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Epigenetic Remodeling in Innate Immunity and Inflammation

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Abstract

The innate immune response is a rapid response to pathogens or danger signals. It is precisely activated not only to efficiently eliminate pathogens but also to avoid excessive inflammation and tissue damage. *cis*-Regulatory element–associated chromatin architecture shaped by epigenetic factors, which we define as the epiregulome, endows innate immune cells with specialized phenotypes and unique functions by establishing cell-specific gene expression patterns, and it also contributes to resolution of the inflammatory response. In this review, we focus on two aspects: (*a*) how niche signals during lineage commitment or following infection and pathogenic stress program epiregulomes by regulating gene expression levels, enzymatic activities, or gene-specific targeting of chromatin modifiers and (*b*) how the programed epiregulomes in turn mediate regulation of gene-specific expression, which contributes to controlling the development of innate cells, or the response to infection and inflammation, in a timely manner. We also discuss the effects of innate immunometabolic rewiring on epiregulomes and speculate on several future challenges to be encountered during the exploration of the master regulators of epiregulomes in innate immunity and inflammation.

1. INTRODUCTION

During cell development and following pathogen and inflammatory cytokine stimulation, regional tissue-specific niches establish cell-specific functions of innate immune cells endowed with the ability to sense pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) released from damaged cells. These cells elicit germ line–encoded pattern recognition receptor (PRR)-, C-lectin-, and cytokine-centered signaling pathways, leading to production of various innate immune effectors to eliminate the invading pathogens and the damaged host cells (1–3). Cell-specific functions correspond to cell-specific phenotypes. These phenotypes of innate immune cells are plastic, and the innate immune cells undergo transition from a quiescent to an activated phenotype in response to pathogen infection or danger signals. Following stimulation, the activated innate immune cells are converted to a repressive phenotype to resolve inflammation and prevent tissue damage (4). On the other hand, cytokine priming and pretreatment with special components from microorganisms cause the innate cells to take on a tolerant phenotype or trained phenotype, which is the basis for innate immune memory (5).

There are many pathological conditions associated with dysregulation of the innate immune response, including not only persistent pathogen infection and superinfection but also immunoparalysis and chronic inflammation. During infection, pathogens utilize strategies to dysregulate innate immune defense functions so as to survive and persist within hosts (6). A dysregulated innate immune response and uncontrolled inflammation can result in disease, even leading to host death during infection (7, 8), as recently seen in patients with severe manifestations of coronavirus disease 2019 (COVID-19) who suffered from acute respiratory distress syndrome (9, 10). Moreover, both pathogen infection and sterile inflammation can also train the innate cells to constantly or excessively produce proinflammatory mediators or to be unresponsive, leading to varieties of inflammatory diseases in a tissue-specific manner, such as rheumatoid arthritis, atherosclerosis, and neurodegenerative diseases (11). Furthermore, pathophysiological niche signals can also reprogram the pathological phenotype of innate cells, like tumor-associated macrophages (12). Thus, we may identify targets for treating inflammatory diseases where the innate immune response is dysregulated by revealing the molecular mechanisms for innate effector functions and identifying the regulators of innate immunity and inflammation.

Specific phenotypes of innate immune cells rely on complex cell-specific gene expression patterns regulated at multiple levels, from gene transcription and posttranscription to translation and posttranslation. Furthermore, tissue niche–mediated and infection signal–mediated metabolic rewiring contributes to the establishment of these cellular phenotypes (13). Cell-specific chromatin architecture, which controls the transcription activities of genes, plays essential roles in phenotype determination and functional transformation for innate immune cells. DNA methylation, histone modification, ATP-dependent chromatin remodeling, and chromatin looping, mediated by chromatin modifiers including enzymes and noncoding RNAs [especially long noncoding RNAs (lncRNAs)], are due to the epigenome, which determines chromatin architecture (14).

Unlike the fixed genotype, the chromatin architecture is endowed with plasticity owing to reversible writing and erasing chromatin modifications and regulable chromatin remodeling and looping, which are determined by the expression levels, enzymatic activities, and gene-specific targeting of chromatin modifiers as well as cofactor and substrate availabilities. Here, we define the epiregulome as chromatin architecture–shaping factors, including specific DNA and histone modifications and nucleosome position regulated by chromatin modifier–mediated epigenetic mechanisms. The epiregulome determines the hierarchical organization, accessibility, and activities of

cis-regulatory elements (CREs), such as promoter elements, enhancers, and insulators, which were previously termed the regulome (15–17). Thus, the reversible epiregulome establishes cell-specific gene expression patterns at both the transcription and posttranscription levels, determining the plasticity of innate immune cells in both innate cell development and the innate immune response (**Figure 1**). Therapeutic targeting of chromatin modifiers involved in regulating the innate immunity–specific epiregulome with their specific inhibitors and activators will contribute to the prevention and treatment of inflammation-related diseases.

The two key questions of epigenetic regulation in innate immunity are how tissue microenvironmental niche signals change the epiregulomes and how the changed epiregulomes in turn establish and change the phenotype and function of innate immune cells. In this review, we summarize advances in addressing these two questions in the context of (*a*) niche signal–mediated establishment of innate cell–specific function during cell development and specification and (*b*) infection- and danger signal–mediated phenotypic transition and metabolic rewiring of innate cells during training, initiation, and resolution of inflammatory innate immunity in response to pathogen infection or pathological stresses.

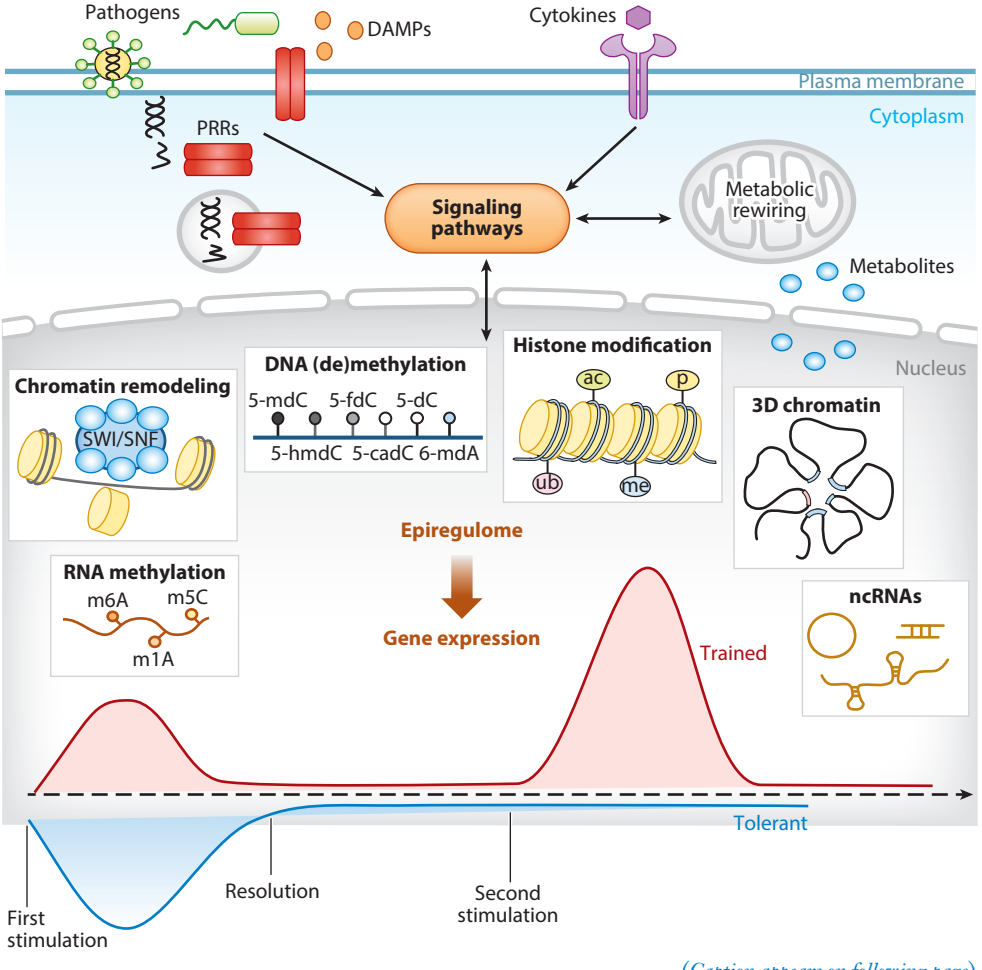

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Figure 1 (*Figure appears on preceding page*)

The cell differentiation– and pathogen infection–programed epiregulome establishes cell-specific gene expression patterns in innate and trained immune responses. During lineage commitment, the regional tissue niche signals establish an innate cell–specific phenotype and function in preparation for pathogen sensing and activation of innate immunity. Infection and sterile triggers activate PRRs and cytokine signaling pathways and initiate the innate immune response. Concomitantly, anti-inflammatory mechanisms are also provoked to properly restrain and resolve inflammation in a timely manner to prevent self-tissue damage. Furthermore, the innate immune system also exhibits adaptive memory evoked by primary insults, which leads to an amplified response (*red curve*) or tolerance (*blue curve*) upon rechallenge after resolution to a naive-like state; this is well-known as trained immunity or endotoxin tolerance. To fulfill the delicate balance of the innate immune response, niche and infection signals and the accompanying metabolic rewiring with changed levels of varieties of metabolites shape an innate immunity–specific epiregulome, including DNA/RNA methylation and demethylation, histone modifications, and multiscale structure of chromosomes in *cis*-regulatory elements. The epiregulome in turn precisely regulates the expression of innate signaling sensors, transducers and regulators, and innate immune effectors at the transcriptional and posttranscriptional levels. Representative DNA methylation and its oxidation forms are 5-mdC, 5-hmdC, 5-fdC, 5-cadC, and 6-mdA. Representative RNA methylation forms are m6A, m1A, and m5C. Representative histone modifications are ubiquitination, acetylation, methylation, and phosphorylation of specific amino acids in histones. Abbreviations: ac, acetylation; DAMP, danger-associated molecular pattern; 5-cadC, 5-carboxyl-deoxycytosine; 5-dC, 5-deoxycytosine; 5-fdC, 5-formyl-deoxycytosine; 5-hmdC, 5-hydroxy-deoxymethylcytosine; 5-mdC, 5-methyl-deoxycytosine; me, methylation; m1A, N^1-methyladenosine; m5C, 5-methylcytosine; m6A, N^6-methyladenosine; ncRNA, noncoding RNA; p, phosphorylation; 6-mdA, N^6-methyl-deoxyadenosine; PRR, pattern recognition receptor; SWI/SNF, switch/sucrose nonfermentable; ub, ubiquitination.

