Published in final edited form as:
Trends Immunol. 2013 May ; 34(5): 216–223. doi:10.1016/j.it.2012.11.001.

Epigenetic regulation of macrophage polarization and function

Lionel B. Ivashkiv
Arthritis and Tissue Degeneration Program, Hospital for Special Surgery, Immunology and Microbial Pathogenesis Program, Weill Cornell Graduate School of Medical Sciences, New York, NY 10021, USA

Abstract

Macrophage polarization refers to development of a specific phenotype important for tissue homeostasis or host defense in response to environmental cues. Environmental factors that induce macrophage polarization include cytokines and microbial factors produced by pathogens or commensal microbiota. Signaling pathways utilized by these polarizing factors have been well characterized, but it is less clear how signals are converted into complex and sustained patterns of gene expression, and how macrophages are reprogrammed during polarization to alter their responses to subsequent environmental challenges. Emerging evidence, reviewed here, suggests an important role for epigenetic mechanisms in modulating and transmitting signals during macrophage polarization and reprogramming. Deeper understanding of epigenetic regulation of macrophage phenotype will enable development of gene-specific therapeutic approaches to enhance host defense while preserving tissue integrity and preventing chronic inflammatory diseases.

Keywords

chromatin; epigenetics; macrophage; signaling; transcription

Introduction

Macrophages are innate immune cells present in most tissues. Under physiological conditions, macrophages promote tissue homeostasis by producing trophic factors, clearing debris, and preventing excessive inflammation in response to environmental stresses [1]. Infection or tissue injury activates macrophage host defense functions that include microbial killing and production of cytokines and chemokines. Activated macrophages polarize towards various functional phenotypes depending on the pathogen and cytokines expressed in the microenvironment (reviewed in [2–5]). The best characterized macrophage activation phenotypes are classical activation (also termed M1) induced by interferon (IFN)-γ and microbial products such as Toll-like receptor (TLR) ligands, and alternative activation (M2) induced by the T helper (Th)2 cytokines interleukin (IL)-4 and IL-13. M1 macrophages are effective at host defense and clearing pathogens, and M2 macrophages are important for resolution of inflammation and tissue repair. The classical M1 and M2 activation phenotypes represent two ends of a functional spectrum of macrophage polarization states that are

Corresponding author: Ivashkiv, L.B. (ivashkivl@hss.edu).

Note added in proof

Epigenetic marking of human monocytes with increased H3K4me3 after vaccination with bacille Calmette-Guerin has been recently demonstrated in Kleinnijenhuis, J. et al. (2012) *Proc. Natl. Acad. Sci. U.S.A.* 109, 17537-17542.

induced by multiple factors and are characterized by expression of transcriptional modules that underlie specialized functions [2–5].

The cytokines, signaling pathways, and transcription factors that induce macrophage polarization states have been extensively studied and previously reviewed [2–5]. Recently it has become clear that the signaling pathways and transcription factors important for macrophage polarization induce epigenetic changes, as exemplified by alterations in chromatin states [6–8]. Conversely, the epigenetic landscape established during macrophage development guides and limits the impact of signaling pathways and transcription factors, thus determining the pattern of gene expression and functional outcome [9]. Accordingly, emerging evidence has revealed an important role for epigenetic modulation of chromatin states in regulating macrophage polarization. Herein I review mechanisms of epigenetic regulation of macrophage polarization and the functional consequences for macrophage gene expression and phenotype.

Polarizing factors and macrophage phenotypes

Macrophage polarization states are defined by the inducing stimulus and by the ensuing patterns of gene expression, which determine function ([2–5] and Table 1). *In vivo*, macrophage phenotype is heterogeneous, and multiple polarization states have been described; it is useful to conceptualize these states as existing on a spectrum of overlapping phenotypes and gene expression patterns related to the original classification of M1 and M2 [4,5,10]. Thus, various M1-like macrophages [induced by IFNs, granulocyte macrophage colony-stimulating factor (GM-CSF), lipopolysaccharide (LPS), and other microbial products] are effective at killing microbes and producing inflammatory cytokines, but have the potential to cause toxicity and collateral tissue damage. Core aspects of the M1-like group of phenotypes are high expression of key M1 effector molecules, such as the cytokines tumor necrosis factor (TNF), IL-1, and IL-12, antimicrobial molecules, reactive oxygen and reactive nitrogen intermediates, and IFN-induced genes such as the Th1-recruiting chemokines CXCL9 and CXCL10 [4]. By contrast, M2-related macrophages [induced by IL-4/13, IL-10, transforming growth factor (TGF)-β, glucocorticoids, and immune complexes] promote tissue function under physiological conditions, preserve function during times of stress, restrain and resolve inflammation after infection or injury, and promote repair and wound healing. Core genes expressed by M2 macrophages include scavenger receptors, growth factors [heparin binding epidermal growth factor (HB-EGF) and insulin-like growth factor (IGF)], Th2 chemokines (CCL18 and CCL22), and suppressors of inflammation and immunity such as IL-10 and indoleamine 2,3-dioxygenase (IDO) [2].

