methylation marks can be associated with both gene activation and repression. Combinations of repressive marks with activation marks can also exist next to each other. For instance, regions in the DNA positive for H3 lysine 27 methylation (H3K27Me, a repressive mark) can contain H3 lysine 4 methylation-positive regions (H3K4Me, an activation mark). When they are present together, they appear to have a role in poising genes for transcription (12).

In monocytes and macrophages, several of these HMTs have been studied. The expression of the H3K4 HMT, myeloid lymphoid leukemia 1 (MLL1), is upregulated by the combination of LPS and IFNγ stimulation in human macrophages. MLL1 was found to be involved in the regulation of inflammatory mediator production, particularly CXCL10 (66). Another H3K4 methyltransferase, MLL4, was required for the expression of *Pigp*, responsible for loading proteins on the cell membrane, including CD14, the coreceptor for LPS and other bacterial molecules. MLL4 deletion in mouse macrophages thereby results in a decrease in LPS-induced signaling and gene expression (6). H3K4 methyltransferase, Ash1l, was shown to suppress IL-6 and TNF production in activated macrophages indirectly through regulating the expression of NF-κB regulating protein A20, protecting mice from sepsis (129). H3K4 methyltransferase, SET7, is also involved in inflammatory signaling as gene silencing of SET7 in human THP-1 monocytes inhibited TNF-induced inflammatory gene expression and H3K4 methylation on the promoters of affected genes (77). As a follow-up on this study, it was found that Set7-induced epigenetic changes contribute to vascular dysfunction in patients with type 2 diabetes. It was shown that patients with type 2 diabetes showed Set7-dependent monomethylation of H3K4 on the NF-κB p65 promoter. This epigenetic signature was associated with upregulation of NF-κB and subsequent increased transcription of oxidative stress and inflammatory genes (95).

Smyd2 is an HMT that can methylate both H3K4 and H3K36. In mouse macrophages, it was found that Smyd2 specifically facilitates H3K36 dimethylation at *Tnf* and *Il6* promoters to suppress their transcription (130).

In mouse macrophages, H3K9 HMT, Setdb2, represses the expression of the *Cxcl1* gene and other genes that are targets of the transcription factor NF-κB. In the absence of Setdb2, mice exhibited increased infiltration of neutrophils during sterile lung inflammation and were less sensitive to bacterial superinfection after infection with influenza virus (108). Suv39h2, an HMT that places the repressive mark H3K9me3, was found to methylate H3K9 sites at promoters of inflammatory genes in human monocytes upon treatment with vitamin A (4). Low levels of G9a, an H3K9me2 HMT, are responsible for strong IFN responses in both human and mouse myeloid cells. This is in contrast to other cell types such as fibroblasts and keratinocytes, where G9a-mediated H3K9me2 methylation results in dampened IFN responses (33, 61). G9a was also found to be involved in silencing the *IL1B* promoter in the human THP-1 monocyte cell line (20). Overall, inhibition of HMTs with the broad HMT inhibitor, 5′-methylthioadenosine (MTA), leads to derepression of *Il1b* gene expression during an inflammatory response (122).

Polycomb repressive complexes (PRCs) are complexes that silence transcriptional activity by writing the repressive H3K27me3 mark. The HMTs, Ezh1 and Ezh2, are involved in functioning of PRC2. Silencing of Ezh1, an H3K27 HMT, was shown to suppress TLR-triggered production of cytokines, including IL-6, TNF, and IFN-β, in mouse DCs and macrophages (79). Ezh1 was found to suppress the transcription of *Tollip* by targeting the proximal promoter of *Tollip* and maintaining the high level of trimethylation of histone H3 lysine 27 there. Ezh2 was shown to be recruited to the promoters of Ccl2 and Ccl8 genes in human blood monocytes, resulting in gene silencing by H3K27me3 and thereby controlling the diurnal rhythms of inflammatory ($Ly6C^{hi}$) monocyte numbers (89).