2. BRIEF INTRODUCTION OF THE MAIN ASPECTS OF EPIGENETIC REGULATION AND RECENT ADVANCES

The early definition of the term epigenetics was related to the mechanisms underlying heritable phenotypic change without genotype change: "how gene activity during development causes the phenotype to emerge" (18, p. 396). Thus, epigenetic regulation refers to chromatin adaptation that establishes gene expression patterns and allows them to be inherited. A nucleosome, which consists of 147 bp of DNA wrapped around a histone octamer, is the basic component of chromatin. Beyond sequence-based regulation, the main aspects of epigenetic regulation are dynamic interaction between DNA and histone and their respective modifications and associated molecules.

2.1. DNA Methylation

Most cytosines in the context of CpG sequences in mammalian genomes are methylated in the form of 5-methyl-deoxycytosine (5-mdC), as mediated by DNA methyltransferases (DNMTs) (19). However, some cytosines in non-CpGs are also methylated in stem cells and special tissues such as brain tissue (20, 21). For gene expression regulation, 5-methylcytosines (5-mCs) in CpG islands and the flanking regions and enhancers inhibit chromatin accessibility and silence gene transcription (22). Ten-eleven translocation 1–3 (Tet1–3) proteins oxidize 5-mdC into 5-hydroxy-deoxymethylcytosine (5-hmdC), 5-formyl-deoxycytosine, and 5-carboxyl-deoxycytosine (5-cadC), which act not only as a nexus for DNA demethylation but also as independent modifications for transcription regulation (23, 24). Tet proteins play important roles in both innate and adaptive immunity (25). N^6-Methyl-deoxyadenosine (m6dA) may also exist in mammalian genomes and play regulatory roles, although this is controversial (26, 27). And the production of endogenous m6dA may depend on RNA N^6-methyladenosine (m6A) (28).

2.2. Histone Modification

Varieties of posttranslational modifications (PTMs) decorate histones, especially the N termini of H3 and H4, for epigenetic regulation (29). For example, acetylation of lysine in histone H3 or H4, which is reversibly regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) (30), promotes gene transcription not only by neutralizing the positive charge of the lysine ε-amino group in histone so as to inhibit DNA binding but also by recruiting bromodomain-containing proteins (BRDs). However, functions of acetylation of different lysine sites in histones may differ (31). Acetylation of histone H3 lysine 27 (H3K27ac), together with monomethylation of histone H3 lysine 4 (H3K4me1), defines active enhancers, which interact with promoters through DNA looping to promote gene transcription (32). With the rapid development of analytic mass spectrometry and other techniques, new types of histone modifications along with their readers are being identified, and their potential biological functions are being studied (33, 34).

2.3. Chromatin Organization

Chromatin accessibility is positively or negatively regulated at the nucleosome level, especially by subfamilies of ATP-dependent chromatin remodelers (35). Notably, the switch/sucrose nonfermentable (SWI/SNF) subfamily, which slides and ejects nucleosomes to access local chromatin, is essential for expression of subsets of proinflammatory genes during the response to lipopolysaccharide (LPS) (36). Mechanistically, removal or shift of nucleosomes in enhancers, promoters, and gene bodies is required for DNA binding by a majority of transcription factors and for assembly of large complexes for transcription initiation and elongation, such as the preinitiation complexes and the super elongation complex. These complexes form condensates containing mediators and BRDs through liquid-liquid phase separation of proteins and are observed as dynamic foci using live-cell superresolution microscopy (reviewed elsewhere, such as 37). Furthermore, chromatin can be hierarchically packaged with higher-order organization, such as in chromosome territories and both intrachromosomal and interchromosomal compartments with spatial segregation. As a kind of characteristic chromatin organization, topologically associating domains (TADs) demarcated by CCCTC-binding factor (CTCF) and cohesin mediate internal long-range interaction between CREs for gene transcription regulation, which can be experimentally identified by Hi-C (38).

In addition, the biological functions of other epigenetic regulators have also been revealed in recent years. For instance, lncRNAs can directly mediate epigenetic regulation both in *trans* and in *cis* by interacting with DNA, RNA, or proteins. In some cases, the lncRNA gene locus, but not the lncRNA itself, acts as a CRE to regulate gene transcription (39). Modifications of mRNA, especially m6A and m5C, add another layer of epigenetic regulation at the posttranscription level; the group of such factors is defined as the epitranscriptome (40, 41). All these aspects of epigenetic factors establish a gene-specific epiregulome to regulate the activities of CREs or modulate gene expression at the posttranscription level.

3. NICHE SIGNALS ESTABLISH A CELL-SPECIFIC PHENOTYPE BY PROGRAMING THE EPIREGULOME OF INNATE IMMUNE CELLS

In addition to the skin barrier, innate myeloid and lymphoid cells are key components of the first line of host defense against pathogen infection. Pathogen infection signals can further modulate differentiation of innate immune cells from progenitors in different ways (42, 43). The tissue microenvironmental niche signals precisely direct the development and specification of innate cell

subsets, and even their mutual transition, in a systematic or tissue-specific manner. As for the underlying mechanisms, the establishment and transition of phenotype depend on niche signal–induced reprogramming of the epiregulome in both progenitors and mature cells; this reprogramming is regulated cooperatively by signal-activated transcription factors and chromatin modifiers for setting up cell-specific gene expression patterns (44, 45). Here we focus on the three major innate cell types, macrophages, dendritic cells, and innate lymphoid cells (ILCs), and introduce representative recent discoveries to illustrate how niche signals mediate epigenetic regulation for building innate cell–specific function.

3.1. Macrophage Development and Polarization

Macrophages not only have steady-state function in regulating tissue development but also are a key component of innate immunity because of their copious abilities to secrete inflammatory mediators, regulate activation and polarization of CD4 T cell subsets, ingest pathogens by phagocytosis, scavenge dead cells and cellular debris, and remodel tissues after injury. Because they must adapt to their variety of cellular functions, macrophages are heterogeneous and plastic. These characteristics are regulated by tissue-specific signals during regional tissue location-dependent specification (46).

3.1.1. Tissue-specific macrophage development. Transcription factors play critical roles not only in the core macrophage program established during early development in the yolk sac, fetal liver, and bone marrow but also in niche signal–dependent specification in a tissue-specific manner (47). Tissue-specific enhancer landscapes and promoter accessibility educated by tissue microenvironments are also involved in establishing heterogeneous macrophage identities (48). Using Kupffer cell (KC) depletion followed by repopulation in the mouse as a model system, Sakai et al. (49) revealed a liver microenvironment–dependent macrophage differentiation process in circulating monocytes that become Kupffer-like cells. SMAD4 and RBPJ, activated respectively by TGF-β and Notch ligand from sinusoidal endothelial cells, activate poised enhancers of monocytes to rapidly induce expression of KC lineage–determining factors including LXRα, which in turn activates KC-specific enhancers for gene induction (49). A nonalcoholic steatohepatitis (NASH) diet can induce death of KCs and transition of KCs to scar-associated macrophages. Mechanistically, functional reprogrammed LXRα collaborating with ATF3 changes the enhancer landscape of KCs to induce NASH-responsive gene expression. Unlike the model of experimental depletion of KCs, the monocyte-derived macrophages that compensate for KC loss during diet-induced NASH also bear a NASH-associated enhancer atlas and have a functional phenotype (50). These findings imply that niche signals under both physiological and pathological conditions can induce tissue-specific lineage commitment or phenotype transition of macrophages by reprogramming epiregulomes of both tissue-resident macrophages and migrated progenitors.