Polarization of macrophages in response to various signals [2–5,10] is summarized in Table 1. One emerging concept is that polarization along the lines of M1/M2 activation that occurs during infection also occurs under homeostatic conditions or during stress. Thus, macrophage colony-stimulating factor (M-CSF), which is systemically expressed and broadly required for macrophage differentiation and survival, skews macrophages towards an M2-like phenotype, thereby dampening inflammatory activation and suppressing inappropriate responses to nondangerous environmental stressors [11,12]. Physiological M2-like phenotypes can be induced by microenvironmental factors in a tissue-specific manner (Table 1). For example, studies using mouse models have shown that IL-10 restrains macrophage inflammatory responses to microbial flora in the bowel [13], eosinophil-derived IL-4 suppresses M1 cytokine production in adipose tissue (thereby preserving insulin sensitivity and preventing obesity and metabolic syndrome) [14], cold-stress-induced IL-4 promotes M2-mediated adaptive thermogenesis [15], and receptor activator of NF-κB ligand (RANKL) suppresses inflammatory cytokine production while inducing osteoclast

differentiation in bone [16]. A recent exciting discovery is that in mice the microbiome, comprising commensal microorganisms that colonize body surfaces, promotes a partial and low-grade M1-like phenotype in macrophages throughout the body, including in lymphoid organs [17,18]. This M1-like phenotype is characterized by IFN-β–signal transduction and activator of transcription (STAT)1 signaling and low level expression of IFN response genes that prime macrophages for augmented responses to infectious challenges. Indeed, in the absence of commensal flora and the attendant M1-like priming of macrophages, mice are more susceptible to infection with lymphocytic choriomeningitis virus (LCMV), murine cytomegalovirus (MCMV), and influenza viruses [17,18]. Overall, under homeostatic conditions, the phenotype and threshold of inflammatory activation of macrophages is finely tuned in a tissue-specific manner.

Macrophage polarization also occurs during resolution of inflammation, and during pathology such as tumor-associated immunosuppression or chronic inflammation in rheumatoid arthritis or lupus nephritis [4,5,19–21] (Table 1). Recent work has highlighted that macrophages *in vivo* can exhibit mixed M1/M2 phenotypes, especially in complex pathological settings such as tumors where they are exposed to several potentially opposing polarizing factors (Table 1) [5]; it is not yet clear if such heterogeneity reflects mixed polarization of individual cells or coexistence of macrophages with distinct phenotypes. In addition, it has become clear that polarization states can evolve and change over time [5,10,22]. For example, in the absence of IFN-γ or GM-CSF, TLR and TNF signaling induces only a transient M1 activation state that rapidly transitions to a tolerant state with M2-like features (Table 1) [5,22]. New factors and pathways that contribute to polarization have been recently identified. Myc has been implicated in M2-like polarization [23], whereas Notch-recombinant-recognition-sequence-binding protein at the Jκ site (RBP-J) signaling induces a subset of M1 genes (IL-12, iNOS) by augmenting translation of transcription factor IFN regulatory factor (IRF)8 [24]. Focused tuning of a TLR-induced transcriptional module that encodes a distinct functional subgroup of the M1 program by a heterologous signaling pathway such as Notch provides a paradigm for sculpting the specificity of M1-like responses.

Importantly, epigenetic regulation for macrophage polarization has also been recently revealed. To date, epigenetic changes have been shown to be crucial for initial inflammatory activation (M1) by TLRs [25–33], induction of an IFN response by the microbiome [18] or LPS [34], transition from an M1 to a tolerant/M2-like phenotype or to a tolerant dendritic cell (DC) phenotype after TLR or TNF stimulation [35–39], inhibition by IL-10 [13], M2 polarization by M-CSF and IL-4 [40–42], and polarization towards the osteoclast pathway by RANKL [43]. Epigenetics has helped us understand how specific patterns of gene expression are established, how transient signals are transformed into more sustained patterns of polarized gene expression, and how the epigenetic landscape of a cell, which reflects its history of differentiation and previous environmental stimulation, determines the functional outcome of subsequent environmental challenges. Epigenetically conferred transcriptional memory provides the molecular basis for integration of various polarizing signals into a coherent phenotype, and for reprogramming of macrophages for altered responses to subsequent environmental challenges.