Trimethylated histone H4 lysine 20 (H4K20me3) was found to be another repressive mark for inflammatory gene expression in macrophages. H4K20me3 is deposited at promoters of a subset of inflammatory genes by the HMT, SMYD5, which is part of the NCoR repressor complex. Liver X receptors antagonize TLR4-dependent gene activation by maintaining NCoR/SMYD5-mediated repression. Signal-dependent erasure of H4K20me3 is required for effective gene activation and is achieved by the histone demethylase, PHF2, through recruitment by NF-κB (120).

Histone acetyltransferases

The HATs utilize acetyl CoA as a cofactor and catalyze the transfer of an acetyl group to the ε-amino group of lysine side chains and thereby they neutralize the lysine's positive charge and this action has the potential to weaken the interactions between histones and DNA (9). This leads to more open chromatin, which is associated with increased gene transcription. HATs are known to function in transcription factor complexes. For instance, in NF-κB signaling, it has been shown that the HATs, p300, CBP, and PCAF, are necessary for NF-κB-mediated gene expression (39, 114). The importance of histone acetylation by HATs has been demonstrated for several NF-κB target genes and inhibition of HATs is mainly anti-inflammatory. It is, however, uncertain whether these effects are through epigenetic histone-modifying mechanisms or by targeting other proteins that can also be acetylated (41). Moreover, it was shown that p300 is bound to enhancers controlling LPS-stimulated gene expression in macrophages. In these enhancers, binding sites for LDTF PU.1 coexisted with those for SDTFs such as NF-κB and IRF (40).

Epigenetic Erasers

TET proteins

TET proteins are involved in DNA demethylation and thereby contribute to gene transcription as DNA methylation is repressing transcription. Not much is known about TET proteins in monocytes and macrophages yet. Somatic loss-of-function mutations of Tet2 are frequently observed in patients with myeloid malignancies. Tet2 deficiency in mice delayed HSC differentiation and skewed development

toward the monocyte/macrophage lineage, indicating that Tet2 has a critical role in regulating the expansion and function of HSCs (69). In addition, in human monocytes, it was found that the loss of DNA methylation during differentiation of primary human monocytes was dependent on Tet2 (68). Moreover, Tet2 was found to inhibit *Il6* expression in mouse macrophages (136), but this acts through regulating Hdac2 activity, discussed below.

Histone demethylases

Two families of HDMs have been identified thus far that demethylate methyl-lysines. These are the amine oxidases and Jumonji C (JmjC) domain-containing iron-dependent dioxygenases (47). HDMs remove methyl marks on several lysines. As these histone methylation marks can be both repressive and active, HDMs can be considered as transcriptional activators or repressors.

It was shown that hypoxia induces the expression of Jmjd1a, an H3K9 HDM, both in mouse macrophages *in vitro* (121) and in tumor tissues *in vivo* (93). Although the expression of Jmjd1a was increased, Jumonji enzyme activity was inhibited when oxygen levels decreased in macrophages. This results in a global increase in H3K9 methylation in the cell and more specifically on the promoters of chemokine *Ccl2* and chemokine receptors, *Ccr1* and *Ccr5* (121).

Lysine-specific demethylase 1 (LSD1/KDM1a), which demethylates H3K4 and H3K9, was found to be a crucial epigenetic mediator for the differentiation of several hematopoietic cells, including monocytes and macrophages. In its absence, HSC genes showed increased H3K4 methylation, resulting in a derepression of stem and progenitor cell genes. Failure to silence these genes compromised the maturation of blood cell lineages (65).

In two important articles, De Santa *et al.* showed the importance of H3K27 demethylase Jmjd3 in regulating macrophage phenotypes. Its expression was found to be upregulated by both the bacterial product LPS (29) and the anti-inflammatory cytokine IL-4 (60). As H3K27 methylation is repressive, Jmjd3 can be seen as a transcriptional activator. It was found to contribute to the expression of both inflammatory genes (28) and IL-4 target genes (60, 107) in mouse macrophages. Interestingly, Ishii *et al.* showed that helminth-induced anti-inflammatory macrophages (*i.e.*, M2 macrophages) are regulated by reciprocal changes in activating H3K4 methylation and Jmjd3-mediated repressive H3K27 methylation (60). Using Jmjd3-deficient mice, Satoh *et al.* found that the Jmjd3 is essential for induction of IRF4-dependent bone marrow macrophage M2 differentiation and polarization (107). In addition, inflammatory cytokine induction by the acute phase protein, serum amyloid A, depends on Jmjd3 as silencing of Jmjd3 expression significantly inhibited SAA-induced expression of proinflammatory cytokines (133). Supporting the role of Jmjd3 in the inflammatory gene transcription program, a Jmjd3 and Utx inhibitor also reduces LPS-induced proinflammatory cytokine production by human primary macrophages (72). Whether Jmjd3 exerts these effects through H3K27 demethylation is debatable as Jmjd3 deletion had no (28) or minimal (107) effects on H3K27 methylation of Jmjd3 target genes. Altogether, these studies indicate that Jmjd3 is essential in regulating a wide range of macrophage responses not only to bacterial components but also for differentiation to a protective phenotype.