3.1.2. Macrophage polarization. Macrophages can be polarized into functionally distinct phenotypes: Classically activated (M1) macrophages elicit inflammation for host defense and clear pathogens, and alternatively activated (M2) macrophages resolve inflammation and repair tissue (51). Epigenetic regulation is involved in these processes. Toll-like receptor (TLR) signals mediate M1 activation, and these processes are regulated by chromatin modifiers, which are described in Section 4. Several studies have identified Jumonji C domain–containing protein 3 (Jmjd3) as a positive regulator of M2 activation that upregulates the M2-activating transcription factor interferon regulatory factor 4 (IRF4) through H3K27me3 demethylation (52, 53). Interestingly, Jmjd3 also promotes NLRP3 inflammasome activation by promoting transcription of *Nrf2*, although

GSK-J4, which Huang et al. (54) used to inhibit Jmjd3, can also inhibit Kdm6A, another H3K27 demethylase known as UTX. PPARγ is critical for alternative activation of macrophages during IL-4-induced M2 activation, and PPARγ in a heterodimer with RXR recruits coactivators like p300 and RAD21 to increase chromatin accessibility at M2-associated gene loci for STAT6 (signal transducer and activator of transcription 6) (55, 56). Chromatin modifiers can regulate M2 activation by modulating expression of PPARγ: Protein arginine methyltransferase 1 (PRMT1) promotes PPARγ expression to promote M2-like macrophage polarization by mediating H4R3me2a at the promoter (57), while Dnmt3b promotes inflammation by silencing PPARγ expression via de novo DNA methylation (58). HDAC3 acts with PU.1 to inhibit enhancer activities of M2-specific genes through histone deacetylation in M2-like macrophages (59), while histone acetylase p300 and MLL histone methyltransferases can act with PU.1 and its associated transcription factors to prime and promote enhancer activities of inflammatory genes during M1-like macrophage activation (60, 61). This indicates that different chromatin modifiers can bind the same transcription factor to regulate different gene subsets. On the other hand, IL-4-activated STAT6 can also act with HDAC3 to repress the activity of enhancers in both IL-4-repressed genes and M1-specific genes targeted by p65 for alternative activation of macrophages (62). Furthermore, during the LPS response, HDAC3 acting with activating transcription factor 2 (ATF2) promotes gene transcription independent of enzymatic activity; HDAC3 acting with ATF3 classically inhibits gene transcription with deacetylase activity (63). These findings indicate that the same chromatin modifier can regulate transcription of different classes of genes, even with different transcription-regulation activities, in a transcription factor–dependent manner. In addition to transcription factors, lncRNAs can also mediate gene-specific epigenetic regulation for macrophage polarization. By comparing the lncRNA induction pattern between M1 and M2 activation, researchers (64) identified the lncRNA *PTPRE-AS1* as an IL-4-induced lncRNA. *PTPRE-AS1* acts as an activation-induced repressor of M2 macrophages by inhibiting the MAPK/ERK1/2 signaling pathway. *PTPRE-AS1* can directly bind and recruit WDR5 to enhance the H3K4me3 level at the *PTPRE* promoter, resulting in increased expression of PTPRE (receptor-type tyrosine protein phosphatase ε) (64).

3.1.3. Disease-associated phenotypic reprogramming of macrophages. Macrophage phenotypes are altered with epigenetic changes during disease development in a tissue-specific manner, such as those of foam cells in atherosclerosis (65) and microglia in neurodegenerative disorders (66). Recent studies reveal the contributions of epigenetic dysregulation to proinflammatory phenotypes of macrophages, which prevent wound healing in diabetes. A decrease in H3K9me3 due to decreased expression of Setdb2, or an increase in H4K16ac due to increased expression of histone acetyltransferase (HAT) MOF (males absent on the first), enhances the expression of proinflammatory cytokines as well as xanthine oxidoreductase (XOR) enzyme for proinflammatory metabolite uric acid production, while an increase in H3K4me3, mediated by Mll1, promotes TLR4 expression in macrophages in wounds in chronic diabetes (67–69). The proinflammatory phenotype of macrophages may be established early in progenitor cells, as evidenced by a study where upregulated Dnmt1 in hematopoietic stem cells from type 2 diabetes mice repressed expression of repair macrophage–specific transcription factors such as Klf4 by mediating DNA hypermethylation (70).

The increasing number of immune-relevant stimulants and distinct microenvironment signals complicates functional phenotypes during macrophage polarization. Activators at different anatomic sites and under different physiological or pathological conditions polarize resident or migrated macrophages to different phenotypes with different markers and functions (71, 72), and the dynamic epigenetic information may add a means of dissecting intrinsic macrophage activation

states. Future studies will further reveal whether and how physiological (embryogenesis; pregnancy; and normal maintenance of selected tissues, even including testis and adipose tissues) and pathological (chronic inflammation and tissue repair, metabolic and vascular disorders, infection, and cancer) conditions and processes direct polarization of tissue-resident and monocyte-derived macrophages through epigenetic regulation.

3.2. Development and Specification of Dendritic Cell Subsets

Development of diverse DC subsets including conventional DCs (cDC1s and cDC2s), monocyte-derived DCs, and plasmacytoid DCs (pDCs), which promote different immune effector modules in response to pathogens, is tightly regulated by distinct expression patterns of specific transcription factors (73). The abundance of transcription factors in progenitors and precursors of DCs determines the development and specification of special DC subsets, and the cross regulation of transcription cascades and enhancer landscapes precisely regulates the abundances of special transcription factors. Take transcription regulation of *Irf8* as an example. Expression of PU.1 in myeloid progenitors activates the distal enhancer at the *Irf8* gene locus to mediate chromatin looping between the distal enhancer and the promoter of *Irf8* to induce transcription of *Irf8* in early DC progenitors (74). Furthermore, different enhancers upstream of the *Irf8* gene promoter differentially regulate cDC1 development or specification: A +41-kb enhancer is required for not only pDC development but also pre-cDC1 specification from a common DC progenitor (CDP) because it induces *Irf8* transcription in CDPs in an E protein–dependent manner, while a +32-kb enhancer is essential for the development of cDC1s from pre-cDC1s because it maintains a high expression level of IRF8 in pre-cDC1s (75). Induction of Id2, probably by nuclear factor IL-3 (Nfil3)-mediated repression of Zeb2, extinguishes E protein activity at the +41-kb enhancer, causing a switch to the +32-kb enhancer for efficient cDC1 final development (76). These findings indicate that chromatin modifiers are involved in regulating H3K4me1 or H3K27ac for either de novo establishment or regulation of the activities of *Irf8*-specific enhancers in a transcription factor–dependent or –independent manner at different stages during DC subset development. The activity of transcription factors is also tightly regulated. A lncRNA termed lnc-DC is exclusively expressed in human monocyte-derived DCs with enriched H3K4me3 at its promoter and maintains DC differentiation and homeostasis by binding STAT3 and promoting its phosphorylation and activation (77).

Engagement of various tissue microenvironmental membrane molecules and stimulation of various soluble factors can promote the generation of regulatory DCs or tolerogenic DCs, which play important roles in inducing immune tolerance by negatively regulating T cell responses (78, 79). External signals may also conduct chromatin programing to regulate the development of tolerogenic DCs. Genome-wide H3K4me3 and H3K27me3 profiles were compared between LPS-activated mature monocyte-derived DCs and TGF-β-conditioned tolerized monocyte-derived DCs, revealing that chromatin of TGF-β-conditioned DCs bears more H3K4me3 peaks, which are enriched at genes related to TGF-β activation and the PPARα/RXRα signaling pathway involved in limiting inflammation. Moreover, decreased expression of costimulators is accompanied by increased H3K27me3 in the gene loci of TGF-β-conditioned DCs (80).

The epigenetic regulation of DC subset development and phenotype specification is not as clear as that of macrophages. There remain questions in this field, such as how tissue microenvironmental niche signals mediate epigenetic regulation for DC subset–specific commitment, especially during pathogen infection; how the specification of resident and migratory DCs is regulated at the chromatin level; and whether cell-specific epiregulomes reflect specific functions of DC subsets.

3.3. Innate Lymphoid Cell Development and Plasticity

Although sharing functional and developmental similarities with T cells, ILCs, which do not bear antigen-specific receptors but secrete cytokines in response to infection, are emerging as important effectors of innate immunity, especially for tissue remodeling. Differentiation and maturation of natural killer (NK) cells, a prototypical ILC population, are also governed by transcription factors. Expression of these transcription factors is modulated by enhancers (81), and chromatin modifiers are also involved. Histone H2A deubiquitinase MYSM1 is required for the maturation of NK cells. Mechanistically, MYSM1 binds with Nfil3 at the *Id2* gene promoter to promote Nfil3 targeting and *Id2* transcription, probably by deubiquitinating histone H2AK119 and opening the local chromatin for Nfil3 access (82). DNA methylation levels within the *FCGR3A* promoter are negatively associated with the expression level of CD16a during human NK cell maturation (83).

ILCs besides NK cells and lymphoid tissue–inducer cells are mainly classified into three groups, ILC1s, ILC2s, and ILC3s, depending on both the development pathways regulated by transcription factors and the types of secreted cytokines (84). Although ILCs have similarities with $CD4^+$ T helper (Th) cells in both effector molecules and transcription factors (85), the chromatin structures of ILCs and Th cells undergo different remodeling during development. Divergent accessible regulatory elements at gene loci of ILC subset–specific effectors and regulators appear early at the precursor stages of ILC subsets, while $CD4^+$ T cells undergo dramatic chromatin remodeling during activation and final polarization. Similar *cis*-regulator landscapes are observed between innate and adaptive cells after activation, but ILCs have their unique patterns of CREs, including superenhancers, especially in genes associated with cytokine signaling and innate immune receptors (17, 86). Interestingly, although the CREs are primed before mouse ILC activation, as evidenced by acquired accessibility and H3K27ac during ILC development, and change little after activation by ILC subset–specific cytokines, H3K4me2 at subsets of gene loci related to ILC2 function undergoes expansion during human ILC2 activation (17, 87). This may be because different chromatin modifications are differentially involved in transcription regulation, which adds another layer beyond chromatin accessibility. Moreover, the remodeled epiregulome can further mediate epigenetic remodeling to support ILC development. For example, the Yeats domain–containing protein Yeats4, which is highly expressed in ILCs and their progenitors, can read H3K27ac at the promoter of *Lmo4* in progenitor cells and recruit Dot1l to mediate H3K79me3 there for effective RNA polymerase II–mediated transcription initiation. Loss of expression of Lmo4 impairs the commitment of ILCs (88). Negative chromatin modification is also involved in lineage specification of ILCs. G9a, a writer of the repressive histone modification H3K9me2, promotes ILC2 specification in bone marrow by repressing transcription of ILC3-specific genes (89).