Epigenetics

Epigenetics refers to developmentally or environmentally induced modifications that do not alter the genetic code but instead control how information encoded in DNA is expressed in a tissue- and context-specific manner [44]. Epigenetic mechanisms are typically mediated by post-translational modifications (such as methylation, acetylation, and phosphorylation) of histones and other chromatin proteins that bind DNA, by methylation and

hydroxymethylation of CpG DNA motifs, and by noncoding RNA [45–49]. Epigenetic marks have traditionally been considered to be stable, potentially transmissible to progeny, and to underlie stable differentiation into various tissues and cell types that express markedly different patterns of gene expression, despite containing identical DNA sequences and genomes. Recently it has become clear that epigenetic chromatin marks are dynamically regulated in response to environmental cues. This has resulted in a shift in the usage of epigenetics to include transient changes in chromatin and/or DNA methylation in response to external stimuli that control gene expression [44]. Although epigenetic marks are dynamically regulated, they are typically more stable than the rapidly fluctuating post-translational modifications of upstream 'conventional' signaling proteins. Thus, epigenetic modifications that persist after the original stimulus has resolved provide a mechanism for extending transient short-lived signals into a more stable and sustained cellular response lasting several hours or days (or longer).

A paradigm that has emerged is that the 'epigenetic landscape' of a cell (the sum total and pattern of DNA methylation, chromatin modifications, and proteins pre-bound to gene regulatory regions such as promoters and enhancers) determines accessibility for binding and thus the genomic localization of 'signaling transcription factors' [such as nuclear factor (NF)-κB and STATs] that are activated by acute signals [44,50]. Thus, the pattern of gene expression in response to an environmental stimulus is sculpted by the developmental history of a cell and previous environmental exposures that have shaped the epigenetic landscape. The epigenetic landscape, in turn, can be remodeled in response to acute stimulation and polarizing stimuli. Such remodeling of the epigenetic landscape helps integrate signaling over time and underlies reprogramming of cells to alter their gene expression responses to subsequent stimuli.

Investigation of the epigenetics of macrophage polarization to date has primarily focused on post-translational modification of histones, with more limited analysis of ATP-dependent nucleosome remodeling. There is a multitude of histone modifications, which can be broadly divided into positive and negative marks that promote or suppress transcription, respectively [45,49,50]. Table 2 shows the most widely studied marks relevant for macrophage polarization. These histone marks are 'written' and 'erased' by enzymes termed chromatin regulators. The pattern of histone marks forms a sort of 'code' that is 'read' by additional chromatin regulators and transcriptional coactivators/corepressors to determine the rates of transcription initiation and elongation. Thus, the balance of positive and negative histone marks at gene promoters and distal regulatory elements (termed enhancers) regulates transcription rates. Chromatin states, defined by well-established combinations of histone marks, determine basal transcription rates and the magnitude and kinetics by which a gene locus responds to extracellular stimuli [45,49,50]. A key concept is that gene loci relevant for polarized macrophage phenotypes exist in three broad states (Figure 1, top three panels) [7–9]. First, there is a repressed state characterized by the presence of negative marks [histone 3 lysine 9 trimethylation (H3K9me3) and H3K27me3], absence of positive marks, and a closed chromatin conformation (as defined by inaccessibility of DNA that is tightly incorporated into nucleosomes to nucleases). These genes are refractory to acute induction by activating stimuli. Second, there is a poised state characterized by the presence of activating histone marks (H3K4me3, H3K9,14-Ac), chromatin conformation that is at least partially open, and in some genes, a prebound RNA polymerase II (pol II) that is stalled near the transcription start site (TSS). Transcription at poised genes is restrained by simultaneous presence of the repressive histone marks such as H3K9me3 and H3K27me3, corepressor complexes, and partially closed chromatin that requires additional positive histone marks and ATP-dependent nucleosome remodeling to provide full accessibility for transcription factors. Third, there is an active state characterized by active histone marks, an open chromatin configuration, and ongoing transcription.