Histone deacetylases

Hdac enzymes oppose the effects of HATs and reverse lysine acetylation, an action that restores the positive charge of the lysine and can stabilize the local chromatin architecture, consistent with Hdacs being predominantly transcriptional repressors (9). Class I (Hdac1-3 and 8) and II (Hdac4-7, 9, 10) Hdacs are both Zn^{2+}-dependent enzymes. Class I Hdacs generally reside in the nucleus, except for Hdac3, which can shuttle between the nucleus and cytoplasm, while class II Hdacs are found both in the cytoplasm and nucleus. Class IV has only a single member, HDAC11, while class III (referred to as sirtuins) Hdacs require a specific cofactor for its activity, NAD^+ (112).

Hdac inhibition, in general, is considered anti-inflammatory as broad-spectrum Hdac inhibitors reduce the inflammatory cytokine production in both monocytes and macrophages in response to various inflammatory stimuli (31, 75, 76, 124). These effects are probably independent of direct effects on histone acetylation, but rather due to indirect effects or effects on the acetylation status of other proteins (22, 71). Some specific Hdacs have been studied in more detail in macrophages.

Hdac 1 and 2 were shown to be involved in the IFNβ response during gamma-herpes virus infection. Expression of Hdac1 and 2 was required for IRF3 activation and accumulation of IRF3 at the *Ifnb* promoter in infected primary mouse macrophages (83). Hdac2 was shown to be recruited by Tet2, involved in DNA demethylation, and specifically represses IL-6 expression in dendritic cells (DCs) and macrophages (140).

Hdac3-deficient mouse macrophages are unable to activate a large part of the inflammatory gene expression program, of which the biggest proportion depends on the autocrine IFN-β/STAT1 activation loop (19). Moreover, Hdac3-deficient macrophages were found to be hyper-responsive to the AAM skewing cytokine IL-4 (84). We recently described a previously unrecognized role of Hdac3 in regulating the atherosclerotic phenotype of macrophages. We found that myeloid Hdac3 deficiency promotes collagen deposition in atherosclerotic lesions and thus induces a stable plaque phenotype. The profibrotic phenotype was directly linked to epigenetic regulation of the *Tgfb1* locus upon *Hdac3* deletion. The absence of Hdac3 increased histone acetylation at the *Tgfb* promoter, leading to increased TGF-β production driving smooth muscle cells to increased collagen production. Moreover, in humans, *HDAC3* was the sole Hdac upregulated in ruptured atherosclerotic lesions, Hdac3 associated with inflammatory macrophages, and *HDAC3* expression inversely correlated with profibrotic *TGFB1* expression (57). Besides its effects on inflammation and fibrosis, we found that Hdac3 deletion resulted in an increase of PPARγ and LXR-dependent gene expression. Hdac3 functions in the NCoR repressor complex, repressing PPARγ and LXR responses in the absence of ligands. The absence of Hdac3 derepressed these PPARγ and LXR genes, resulting in less lipid accumulation in the macrophage. Overall, Hdac3 is a key regulator of macrophage phenotypes and thus an interesting target for intervention in several diseases.

In contrast, Hdac4 is associated with anti-inflammatory effects as Hdac4 was shown to inhibit NF-κB activity over proinflammatory genes. In the absence of myeloid Hdac4, more proinflammatory cytokines are expressed in adipose tissue, resulting in insulin resistance and obesity (80). In addition, in human monocyte-derived DCs, Hdac4 was found to be involved in anti-inflammatory effects as it positively regulates the activity of STAT6 and expression of anti-inflammatory genes (134).