After specification in bone marrow, ILCs migrate into tissues. Niche signals in different tissues may drive further maturation and function of ILCs, and even mutual transition between ILC subsets during both steady state and infection. This transitioning is called ILC plasticity and is regulated mainly by transcription factors activated by ILC-specific cytokines (90). Chromatin modifiers are also involved in these processes: The *Rroid* locus acts as a CRE to maintain chromatin accessibility of the *Id2* promoter for IL-15-activated STAT5 deposition, promoting homeostasis and function of ILC1s in a tissue-specific manner (91). IL-1β-primed ILC2s have the ability to phenotypically switch into ILC1s in response to IL-12, due to decreased repressive H3K27me3 and increased positive H3K9ac at promoters of ILC1-specific genes, such as *IFNG* for transcription initiation (92). lncKdm2b, a divergent lncRNA highly expressed in intestinal ILC3s, sustains the peripheral proliferation of ILC3s by promoting transcription of Zfp292. Mechanistically, lncKdm2b mediates interaction between the chromatin organizer Satb1 and the nuclear remodeling factor (NURF) complex at the *Zfp292* promoter. Loss of Zfp292

impairs maintenance of ILC3s, although the underlying mechanism needs further investigation (93). Qi et al. (94) found that Brg1 not only promotes differentiation of NKp46$^+$ ILC3s from NKp46$^-$ ILC3s by enhancing transcription of *Tbx21* but also represses the proinflammatory property of ILC3s by inhibiting transcription of *Csf2*. Their findings also indicate a dichotomous engagement of Brg1 in transcription regulation. Thus, tissue niche signals during steady state and infection can remodel the epiregulome and induce subset-specific gene expression patterns for maturation, maintenance, and function of ILC subsets. However, the epigenetic mechanisms for development of ILC subsets are still elusive, especially those for the transition between ILC subsets in the context of a special microenvironment. For example, how do the niche signals break up the cell-specific epiregulome previously established during cell development for phenotype switching, and which chromatin modifiers act cooperatively with signal-induced transcription factors to mediate gene-specific transcription regulation?

As described above, transcription factors and chromatin modifiers set up a cell type–specific epiregulome for phenotype establishment during lineage commitment in response to niche signals from a tissue-specific microenvironment during both steady state and infection. There are a well-established transcription factor network and lineage-determining transcription factors. Considering that chromatin states determine the accessibility of transcription factors that regulate gene transcription with the help of chromatin modifiers, further investigation is needed to determine whether there are lineage-determining chromatin modifiers and whether the repression or overexpression of specific chromatin modifiers can program the progenitors to form specific innate cell types and mediate transitions among subsets of innate immune cells in a transcription factor–dependent or even transcription factor–independent manner.

4. THE EPIREGULOME OF INNATE IMMUNE CELLS PROGRAMED BY SIGNALING DURING PATHOGEN INFECTION DELICATELY BALANCES THE INNATE IMMUNE RESPONSE

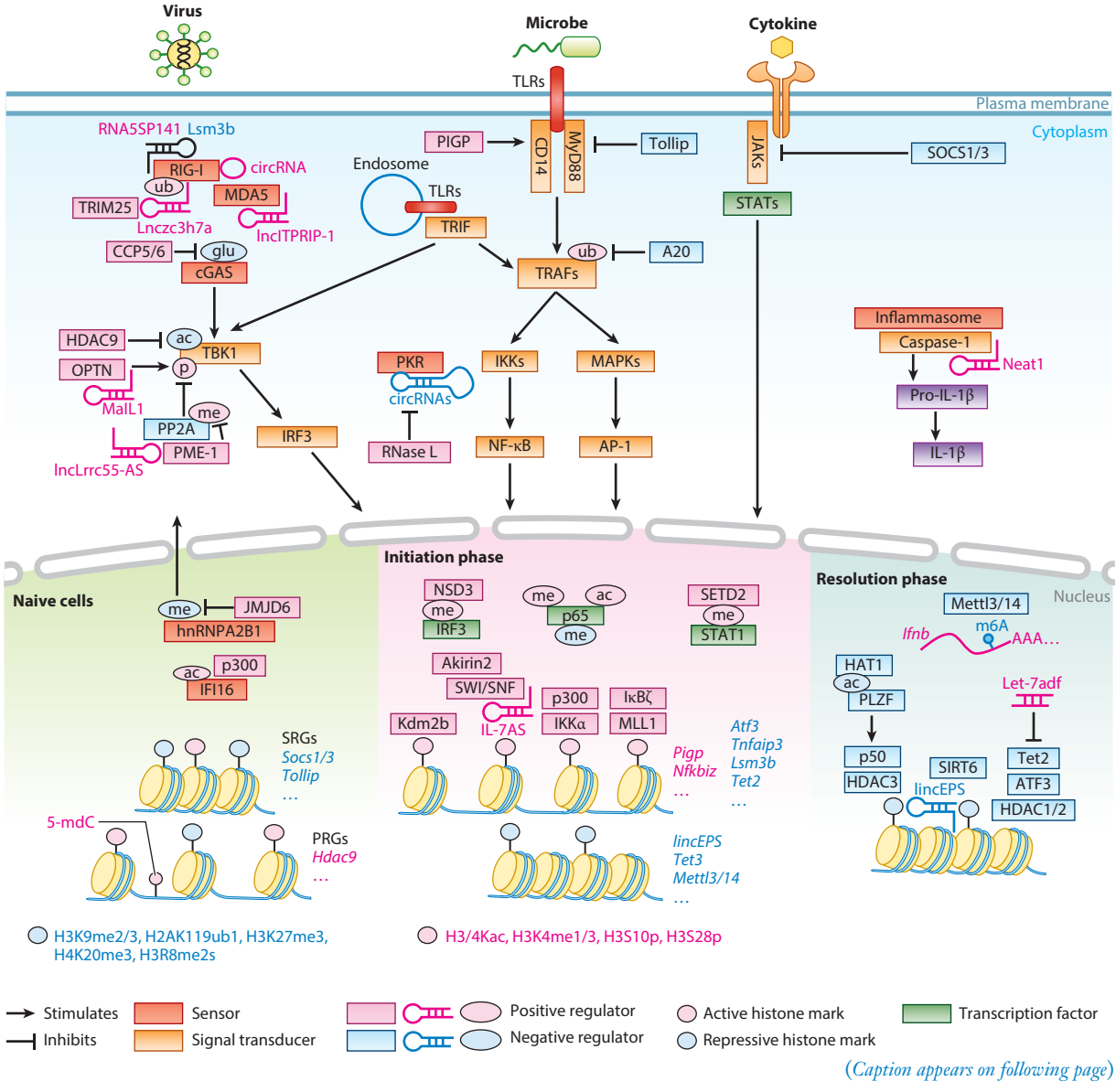
During pathogen infection, innate immune cells recognize pathogens through interweaved PRRs and C-lectin signaling systems, and they initiate an innate immune response characterized by induction of innate immune effectors, including proinflammatory cytokines and antipathogen molecules. To prevent tissue damage due to constant or unresolved inflammation, negative regulation takes hold during the late phase of the innate immune response to resolve inflammation. Signals induced by the interaction between pathogens and innate immune cells are transduced into the nucleus and program the epiregulome of naive innate immune cells by regulating the expression, activity, and gene-specific targeting of chromatin modifiers. This in turn maintains a delicate balance of the innate immune response at multiple molecular levels, from signaling transduction in the cytoplasm to transcription regulation in the nucleus (**Figure 2**).

4.1. Chromatin Modifier–Mediated Regulation of Pathogen Sensing and PRR Signaling Pathways

Expression and activities of pathogen sensors and PRR-signaling transducers and their regulators are tightly regulated at multiple molecular levels to effectively initiate the innate immune response. Chromatin modifiers are involved in not only nonclassically regulating the function of proteins and but also classically mediating epigenetic regulation.