Acute M1 macrophage activation by TLRs

TLR signaling leads to activation of mitogen-activated protein kinases (MAPK)s, NF-κB and IRFs and induction of downstream genes encoding inflammatory cytokines such as TNF, IL-1β, IL-6, IL12 p40, and chemokine CXC ligand (CXCL)10 that form the core of an acute M1 response [7–9]. Although the detailed mechanisms of activation of these genes are not identical, several common principles concerning epigenetic regulation have emerged [3,6,8,9,44] (Figure 1). An epigenetic landscape at these gene loci is established during macrophage differentiation whereby master transcription factors such as PU.1 and CCAAT/enhancer binding protein (C/EBP)α bind to and open the regulatory regions (promoters and enhancers) of these genes [51–54]. Thus, even in resting macrophages, gene promoters are marked by basal permissive histone marks (H3K4me3 and H3K-Ac) and a nucleosome-depleted region upstream of the TSS [25,28,29,31]. Enhancers are marked by PU.1, H3K4me1, and open chromatin, as demonstrated by hypersensitivity to DNase I digestion. There may be basal low level transcription, and some genes (such as *Tnf*) are occupied at baseline by pol II that is paused in the vicinity of the TSS. Basal transcription rates can be set by prebound primer factors, including JunB, activating transciption factor (ATF)3, and IRF4, which also may serve as beacons to direct recruitment of additional factors after cell stimulation [55]. In the absence of TLR signaling, inflammatory cytokine gene transcription is restrained (and thus genes kept in a 'poised' state) by gene-specific repressive mechanisms. These include occupancy of gene loci by repressors such as B cell leukemia (BCL)6 and nuclear receptors that recruit corepressor complexes that contain histone deacetylases (HDACs) and histone demethylases that limit the amount of positive histone marks [6,26]. Inflammatory gene loci also contain the negative histone marks H3K9me3 [56–58], H3K27me3 [27,59], and H4K20me3 [33], and chromatin accessibility of genes such as *Il12b* is limited by occlusive positioning of nucleosomes [31]. Furthermore, TLR stimulation results in the release of the aforementioned epigenetic 'brakes', for example dismissal of BCL6 and corepressors from gene loci and concomitant induction or activation of demethylases such as JMJD3, JMJD2d, AOF1, and PHF2 that erase the negative histone marks H3K27me3, H3K9me3, and H4K20me3 [27,33,57,58]. In addition to histone modifications, induction of a subset of genes that includes *Il6* and *Il12b* requires nucleosome remodeling by the ATP-dependent complex BAF (also termed SWI/SNF) [31,32]. This epigenetic remodeling facilitates recruitment of signaling transcription factors such as NF-κB, an increase in positive histone marks such H3S10-P, H4-Ac and H3K4me3, and release of paused pol II [25,29] to promote transcription elongation. Enhancers are also activated, as demonstrated by recruitment of the histone acetyltransferase (HAT) p300, increased histone acetylation, binding of signaling transcription factors, and transcription of enhancer RNA [51–53,60]. Except for induction of histone phosphorylation by MAPK cascades via mitogen- and stress-activated kinases (MSKs) and recruitment of HATs p300/CBP by signaling transcription factors such as NF-κB and STATs, little is known about how TLR-induced signals are propagated to chromatin and histones.

Together, the aforedescribed epigenetic events regulate the magnitude and kinetics of gene induction in response to environmental signals, and provide mechanisms for gene-specific regulation downstream of canonical and relatively invariant signaling pathways. A recent exciting advance is the development of therapeutic compounds that suppress M1 gene induction and inflammation by targeting chromatin regulators. A small molecule inhibitor of bromodomain and extra terminal domain proteins (iBET) that disrupts interaction of BET proteins with acetylated histones selectively blocks expression of a subset of TLR4-induced genes and demonstrates efficacy in mouse models of endotoxin toxicity and polymicrobial sepsis [61]. iBET and related compounds JQ1 and iBET151 strongly suppress Myc expression and also show great promise for the treatment of Myc-driven cancers such as acute myeloid leukemia (AML) and multiple myeloma ([62] and references therein).

Inhibitors of histone demethylase lysine-specific demethylase (LSD)1 may be effective in the treatment of AML [63]. Compounds that inhibit JMJD3 and related ultrathorax (UTX) histone demethylases broadly suppress TLR-induced expression of inflammatory cytokines [59]. HDAC inhibitors, which paradoxically suppress inflammatory gene expression by unknown but likely indirect mechanisms [64], have shown efficacy in a Phase I trial in juvenile inflammatory arthritis [65]. One surprise has been the relative lack of toxicity of small molecule inhibitors of chromatin-regulating enzymes that are broadly expressed and involved in expression of multiple genes. This most likely reflects an intricate network of chromatin regulators that cooperate to fine tune gene expression in a gene-specific manner. Thus, targeting chromatic regulators represents an exciting area of therapeutic development that offers potential for gene-specific and patient-specific therapy. Chromatin marks can be long-lived; therefore, such therapies hold promise for longer-term effects and even induction of remission in patients with chronic inflammatory conditions.