Hdac5 was associated with a proinflammatory macrophage phenotype as Hdac5 overexpression in RAW264.7 cells significantly elevated secretion of TNF and other inflammatory mediators (98); the same accounts for Hdac7 (113) in RAW264.7 cells. In addition, pharmacological inhibition of Hdac6 was shown to decrease the inflammatory potential of macrophages *in vitro* (132) and to improve survival in a sepsis model (78).

In mouse macrophages, Hdac9 was found to be upregulated during macrophage differentiation (16). Interestingly, a GWAS study identified an *HDAC9* variant to be associated with ischemic stroke (11). Mouse macrophages lacking Hdac9 express less inflammatory genes and show increased expression of genes involved in lipid handling. In the absence of Hdac9, histone acetylation at the *Pparg1*, the *Abca1*, and the *Abcg1* locus increases, resulting in enhanced cholesterol efflux and decreased lipid accumulation in macrophages (16).

Epigenetic Readers

Epigenetic readers contain bromodomains, malignant brain tumor domains, chromodomains, and tudor domain proteins. The latter three groups of enzymes recognize histone methyl marks, but are to our knowledge not yet studied in monocytes or macrophages. Bromodomain and extraterminal (BET) proteins are important epigenetic readers of histone acetyl marks. Blockade of the recruitment of BET proteins to acetylated histones suppresses BET-mediated transcription. The BET family is a distinct group of bromodomain proteins that includes BrdT, Brd2, Brd3, and Brd4. BET proteins recruit P-TEFb to the promoter, which in turn phosphorylates RNA polymerase II, leading to RNA elongation (136). LPS-induced gene expression in macrophages is generally associated with increased histone acetylation on proinflammatory genes. It was observed that Brd2 and Brd4 are associated with LPS-induced genes (10). Synthetic, acetylated histone mimics, such as I-BET151, inhibit the expression of inflammatory secondary response genes in mouse macrophages. The effects of BET inhibition were selective to a subset of genes and leave other important inflammatory mediators such as TNF unaffected. Besides hypoinflammatory effects *in vitro*, I-BET151 also protects mice in LPS-induced endotoxic shock and bacteria-induced sepsis (90). Similar observations were made when studying the effects of JQ1, another BET inhibitor, on type I interferon-induced gene expression. Brd4 was found to be recruited to IFN-stimulated genes (ISGs) after IFNβ stimulation; Brd4 then recruits P-TEFb to initiate RNA elongation. JQ1 represses the IFN-induced gene expression by inhibiting Brd4-acetyl histone binding (97, 100). Besides, in acute inflammatory models, BET inhibition was found to be protective in autoimmune disease by dampening the production of proinflammatory cytokines in T cells (8, 81). Moreover, BET inhibition improves survival in cancer mouse models by inhibiting cell proliferation (26, 30, 36, 143).

Therapeutics and Future Perspectives

The regulation of monocyte-to-macrophage differentiation and activation is tightly regulated by histone-modifying enzymes, leads to major changes in the epigenetic landscape, and can be beneficially altered by targeting epigenetic enzymes through inhibition of enzymatic activity or gene deletion. Therefore, inhibition of these enzymes may be a therapeutic tool to alter monocyte and macrophage phenotype and control inflammatory processes in human diseases (87). Hdac inhibitors are the most extensively studied epigenetic pharmaceuticals and are currently thoroughly tested in clinical trials for the treatment of various cancers (3). Additionally, novel and more specific inhibitors have been developed over the last couple of years. For example, both Jmjd3/Utx inhibitors and BET inhibitors, blocking the reading of acetylated histone residues, were shown to impair the inflammatory program (72, 90). Identification of small-molecule epigenetic drugs targeting the inflammatory repertoire in disease is one of the key challenges in the upcoming years. Moreover, there is a need of cell-specific targeting of these epigenetic inhibitors. Epigenetic enzymes function in different transcription factor complexes, depending on the cell type and function, and inhibition may therefore lead to diverse effects in various cell types. Hdac3 exemplifies this in atherosclerosis. Targeting Hdac3 in macrophages was shown to be beneficial for atherosclerosis outcome (57), while deletion in endothelial cells worsens outcome (139).