4.1.1. Classical regulation at the chromatin and mRNA levels to initiate PRR signaling. A cell-specific epiregulome guarantees the ability of innate immune cells to rapidly respond to

pathogen infection. Mediators and regulators of PRR signaling pathways should be effectively expressed by innate immune cells. On one hand, as described in Section 3, during development and specification of innate immune cells, a cell-specific enhancer landscape is established cooperatively by transcription factors and chromatin modifiers in response to niche signals for transcription activation. On the other hand, special chromatin modifiers mediate gene-specific regulation of both negative and positive regulators of PRR signaling: In macrophages, Kmt2b promotes transcription of *Pigp* for effective CD14 anchoring by writing H3K4me3 at the promoter (95); Ezh1 and Ezh2 inhibit transcription of negative regulators *Tollip* and *Socs3*, respectively, in TLR signaling pathways by writing H3K27me3 at the promoters (96, 97); and Kdm5a inhibits transcription of *Socs1*



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Epigenetic regulation of pattern recognition receptors/cytokine signaling pathways and immune effector expression in the innate immune response. To balance the innate immune response, sensors and signal transducers are positively or negatively regulated at the protein level by cytoplasmic long noncoding RNAs or nonclassical posttranslational modifications. Epigenetic regulation adds another layer. From initiation to resolution of the inflammatory innate response, chromatin remodeling, erasing or adding repressive and active histone marks, and methylation at the DNA/RNA levels are largely involved. First, in naive cells, these epigenetic modifications regulate the regulators of signaling pathways and repress innate immune effectors. Second, at the initiation phase, they not only induce gene transcription of (*i*) proinflammatory cytokine genes categorized as PRGs and SRGs based on the dependence on chromatin remodeling for transcription initiation and (*ii*) positive (*pink*) and (*iii*) negative (*blue*) regulator genes, but also repress gene transcription of the negative regulators. Third, at the resolution phase, they repress proinflammatory cytokines. Abbreviations: ac, acetylation; AP-1, activating protein-1; ATF3, activating transcription factor 3; CCP5/6, cytosolic carboxypeptidase 5/6; cGAS, cyclic GMP–AMP synthase; circRNA, circular RNA; 5-mdC, 5-methyl-deoxycytosine; glu, glutamylation; HAT1, histone acetyltransferase 1; HDAC9, histone deacetylase 9; H3K4me3, histone H3 lysine 4 trimethylation; IFI16, IFN-γ-inducible protein 16; IκBζ, inhibitor of nuclear factor kappa B zeta; IKK, inhibitor of nuclear factor kappa B kinase; IRF3, interferon regulatory factor 3; JMJD6, jumonji domain containing 6; Kdm2b, lysine demethylase 2b; lincEPS, long intergenic noncoding EPS; MAPK, mitogen-activated protein kinase; MDA5, melanoma differentiation-associated gene 5; me, methylation; Mettl3/14, methyltransferase-like 3/14; MLL1, mixed-lineage leukemia 1; m6A, N^6-methyladenosine; MyD88, myeloid differentiation primary response protein 88; NF-κB, nuclear factor kappa B; NSD3, nuclear receptor binding SET domain protein 3; OPTN, optineurin; p, phosphorylation; PIGP, phosphatidylinositol glycan anchor biosynthesis class P; PKR, protein kinase R; PLZF, promyelocytic leukemia zinc-finger protein; PME-1, phosphatase methylesterase 1; PP2A, protein phosphatase 2A; PRG, primary response gene; RIG-I, retinoic acid–inducible gene I; SETD2, SET domain–containing 2; SIRT6, surtuin 6; SOCS, suppressor of cytokine signaling; SRG, secondary response gene; STAT, signal transducer and activator of transcription; SWI/SNF, switch/sucrose nonfermentable; TBK1, TANK-binding kinase 1; Tet, ten-eleven translocation; TLR, Toll-like receptor; Tollip, Toll-interacting protein; TRAF, TNF receptor–associated factor; TRIF, Toll/IL-1 receptor domain–containing adapter inducing IFN-β; TRIM25, tripartite motif containing 25; ub, ubiquitination.

for NK cell activation by IL-12 by erasing H3K4me3 at the promoter (98). Epitranscription regulation is also involved, such as in Mettl3-mediated m6A promoting translation of costimulators and TLR4-signaling adaptors and cathepsins for DC activation and function (99, 100). However, mechanisms underlying gene-specific epigenetic regulation need to be further investigated.

4.1.2. Nonclassical regulation at the posttranscription level for PRR-signaling initiation. Chromatin modifiers can shuttle between the cytoplasm and nucleus to write and erase PTMs of proteins in PRR signaling, from sensors to transducers to transcription factors: JMJD6-mediated demethylation of R226 arginine of the nuclear DNA sensor hnRNPA2B1 is required for its cytoplasmic export (101). p300 mediates acetylation of IFI16, promoting its cytoplasmic localization so as to transduce an antiviral signal (102). CCP5/6 erase monoglutamylation and polyglutamylation of cyclic GMP–AMP synthase (cGAS) to promote DNA affinity of cGAS and its enzymatic activity (103). HDAC9, transcription of which is enhanced by Dnmt3a-mediated DNA methylation at its distal promoter, maintains the deacetylation state of TBK1 for its full activation during virus infection (104); methylation and acetylation on lysine and arginine of p65 are implicated in regulating its stability and activity (105, 106). STATs and IRF3 are also targets of chromatin modifiers (107); for example, Setd2-mediated K525 monomethylation of STAT1 and Nsd3-mediated K366 monomethylation of IRF3 promote phosphorylation of the two transcription factors in antiviral innate signaling (108, 109).

Cytoplasmic lncRNAs also take part through RNA-protein interactions. For retinoic acid–inducible gene 1 (RIG-I) signaling, a virus infection–induced host ncRNA and the foreign intron-programed circular RNAs can interact with and activate RIG-I (110, 111). Further, lnczc3h7a can serve as a molecular scaffold to promote TRIM25-mediated, K63-linked ubiquitination of RIG-I, enhancing RIG-I signaling (112). On the other hand, during the late phase of virus infection, interferon-induced self-lncRNA–Lsm3b can act as a negative competitor for viral RNAs by interacting with RIG-I to terminate the antiviral innate response in a timely manner (113).

The different functions may be due to different lncRNA structures and interaction domains for RIG-I binding, and the protein status of RIG-I before and after sensing viral RNAs may also contribute to this discrepancy. lncITPRIP-1 can bind the C terminus of MDA5 and act as a cofactor to promote its oligomerization and activation (114). Neat1, cytoplasmic translocation of which is induced by an inflammasome activation signal, promotes inflammasome assembly and subsequent procaspase-1 processing by interacting with the caspase-1 p20 domain (115). Phosphorylation of transcription factors is also indirectly regulated by cytoplasmic lncRNA in a third-party-dependent manner (116, 117). Nonclassical regulation also takes place in the cytoplasm to erase repressive factors. Imperfect RNA duplexes formed in endogenous circular RNAs can act as inhibitors of double-stranded RNA (dsRNA)-activated protein kinase R (PKR), while the early innate immune response induces degradation of circular RNAs by RNase L, leading to activation of PKR to amplify innate immunity (118). Mechanisms underlying the regulation of RNase L–mediated degradation of circular RNA before and after innate immune activation, and even during resolution of inflammation, may be another key point for this model. Identifying common RNA structures or motifs for RNA-protein interaction may be the greatest challenge in this field.

4.2. Chromatin Modifier–Mediated Transcription Induction of Innate Immune Effectors

Transduction of PRR signals into the nucleus by activated transcription factors can initiate robust transcription of innate immune effectors, especially proinflammatory cytokines. These processes include remodeling local chromatin structure, erasing repressive chromatin markers, and writing positive chromatin marks for polymerase II–mediated transcription initiation and elongation. The three processes are not fulfilled sequentially; they are concurrent and dependent on each other.

4.2.1. Chromatin remodeling and erasing repressive chromatin marks. ATP-dependent chromatin remodeling by the SWI/SNF complex is required for induction of transcription of secondary response genes (SRGs). In contrast, primary response genes (PRGs) have open chromatin structures and bear positive histone modifications at their promoters in naive cells (36, 119, 120). Several chromatin modifiers can promote chromatin remodeling: Akirin2, Kdm2b, and lncRNA IL-7-AS promote chromatin remodeling at promoters of SRGs such as *Il6* (121–123). The potential initiator of chromatin remodeling has not yet been identified. Among the PRGs, the pioneer transcription factors that can bind nucleosomal DNA (124) are candidates.

Erasing repressive histone modifications is also required for induction of both proinflammatory and antivirus genes: Jmjd2a erases promoter H3K9me3 for *Ifnb* induction (125); Jmjd2d or Kdm1b erases enhancer H3K9me3 or promoter H3K9me2, respectively, for proinflammatory cytokine induction (126, 127); Phf2 erases promoter H4K20me3 deposited by NCoR/SMYD5 for TLR4 target gene induction (128); erasing of the 2A-HUB-mediated promoter H2AK119ub1 promotes transcription elongation of chemokine genes (129); and H3K27me3 erasers Kdm6a/b also promote cytokine transcription (130–132). Although most studies do not systematically categorize PRGs and SRGs when investigating the distribution patterns and effects of repressive histone marks for transcription regulation, repressive histone marks are largely involved in transcription repression of SRGs. Repression of the epigenetic repressors is another way to amplify the innate immune response; e.g., TLR signaling represses expression of long intergenic noncoding RNA–EPS, which inhibits transcription of proinflammatory genes by maintaining a repressed chromatin state (133). The B type of carbonic anhydrase 6 interacts with PRMT5 and inhibits PRMT5-mediated repressive histone mark H3R8me2s [symmetric dimethylation of Arg8 (R8) on histone H3] at the *Il12b* promoter (134).

4.2.2. Writing positive chromatin markers. Positive histone marks, active enhancers, and lncRNAs act cooperatively for efficient transcription initiation and elongation of innate immune genes during the early phase of the innate immune response. H3 and H4 acetylation written by GCN5, PCAF, or p300 is required for efficient transcription of innate immune effectors, and acetylation of different lysine sites in H3 and H4 plays different roles in transcription initiation and elongation (120, 135). To further promote histone acetylation, factors promoting histone deacetylation are inhibited; e.g., expression of Tet3, which acts with HDAC1 to inhibit transcription of IFN-β, is inhibited by antiviral signaling (136). On the other hand, acetylation of nonhistone proteins such as signal transducers and regulators can also play negative roles in cytokine induction (137), and HDAC inhibitors repress expression of proinflammatory cytokines, especially during the early phase of inflammation (138, 139).