TLR-induced expression of core M1 inflammatory cytokines is transient, and gene expression is rapidly repressed to near baseline levels [22]. In contrast to activation, little is known about mechanisms of gene repression (Figure 1, bottom panel). Nuclear receptors, TLR-induced transcriptional repressors ATF3 and hairy and enhancer of split (Hes)1, feedback inhibitors induced by IL-10, and the p50 NF-κB subunit can recruit corepressor complexes that contain HDACs and histone demethylases and decrease gene expression [6,13,66–70]. However, the precise mechanisms of action of these repressors and how chromatin states are regulated during deactivation of M1 inflammatory genes are not known. Histone marks can be long lived, and the extent to which positive marks are removed, or whether negative marks are installed, during gene repression is not clear. In addition, there is evidence for a repressive role for the nucleosome remodeler and deacetylase (NURD), which presumably shifts nucleosomes to a configuration that limits access of gene loci to transcription factors and general transcriptional machinery [32,71,72]. Interestingly, deactivation of cytokine gene expression is delayed by the M1-promoting cytokines IFN-γ and GM-CSF, which work in part by suppressing expression of transcriptional repressors such as Hes1 [68,73]. Greater understanding of epigenetic mechanisms that mediate repression of inflammatory cytokine gene expression represents an important area for future research that holds promise for development of new therapeutics.

Priming of the M1 state

An important aspect of full M1 activation is that IFNs prime macrophages for augmented and sustained expression of inflammatory cytokine genes in response to pathogen-associated molecular patterns (PAMPs) and inflammatory cytokines. Low homeostatic levels of IFN-β and downstream Janus kinase (Jak)–STAT signaling maintain macrophages in a primed state of increased readiness to respond rapidly and strongly to infectious challenges (reviewed in [74]). Strikingly, recent reports have shown that homeostatic IFN signaling that calibrates macrophage responses to pathogens and inflammatory factors is induced by commensal microbiota [17,18]. Although the microbiome-derived molecules that induce tonic IFN signaling and thus homeostatic priming are not known, it is clear that priming induces increased positive H3K4me3 marks at *Ifnb*, *Il6*, and *Tnf* promoters, which correlates with increased promoter occupancy by NF-κB p65 and pol II upon cell stimulation. Interestingly, poising of IFN response genes is targeted by the influenza A protein NS1, which mimics H3K4-containing peptides and thereby suppresses the positive functions of H3K4me3 by blocking interactions with readers of this epigenetic mark [75]. Another mechanism that primes macrophages and DCs for strong IFN responses is maintenance of low levels of negative H3K9me3 marks at IFN response gene loci, in contrast to G9a-mediated H3K9me3 methylation that dampens IFN responses in fibroblasts and keratinocytes [56]. Overall, these studies help resolve the longstanding mystery of what drives tonic IFN signaling *in vivo*,

explain at least in part why inflammatory cytokine genes are poised for rapid and strong activation, and clearly implicate epigenetic regulation in establishing set points for cytokine production and thus calibrating innate immune responses.

Several TLR ligands and TNF induce an autocrine IFN-β–Jak–STAT loop that is an important component of M1 activation [3,76]. Induction of *Ifnb* and the downstream IFN response is strongly dependent on HDAC3 [34]. Strikingly, in HDAC3-deficient macrophages LPS-induced histone acetylation at the *Ifnb* promoter is almost completely abolished, whereas recruitment of p65 and IRF3 remains intact. This paradoxical effect of diminished histone acetylation in the absence of HDAC3 likely reflects an indirect effect, whereby deletion of HDAC3 derepresses various genes, including *Ptgs1*, whose products repress *Ifnb* [34]. HDAC3 is also required for LPS-mediated induction of additional inflammatory genes, such as *Il6*. Concordantly, HDAC inhibitors suppress induction of various inflammatory and IFN target genes [64]. This requirement for histone deacetylation for inflammatory gene expression is not understood, and may reflect either indirect effects or a requirement for a cycle of coupled histone acetylation and deacteylation for efficient gene transcription [77].

IFN-γ is the most potent M1-activating cytokine and potentiator of a TLR-induced classical inflammatory activation state. Epigenetic mechanisms by which IFN-γ drives M1 activation have not been clarified. IFN-γ and STAT1 have been implicated in nucleosome remodeling and opening of chromatin [35,78] and may prime formation of new enhancers that augment gene expression (Qiao and Ivashkiv, unpublished). An important area for future research will be to elucidate epigenetic mechanisms utilized by IFN-γ during M1 polarization.