Technology is making it increasingly possible and affordable to characterize the epigenome in low cell numbers and on a larger scale. It will be very interesting to study how changes in the epigenome associate with disease. It was recently shown that monocytes isolated from patients with systemic lupus erythematosus (SLE) show clear changes in their enhancer landscape and that these changes particularly occur in regions associated with interferon responses, a critical hallmark of the inflammatory profile in SLE and a target for intervention in this disease (115). It is expected that many research programs will focus on comprehensive mapping of the epigenome in human disease to better understand disease development and possibly to identify specific epigenetic markers for disease, disease stages, and effectiveness of interventions. The epigenome turns out to be very dynamic and is highly influenced by environmental or lifestyle factors, such as smoking or excessive food consumption. This puts epigenetic processes at the interplay between genetic susceptibility and environment. Over the past few years, studies have focused on how external factors change the epigenetic landscape and we are now beginning to understand the consequences for cellular functioning.

The way genetic variation contributes to differences in epigenetic regulation and cellular responses and consequent susceptibility to disease has been greatly underappreciated. Exome sequencing focused mainly on variation in coding regions of the genome. The majority of GWAS loci, however, are located in noncoding DNA (54). Future genetic studies will focus more and more on genetic variation in enhancers as this will highly contribute to unraveling the molecular mechanisms underlying disease. In autoimmune disease, the

majority of causal disease variants mapped to enhancer-like elements (34), which implies that phenotypic consequences of such variation are largely due to effects on regulation of gene expression. Recent studies in monocytes and macrophages showed that genetic variation in enhancer elements highly contributes to enhancer activity, gene expression, and disease outcome (35, 53). Moreover, genetic variants in enhancer regulatory regions alter the effectiveness of the antidiabetic drug, rosiglitazone (117), indicating that genetic variation determines individual disease risk and drug response. New research studies in the coming years should lead to a better understanding of mechanisms by which genetic variation in enhancer regions influences disease risk and identification of the pathways that are regulated by specific enhancers. Overall, the recent acceleration of technological developments for genome analysis has allowed major improvements on our insights into the epigenetic repertoire underlying functioning of monocytes and macrophages in health and disease. Targeting epigenetic processes and identifying epigenetic profiles that underlie pathology may offer great new opportunities for enhanced patient stratification, personalized medicine, and innovative new approaches for treatment of disease.

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Abbreviations Used

AAMs = alternatively activated macrophages
BCG = Bacille Calmette-Guérin
BET = bromodomain and extraterminal
BRD = bromodomain
CAMs = classically activated macrophages
ChIP = chromatin immunoprecipitation
CMP = common myeloid progenitor
CSF-1 = colony-stimulating factor 1
DC = dendritic cell
DNMT = DNA methyltransferase
eRNA = enhancer RNA
GMP = granulocyte–monocyte progenitor
GWAS = genome-wide association study
HAT = histone acetyltransferase
HDAC = histone deacetylase
HDM = histone demethylase
HMT = histone methyltransferase
HSC = hematopoietic stem cell
IFNγ = interferon γ

Abbreviations Used (Cont.)

IL-10 = interleukin 10
IRF = interferon regulatory factor
ISGs = IFN-stimulated genes
LDTF = lineage-determining factor
LPS = lipopolysaccharide
LSD1 = lysine-specific demethylase 1
LXR = liver-x-receptor
MBT = malignant brain tumor
MLL1 = myeloid lymphoid leukemia 1
MTA = 5′-methylthioadenosine
NF-κB = nuclear factor κB
PPARγ = proliferator-activated receptor γ
PRC = polycomb repressive complex
PRMT = protein arginine N-methyltransferase
SAM = S-adenosyl methionine
SDTF = signal-dependent transcription factor
SLE = systemic lupus erythematosus
SNP = single-nucleotide polymorphism
STAT = signal transducer and activator of transcription
TET = ten-eleven translocation
TGFβ = transforming growth factor β
TLR = Toll-like receptor
TNF = tumor necrosis factor
TSS = transcription start site