MyD88 signaling–induced IκBζ increases H3K4me3 levels at the promoters of SRGs (140). MLL1 in the COMPASS complex writes H3K4me3 in proinflammatory cytokines, and MLL1 loss decreases promoter H3K4me3 levels and expression of proinflammatory cytokines in LPS-activated macrophages (141). Dot1l-mediated H3K79me2/3 selectively promotes transcription of *Il6* and *Ifnb* in macrophages in response to PRR signaling (142). Signaling transducers may also act as chromatin modifiers to mediate chromatin modifications. TLR signaling induces histone phosphorylation H3S10p and H3S28p at promoters of proinflammatory genes in a p38- and nuclear IKKα–dependent manner (143–145). lncRNAs including enhancer RNA (eRNA) transcribed from genomic regions defined as enhancers can also promote inflammation initiation by mediating gene-specific epigenetic regulation (61, 146, 147).

Further investigation is needed to determine whether chromatin remodeling and kinds of chromatin markers deposited in one gene locus act independently or interdependently for effective induction of innate immune effectors and whether there is a decisive factor controlling or triggering this series of epigenetic processes.

4.3. Epigenetic Regulation for Restraining and Resolving the Innate Immune Response

To prevent overactive inflammation and tissue damage, the innate immune response always needs to be precisely controlled and quickly transitioned into a resolution phase after pathogen elimination. Thus, a lot of negative regulators are simultaneously induced or activated with innate immune effectors, such as negative regulators in PRR signaling pathways (4). Controlling or shutting down PRR signaling pathways is not enough to control or shut down the expression of proinflammatory cytokines, and epigenetic repression adds another layer not only by enhancing the expression of negative regulators of PRR signaling and indirectly controlling gene transcription but also by directly controlling or shutting down transcription of proinflammatory cytokines.

4.3.1. Upregulating transcription of negative regulators of PRR signaling and indirect transcription repression. For PRR signaling, A20 mediates K63 deubiquitylation of NF-κB essential modulator (NEMO) and TNF receptor–associated factor 6 (TRAF6) to shut down TLR signaling. Ash1l promotes TLR-signaling-induced expression of A20 because it writes H3K4me3 at the promoter of *Tnfaip3* (encoding A20) (148). For indirect transcription repression, although symmetric R30 dimethylation of p65 mediated by PRMT5 increases p65 affinity for DNA (149), PRMT1 mediates asymmetric R30 dimethylation of p65 to inhibit the DNA binding ability of p65, controlling TNF-α-induced activation of NF-κB (150). HAT1 can acetylate and enhance activity of transcription factor PLZF, promoting the assembly of a repressive complex including HDAC3 and p50 for transcription inhibition of proinflammatory genes (137).

4.3.2. Direct epigenetic repression of proinflammatory genes. There are several models of epigenetic repression. First, active transcription factors can also recruit epigenetic repressors to restrain transcription of their target gene. For example, p65 recruits Sirtuin 6 (SIRT6) to promote histone deacetylation of its target promoters (151). Second, repressive transcription factors are activated and recruit epigenetic repressors. ATF3, a TLR4-activated transcription factor, acts cooperatively with HDACs to restrain transcription of NF-κB target genes through histone deacetylation (152). Third, expression of epigenetic repressors is induced to mediate inflammation resolution. Tet2, expression of which is increased early after LPS stimulation, selectively inhibits transcription of IL-6 via HDAC-mediated histone deacetylation during the late phase of the LPS response (139). To promote transcription of *Il6* during the resolution phase of the innate immune response, Let-7adf restrains the expression of Tet2 (153). With increased expression during virus infection, m6A writers Mettl3 and Mettl14, and readers Ythdf2 and Ythdc1, mediate m6A modification of *Ifnb* mRNA and destabilize it (154).

Mechanisms underlying the selective epigenetic regulation of innate immune effectors, gene loci of which bear similar binding sites for proinflammatory transcription factors such as NF-κB and AP-1, and whether this selectivity is based on different chromatin status determined during lineage commitment need to be further investigated. Feedback regulation by signals from autocrine and paracrine cytokines during the dynamic epigenetic regulation of the innate immune response also warrants further analysis.

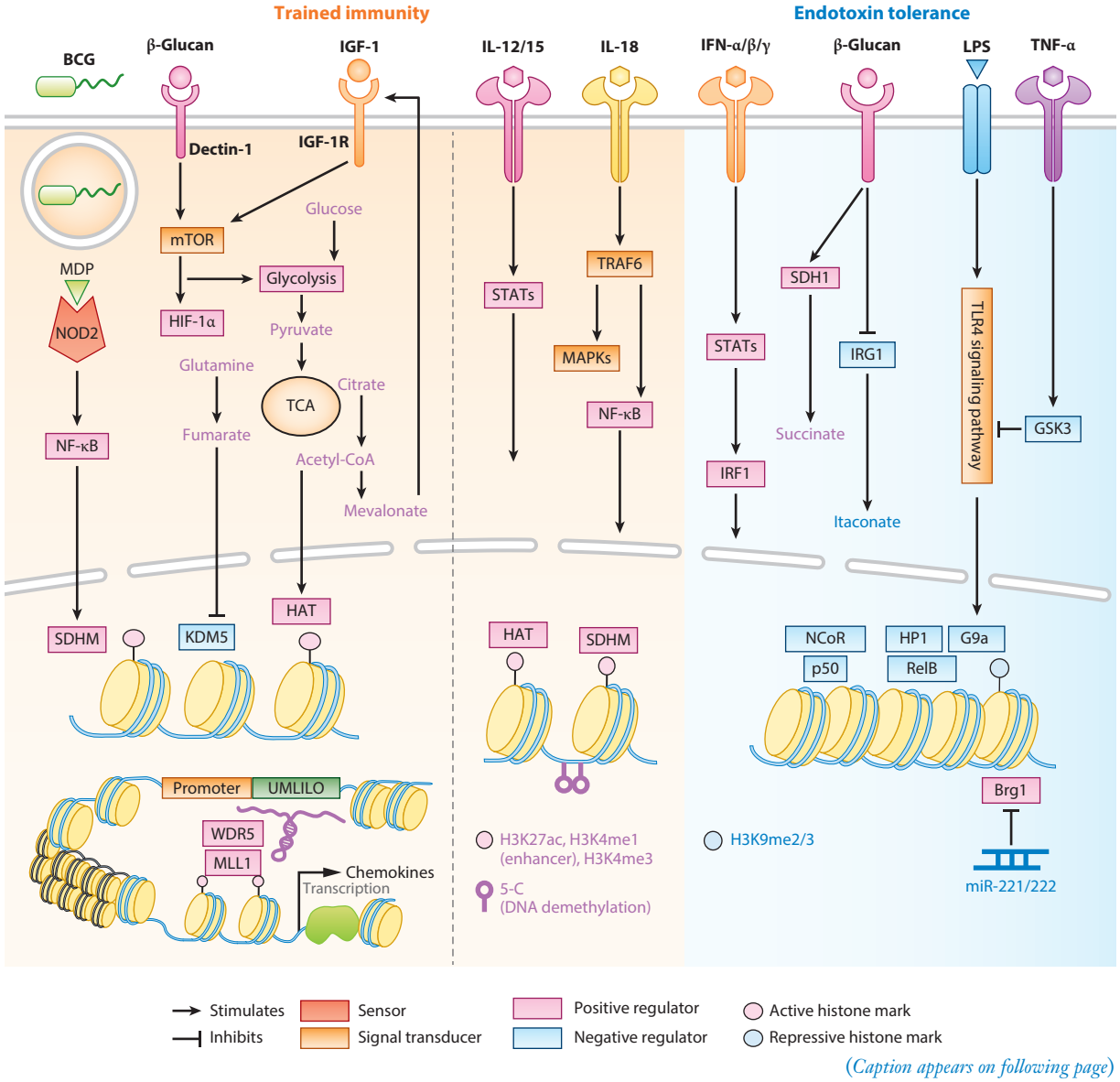
5. TRAINING- AND PRIMING-MEDIATED PROINFLAMMATORY EPIREGULOME FOR INNATE IMMUNE MEMORY

Immunological memory induced by initial insults is no longer understood to be unique to adaptive immunity. Trained immunity refers to enhanced responsiveness to subsequent triggers after initial activation of the innate immune response (155). Adaptive features of macrophages were initially observed during investigations of IFN-γ priming and endotoxin tolerance. Subsequent studies have revealed both specific and nonspecific protection against reinfection in different types of innate immune cells (like monocytes, DCs, NK cells, and ILCs, and even bone marrow progenitor cells) due to primary activation by stimulants ranging from microorganism-derived agents like the fungal cell wall component β-glucan; LPS; and the bacillus Calmette–Guérin (BCG) vaccine, which is the live attenuated vaccine against *Mycobacterium tuberculosis*, to cytokines and endogenous danger molecules like oxidized low-density lipoprotein (5). Additionally, recognition of cytomegalovirus (CMV) by antigen-specific receptors can elicit memory NK cells and ILCs (156–158). Training-mediated epiregulome programing is the key mechanism involved in these processes (**Figure 3**).