M1 to M2 transition during tolerization

Acute activation of macrophages by TLR ligands or TNF is transient and is followed by a state of tolerance [22]. Tolerant macrophages exhibit a selective defect in the induction of a subset of genes, including inflammatory cytokine genes. By contrast, other genes, for example, those encoding antimicrobial products and select chemokines and M2 products are expressed. Recent work has clarified that epigenetic mechanisms likely explain the gene-specific nature of tolerance [35–39,70,79]. Tolerized genes exhibit decreased chromatin accessibility, as assessed by restriction accessibility assays, and diminished recruitment of transcription factors such as p65. The molecular explanation for diminished accessibility involves decreased TLR-induced recruitment of Brg1-containing nucleosome remodeling complexes, and complex changes in histone acetylation and methylation. By contrast, non-tolerized genes maintain an open chromatin state. The signals that lead to tolerance are not clear, although newly transcribed gene products are important to establish tolerance [36], and glycogen synthase kinase (GSK)3 plays a key role in TNF-induced tolerance [38]. Interestingly, IFN-γ prevents tolerance by preserving expression of the receptor-interacting protein 140 (RIP140) coactivator and promoting TLR-induced chromatin accessibility upon secondary TLR challenge [35,79]. Epigenetic mechanisms that regulate polarization of macrophages to a tolerized state need to be further clarified by genome-wide analysis of chromatin states and enhancers, and identification of key transcription factors and chromatin remodeling complexes that regulate the tolerization process.

M2 alternative activation

Alternative activation of macrophages in response to chitin or helminth infection *in vivo* is mediated by histone demethylase JMJD3, which facilitates expression of the key M2-promoting transcription factor IRF4 by removing negative H3K27me3 marks at the *Irf4* locus [42]. JMJD3 is required for M2 gene expression in M-CSF-cultured bone-marrow-derived macrophages, although genome-wide chromatin immunoprecipitation followed by

high throughput sequencing (ChIPseq) analysis indicated that most M2 genes are not direct targets of JMJD3 [42]. The role of JMJD3 in alternative activation was independently identified in a study showing that JMJD3 expression is induced by IL-4 in a STAT6-dependent manner [40]. In that study, ChIP-PCR analysis detected direct JMJD3 binding at M2 genes such as *Chi3l3*, *Retnla*, and *Arg1*. These differences in the exact mechanism of JMJD3 action are not mutually exclusive and may reflect differences in sensitivity of experimental approaches and culture conditions. Interestingly, JMJD3 is also important for increasing nuclear factor of activated T cells (NFAT)c1 expression and thus promoting RANKL-induced differentiation down the alternative osteoclast pathway [43]. Overall, these studies clearly implicate JMJD3 in M2/alternative polarization, while suggesting a lesser role in M1 activation. By contrast, HDAC3 acts as a brake on IL-4-induced M2 polarization by deacetylating putative enhancers of IL-4-induced M2 genes [41]. Thus, both histone methylation and acetylation are important for M2 polarization.

Outstanding questions

It has become clear that epigenetic modification of chromatin plays an important role in macrophage polarization and function. Pre-existing chromatin marks deposited during macrophage differentiation interpret, calibrate, and transmit environmental signals to determine the magnitude and specificity of gene expression, and thus macrophage phenotype. One interesting question relates to the stability of epigenetic marks induced during macrophage activation, and the impact of epigenetic changes on macrophage responses to subsequent changes in the environment. At the least, epigenetic mechanisms stabilize transient signals into a polarized phenotype that is sustained over several days, which may correspond to the lifespan of many macrophages *in vivo*. However, macrophage phenotypes exhibit considerable plasticity and evolve in response to changes in the environment [5,10,80]. Epigenetic marks can slow down such shifts in phenotype, and evidence reviewed here supports the exciting hypothesis that epigenetic changes fundamentally reprogram macrophages to exhibit altered gene expression programs in response to environmental stimuli. Such reprogramming would allow transcriptional memory to shape macrophage phenotype in response to environmental changes, and may contribute to the complex mixed M1/M2 phenotypes that have been observed *in vivo*. An additional important question is whether chromatin regulators impart specificity to polarization by gene-specific regulation, or whether they broadly open chromatin to facilitate the function of ‘master transcription factors’ that instruct polarization in response to specific signals. The congruent functions of HDAC3 in promoting M1-like IFN responses while suppressing IL-4-induced M2 polarization indicates a role for this chromatin regulator in imparting polarization specificity. By contrast, JMJD3 is important for M2 polarization, but also promotes M-CSF- and RANKL-induced osteoclast differentiation and, together with related UTX, is required for effective induction of multiple M1 genes by LPS. These roles of JMJD3 are not necessarily paradoxical, but may reflect a function of broadly opening chromatin to facilitate responses to environmental factors such as IL-4, M-CSF, RANKL, and LPS that specify polarization.