5.1. LPS Training Establishes a Tolerant State of Macrophages at the Chromatin Level

LPS tolerance, which recapitulates features of sepsis-associated immunoparalysis, is a form of repressive innate immune memory that blunts subsequent responses to infection. Constant LPS stimulation induces macrophage tolerance characterized by repression of transcription of proinflammatory genes but priming of genes encoding antimicrobial molecules in macrophages. TLR4 signal–induced epigenetic reprogramming, especially gene-specific histone acetylation regulation in tolerant cells, is the key mechanism (159, 160). Transcription silencing mediated by the p50-recruited NCoR complex, which has histone deacetylation activities; the RelB-recruited histone H3 lysine 9 methyltransferase G9a; and heterochromatin protein 1 (HP1) contributes to

the tolerant state in a gene-specific manner (161–163). Additionally, factors promoting chromatin accessibility can be inhibited during tolerance. miR-221 and miR-222 can repress chromatin remodeling at promoters of proinflammatory genes by directly inhibiting the mRNA level of Brg1 and indirectly inhibiting STAT1/2 activity (164). Relieving or reversing epigenetic repression can end the tolerant state. β-Glucan training derepresses gene transcription by restoring H3K27ac deposition at gene enhancers and promoters in tolerant macrophages (165). β-Glucan also inhibits IRG1 transcription and the production of itaconate, a repressor of the innate immune response, and restores the expression of SDH1 for succinate production by regulating the promoter H3K27ac level, another mechanism for β-glucan to reverse tolerance (166).



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

The epiregulome determines the phenotypes of innate memory cells by regulating gene-specific transcription. Innate memory cells are established in a cell-specific and stimulant-specific manner via epigenetic mechanisms. For endotoxin tolerance (*blue*), LPS-activated p50 and RelB act cooperatively with epigenetic repressors to erase positive histone marks or add H3K9me2/3. miRNAs inhibit the positive chromatin remodelers, such as Brg1, leading to condensed chromatin at proinflammatory gene loci. TNF-α indirectly inhibits TLR signals and chromatin accessibility via GSK3. IFN-γ and IFN-α/β can partially reverse a tolerant chromatin state in a STAT1- and IRF1-dependent manner, and β-glucan can reverse tolerance by indirectly repressing the itaconate level but increasing the succinate level via regulation of the expression of their respective enzymes. For positive priming and training (*orange*), during cytokine priming, proinflammatory cytokines induced by virus infection increase chromatin accessibility by adding active histone marks with HATs and SDHM or by promoting DNA demethylation at the enhancers or promoters of genes such as *Ifng* in natural killer cells or innate lymphoid cells. During β-glucan and BCG training, dectin-1 and NOD2 signaling pathways indirectly promote deposition of active histone marks by increasing the level of fumarate, which inhibits the activities of KDM5 family members, and acetyl-CoA for histone acetylation. Furthermore, training signals can upregulate the transcription of immune gene–priming lncRNAs, which in turn promote transcription of chemokine genes by recruiting the MLL1/WDR5 complex for H3K4me3 in chromatin topologically associating domains. The gray dashed line separates cytokine-induced (*right*) and pathogen-induced (*left*) trained immunity. Abbreviations: BCG, bacillus Calmette–Guérin; Brg1, brahma-related gene 1; 5-C, 5-cytosine; GSK3, glycogen synthase kinase 3; HAT, histone acetyltransferase; HIF-1α, hypoxia-inducible factor 1α; HP1, heterochromatin protein 1; H3K4me3, histone H3 lysine 4 trimethylation; IGF-1R, insulin-like growth factor 1 receptor; IRF1, interferon regulatory factor 1; IRG1, immune-responsive gene 1; KDM5, lysine demethylase 5; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MDP, muramyl dipeptide; MLL1, mixed-lineage leukemia 1; mTOR, mammalian target of rapamycin; NCoR, nuclear receptor corepressor; NF-κB, nuclear factor kappa B; NOD2, nucleotide-binding oligomerization domain–containing protein 2; RelB, v-rel reticuloendotheliosis viral oncogene homolog B; SDHM, *S*-adenosyl methionine–dependent histone methyltransferase; STAT, signal transducer and activator of transcription; TCA, tricarboxylic acid; TLR4, Toll-like receptor 4; TRAF6, TNF receptor–associated factor 6; UMLILO, upstream master lncRNA of the inflammatory chemokine locus; WDR5, WD repeat–containing protein 5.

Moreover, constant stimulants derived from microorganisms can also mediate gene-specific transcription repression to affect macrophage function through epigenetic mechanisms. Influenza virus infection represses transcription of *Cxcl1* via Setdb2-mediated H3K9me3 in an IFN-β-dependent manner. Decreased infiltration of neutrophils due to repressed transcription of *Cxcl1* is an important mechanism for susceptibility to bacterial superinfection after virus infection (167). Interestingly, influenza infection can also induce long-term innate memory of monocyte-derived alveolar-like macrophages but not resident alveolar macrophages for one month, characterized by increased chromatin accessibility in the *Il6* locus and elevated expression of IL-6 for conferring protection from *Streptococcus pneumoniae* infection (168). Virus infection may have different short-term and long-term effects on macrophages, depending on the macrophage subset.

5.2. Cytokine Signals Promote Innate Memory in a Cell- and Signal-Specific Manner Through Epigenetic Mechanisms

Proinflammatory cytokines train ILCs during CMV infection and prime myeloid cells for subsequent activation and tolerance. Changes at the chromatin level are involved.

5.2.1. Cell-specific priming of innate lymphoid cells. ILCs can be primed by cytokines in a cell-specific manner with epigenetic remodeling as the potential mechanism. NK cells preactivated by IL-12 and IL-18 can act as memory-like NK cells in vivo for weeks after the initial priming, characterized by a phenotype similar to that of naive NK cells but enhanced expression of IFN-γ upon restimulation (169). Cytokine priming–induced DNA demethylation of conserved noncoding sequences 1 (CNS1) of the *IFNG* locus in NK cells contributes to the memory-like character (170). Pairwise comparison of chromatin accessibility in naive and memory NK cells during mouse cytomegalovirus (MCMV) infection showed that an interferon-stimulated response

element (ISRE)-like sequence was enriched in accessible memory peaks, indicating that JAK-STAT signaling is critical for establishing the innate memory–specific chromatin states (171). IL-15 contributes to increased enhancer accessibility and gene expression levels of *IFNG* and *VEGFA* in pregnancy trained decidual NK cells from women who have had multiple pregnancies (172). MCMV-specific antigen but not IL-12 and IL-18 signaling is the key stimulator for memory formation of ILC1s with enhanced IFN-γ expression after MCMV reinfection, and genome-wide chromatin remodeling was also induced in this process (158).

5.2.2. Cytokine-specific priming of myeloid cells. Different proinflammatory cytokine signals can induce distinct innate memory in the same cell type. TNF-α priming induces tolerance both by inhibiting NF-κB signaling and by decreasing positive histone modifications, including H3K4me3 and H4Ac, and chromatin accessibility in proinflammatory cytokine gene loci in a GSK3-dependent manner. Type I interferon can partially abrogate TNF-α-induced tolerance in a gene-specific manner by inducing IRF1 targeting and inducing positive epigenetic marks even in response to a weak LPS signal (173, 174). On the other hand, IFN-γ priming can both enhance transcription of proinflammatory cytokines and repress TLR4-signaling-induced feedback inhibitors to prevent endotoxin tolerance by regulating chromatin accessibility and histone acetylation levels at gene enhancers and promoters during the LPS response (175–177). Moreover, in vivo priming of tissue-specific macrophages by IFN-γ was also investigated, although the effect was on a different gene set. During respiratory viral infection, priming of alveolar macrophages by $CD8^+$ T cell–derived IFN-γ is critical for antibacterial trained immunity because it increases expression of neutrophil-specific chemokines and enhances neutrophilia, although the epigenetic mechanism has not yet been revealed (178). During pathogen infection, enhanced myelopoiesis amplifies the number of innate cells, including monocytes, in response to proinflammatory cytokine signaling. Tet2 is required for promoting infection-induced myelopoiesis during systematic infection because it promotes IL-3 signaling, and mechanistically, Tet2 inhibits expression of Socs3 at the posttranscription level because it promotes demethylation of 5-mC for mRNA degradation (179, 180). Furthermore, LPS can induce memory of hematopoietic stem cells characterized by increased myelopoiesis and immune gene responsiveness to secondary stimulation via increasing accessibility of enhancers in a C/EBPβ-dependent manner (181).

The different priming effects of cytokines on specific innate cells may depend on specific transcription factors and chromatin modifiers. How these regulators cooperatively establish or reverse the stable chromatin states in naive cells or previously activated cells and how they maintain the reprogrammed epigenome for a long time need to be further investigated.

5.3. Epigenetic Regulation in Microorganism-Mediated Trained Immunity

Exposure of the innate immune system to pathogens and pathogen-derived immunostimulatory agents can train innate cells to perform better during reinfection, even with different types of pathogens. The training signals can educate the chromatin state at the gene transcription level. β-Glucan-dectin-1- and BCG-NOD2-mediated training can enhance the transcription of proinflammatory cytokines in monocytes and macrophages during reinfection by increasing H3K4me3 levels at gene promoters, H3K4me1 levels at enhancers, and histone acetylation at both elements. Methylthioadenosine (MTA), an inhibitor of *S*-adenosyl methionine (SAM)-dependent methylation, can inhibit the training effect, further confirming that histone methylation is involved, although SAM-dependent DNA, RNA, and nonhistone methylation may also be inhibited by MTA (182–184). As a constant pool to produce short-lived monocytes, hematopoietic stem cells and

multipotent progenitors can also be trained by β-glucan and BCG with a stable proinflammatory epiregulome inherited by macrophages during lineage commitment (42, 185). Recently, researchers using Hi-C to chart TADs found a group of immune gene–priming lncRNAs that are encoded in the same TADs as proinflammatory genes, especially those encoding chemokines. These lncRNAs upregulated by β-glucan can act in *cis* to guide the histone methylase complex WDR5-MLL1 to chemokine promoters and to increase H3K4me3 levels in TADs, adding a new clue for gene-specific epigenetic regulation in trained immunity (186). Rewired metabolic pathways also contribute to promoting epigenetic programing in trained monocytes: mTOR- and HIF-1-α-mediated aerobic glycolysis provides both acetyl-CoA for histone acetylation and mevalonate, which is a key mediator of training because it promotes activation of IGF-1-R and mTOR. MTA can partially inhibit this metabolic programing; glutaminolysis produces fumarate, which inhibits the activity of histone demethylases of KDM5 family members to increase H3K4me3 levels (187–189).