A key question in the field is which of the multitude of histone marks play an important functional role in determining gene expression. Although biochemical, pharmacological and genetic studies have demonstrated important functions for several chromatin regulators, it is possible that many histone marks are deposited as a consequence of transcription factor binding and have limited functional significance. Transcription factors that bind to promoters and enhancers play a key regulatory role, but binding of many signaling transcription factors is more dynamic than turnover of chromatin marks [55], which thus can extend and stabilize signals. Cooperation and reciprocal interactions between transcription factors that bind gene loci in a DNA sequence-specific manner and various coactivators,

corepressors, and chromatin regulators that are recruited to regulatory sites to modify chromatin will determine gene expression patterns and macrophage phenotype.

Concluding remarks

Investigation of epigenetic regulation of macrophage polarization and function is at an early stage and there are many exciting areas for future research. It will be important to explore additional epigenetic mechanisms, such as DNA (hydroxyl)methylation and the role of miRNA and various classes of noncoding RNA, and to gain insights into the role of enhancers and 3D chromatin conformation. In addition, it will be important to extend epigenetic analysis to human macrophages, which differ in several important aspects from mouse macrophages [10,81]. This includes investigation of the role of epigenetic regulation of macrophages in disease states, particularly in sustaining chronic inflammation and in mediating the interactions of genes and environment that lead to disease. Interestingly, many single nucleotide polymorphisms associated with autoimmune/inflammatory diseases occur in regulatory regions that are subject to epigenetic regulation [45,54,82,83,84]. Finally, recent breakthroughs have opened a new door to epigenetic therapy of cancer and inflammatory diseases, which has the potential to be gene-specific while exhibiting sustained effects that hold promise for inducing long-term disease remission.

Acknowledgments

This work was supported by grants from the NIH. I thank Laura Donlin, Xiaoyu Hu, Sung-ho Park and Inez Rogatsky for reading the manuscript.

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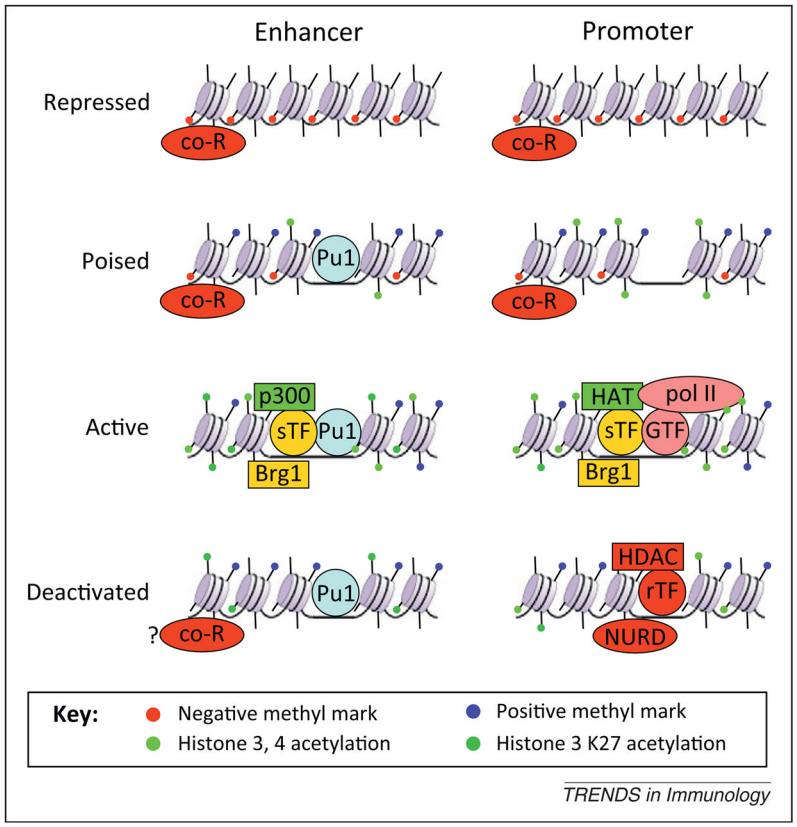

Figure 1.

Epigenetic regulation of inflammatory cytokine gene loci in macrophages. **(a)** In cells that do not express inflammatory cytokines, corresponding gene loci exhibit inaccessible chromatin, occupancy by transcriptional repressors and corepressors (co-R), and negative histone marks. **(b)** During macrophage differentiation master transcription factors (also termed pioneer factors) such as Pu1 bind to cytokine gene promoters and enhancers and facilitate the opening of chromatin (as determined by DNase I hypersensitivity) by nucleosome remodeling and histone acetylation, and promote positive methyl marks (H3K4me3 at promoters and H3K4me1 at enhancers). Genes are maintained in a poised state of low or nonproductive basal transcription but high responsiveness to extracellular stimuli by a balance between positive and negative epigenetic marks. **(c)** Stimulation of macrophages by Toll-like receptor (TLR) ligands leads to release of corepressors, increased histone acetylation, additional nucleosome remodeling by Brahma-related gene (Brg)1 (as determined by restriction enzyme accessibility assays), and recruitment of signaling transcription factors (sTFs) such as nuclear factor (NF)-κB. This results in increased recruitment of general transcription factors (GTFs) and RNA polymerase II (pol II), and active transcription. Enhancers of active genes are characterized by occupancy by p300, H3K27-Ac, and low levels of transcription of noncoding enhancer RNA. **(d)** Mechanisms by which inflammatory genes are deactivated are not well understood, but include occupancy by transcriptional repressors (rTFs), decreases in histone acetylation mediated by histone deacetylases (HDACs), and nucleosome remodeling by the nucleosome remodeling and deacetylation (NURD) complex that also contains HDACs. This figure represents a composite; regulation of individual genes varies and gene-specific mechanisms are not depicted.

Table 1

Macrophage polarization at various tissue sites during homeostasis and after activation[a,b]

	Inducing factor	Site	Phenotype	Transcription factor	Epigenetic regulation
Infectious challenge ('activation')	IFN-γ + TLR	Infection	M1	STAT1, NF-κB	
	IFN-γ	Th1 response	$M1^{partial}$	STAT1	
	TLR ligand	Infection	$M1 \rightarrow M2^{tol}$	NF-κB, IRF5, IRF8	H-Ac, BAF, HDAC3, JMJD3, BCL6, NURD
	TNF	Infection	$M1 \rightarrow M2^{tol}$	NF-κB	
	GM-CSF	Th17 response	M1-like	STAT5, IRF5	
	IL-4/13	Th2 response	$M2^{classic}$	STAT6, IRF4	H3K27me3, JMJD3, HDAC3
Homeostasis	M-CSF	Systemic	$M2^{partial}$	IRF4	H-Me, JMJD3,
	Microbiota	Systemic	$M1^{low}$	STAT1/2	H3K4me3
	IL-10	Gut	M2	STAT3, NFIL3	H4-Ac, HDAC3
	IL-4, FA	Adipose	$M2^{low}$	STAT6, PPARγ	
	RANKL	Bone	M2-like→OC	NFATc1	H3K27me3, JMJD3
Resolution	IL-10/IC	Infection	M2	STAT3	
	TGF-β	Infection	M2	Smad	
	IL-4/13	Infection	M2	STAT6	H3K27me3, JMJD3, HDAC3
	GCs	Inflammation	M2	GR, GRIP	
Pathology	Multiple	Joints (RA)	M1-like	STAT1	
	Multiple	Lungs (asthma)	M2-like	STAT6	
	Multiple	Kidneys (SLE)	M1/M2		
	Multiple	Tumors	M1/M2	STAT1, STAT6	

[a]Under homeostatic conditions macrophages are polarized by systemic or tissue-specific factors. Further polarization occurs during macrophage activation in response to infections, immune response factors, or stress, and during subsequent resolution of inflammation and tissue healing. These processes are dysregulated in various disease states. Polarizing factors induce signaling and epigenetic mechanisms that cooperate to determine patterns of gene expression and macrophage phenotype and function.

[b]Abbreviations: Ac, acetylation; FA, fatty acid; GC, glucocorticoid; GR, glucocorticoid receptor; GRIP, glucocorticoid receptor-interacting protein; H, histone; K, lysine; Me, methylation; NFIL, nuclear factor, interleukin-3 regulated; PPAR, peroxisome proliferator-activated receptor; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; Smad, S and mothers against decapentaplegic.

Table 2

Histone marks that promote or suppress transcription[a]

	Function	Location	Writer	Eraser
H3K4me3	+	P	MLL	KDM5B
H3K9,14-Ac	+	P,E	HAT (CBP/p300)	HDAC3
H4K5,8,12, 16-Ac	+	P,E	HAT (CBP, MOF)	HDAC
H3K27-Ac	+	E,P	HAT (CBP)	HDAC1,2
H3K4me1	+	E	MLL	LSD1/KDM1A
H3K9me3	−	E,P	G9a	JMJD2
H3K27me3	−	P,E	EZH2 (PRC2)	JMJD3, UTX

[a]Ac, acetylation; CBP, CREB binding protein; E, enhancer; H, histone; K, lysine; KDM, lysine demethylase; me, methylation; MLL, mixed lineage leukemia; P, promoter; PRC2, polycomb related complex 2.