Increased positive histone marks in hundreds of genes categorized into several classes during both LPS tolerance and trained immunity may be regulated by different epigenetic mechanisms and chromatin modifiers beyond specific transcription factors. How training and tolerance signals initiated by the stimulants mediate gene-specific epigenetic regulation at hundreds of promoters and enhancers and how these chromatin markers are stably maintained during cell proliferation and lineage commitment are still elusive. And exploring specific chromatin modifiers promoting or repressing these processes and developing inhibitors of these chromatin modifiers to break or promote the priming effects or memory states may provide clinical clues for treating infectious and inflammatory diseases.

6. METABOLIC REWIRING GUIDES EPIREGULOME PROGRAMING DURING THE INNATE IMMUNE RESPONSE

The Krebs cycle oxidizes not only carbohydrates but also fatty acids and amino acids to produce metabolic intermediates. Furthermore, the high-transfer-potential electron carriers nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) are part of the electron transport chain, which creates a proton gradient for ATP production in a process known as oxidative phosphorylation, and are also products of the Krebs cycle (190). As the central pivot of metabolic pathways, the Krebs cycle is an amphibolic pathway. On one hand, the diversion of nutrients can replenish Krebs cycle intermediates, and on the other hand, Krebs cycle intermediates can feed into various biosynthetic pathways. Furthermore, these metabolic intermediates and their derivatives have additional functions beyond metabolism. Microenvironmental signals such as pathogen infection and inflammation can rewire the Krebs cycle to regulate cellular functions (13). Epigenetic regulation is also modified by metabolic intermediates (**Figure 4**).

6.1. Metabolites Act as Cofactors to Mediate Chromatin Modification

Several metabolites are essential cofactors for chromatin-modifying enzymes that catalyze different kinds of histone, DNA, and RNA modifications and mediate chromatin remodeling. These metabolite-dependent epigenetic mechanisms are largely involved in regulating PRR signaling and transcription dynamics during training, initiation, and resolution of the innate immune response (6, 119). ATP, the final product of oxidative phosphorylation, is required for chromatin remodelers such as Brg1 to increase chromatin accessibility (191). Acetyl-CoA, which is the primary substrate that enters the Krebs cycle, and SAM, which is produced by the methionine pathway, are utilized by HATs such as p300 and GCN5 and SAM-dependent methyltransferase, respectively.

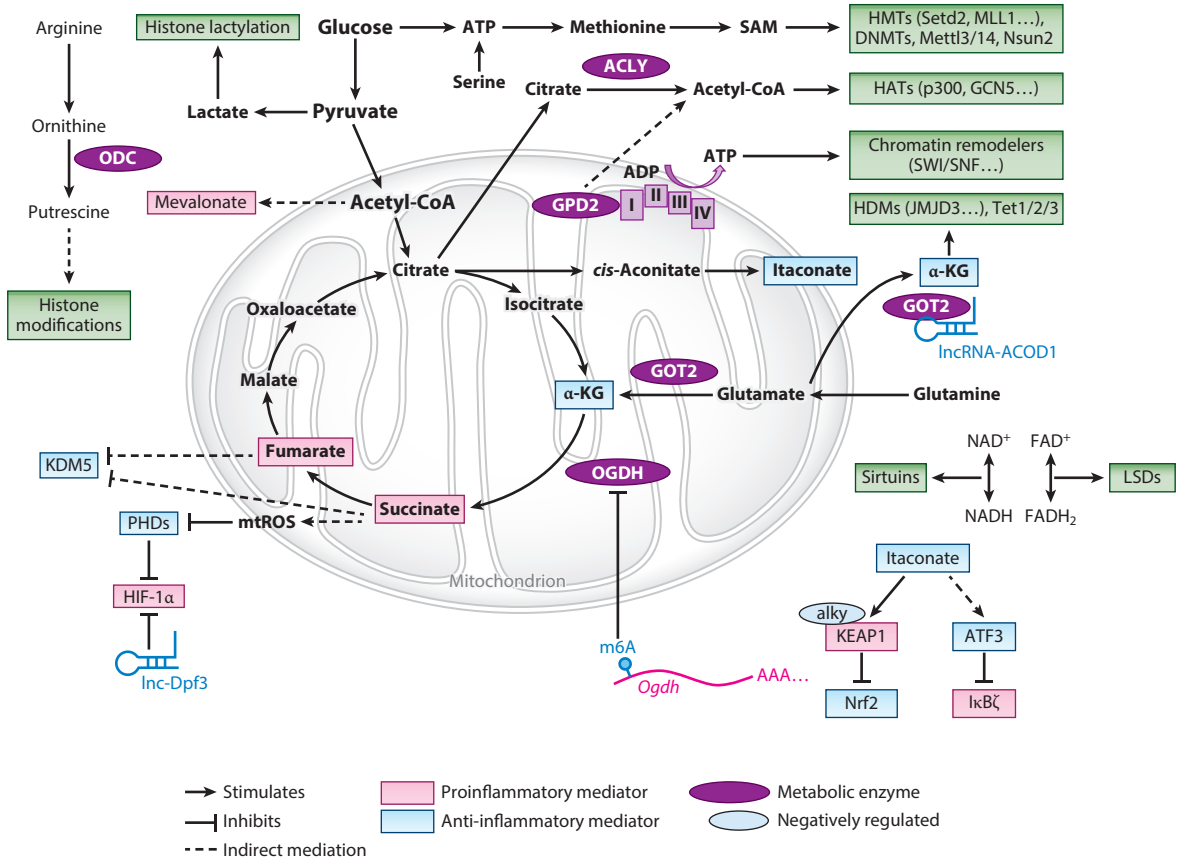


Figure 4

Metabolic rewiring remodels the epiregulome by modulating the activity of chromatin modifiers. Innate signals induce metabolic rewiring characterized by the changed activities of specific metabolic pathways centered around the Krebs cycle, leading to changed levels of specific metabolites. Some are proinflammatory mediators, such as succinate and fumarate, the levels of which increase due to aerobic glycolysis and inhibited glucose oxidation during classical activation of macrophages. Some are anti-inflammatory mediators, such as itaconate and α-KG, the levels of which increase due to increased expression of IRG1 and increased glutaminolysis during alternative activation of macrophages. Several metabolites such as acetyl-CoA, SAM, α-KG, and ATP serve as cofactors of chromatin modifiers, which write or erase acetylation or methylation of DNA, RNA, or proteins or remodel chromatin. Some metabolites can also indirectly mediate epigenetic regulation, probably acting as cofactor competitors or affecting the levels of other metabolites. Exploring new chromatin modifications like histone lactylation may reveal new functions of metabolites. Protein levels of transcription factors that play positive or negative roles in the innate immune response can also be regulated by metabolites: Itaconate alkylates KEAP1 to stabilize Nrf2, IκBζ translation is inhibited via ATF3, and the succinate-mtROS axis stabilizes HIF-1α by inhibiting PHDs. Metabolic enzymes can also be regulated by epigenetic regulators; for example, m6A destabilizes mRNA of OGDH, lnc-Dpf3 inhibits HIF-1α-mediated induction of enzymes in glycolysis, and lncRNA-ACOD1 promotes activity of GOT2. Abbreviations: acetyl-CoA, acetyl coenzyme A; ACLY, ATP-citrate lyase; alky, alkylation; α-KG, α-ketoglutarate; ATF3, activating transcription factor 3; DNMT, DNA methyltransferase; FAD, flavin adenine dinucleotide; GCN5, general control non-depressible 5; GOT2, glutamic-oxaloacetic transaminase 2; GPD2, glycerol 3-phosphate dehydrogenase 2; HAT, histone acetyltransferase; HDM, histone demethylase; HIF-1α, hypoxia-inducible factor 1α; HMT, histone methyl transferase; IκBζ, inhibitor of nuclear factor kappa B zeta; JMJD3, Jumonji C domain–containing protein 3; KDM5, lysine-specific demethylase 5; KEAP1, kelch-like ECH-associated protein 1; lncRNA, long noncoding RNA; LSD, lysine-specific demethylase; Mettl3/14, methyltransferase-like 3/14; MLL1, mixed-lineage leukemia 1; m6A, N^6-methyladenosine; mtROS, mitochondrial reactive oxygen species; NAD, nicotinamide adenine dinucleotide; Nrf2, nuclear factor erythroid 2–related factor 2; Nsun2, NOP2/Sun domain family member 2; ODC, ornithine decarboxylase; OGDH, oxoglutarate dehydrogenase; PHD, prolyl hydroxylase; SAM, *S*-adenosyl methionine; Setd2, SET (suppressor of variegation, enhancer of zeste, trithorax) domain–containing 2; SWI/SNF, switch/sucrose nonfermentable; Tet1/2/3, ten-eleven translocation 1/2/3.

They are utilized as cofactors to transfer an acetyl or methyl group to histones and nonhistone proteins, DNA, and RNA. HATs can use not only acetyl-CoA but also other acyl-CoAs such as propionyl-CoA, butyryl-CoA, crotonyl-CoA, and succinyl-CoA as substrates to mediate histone and nonhistone modifications (192, 193). Nicotinamide adenine dinucleotide (NAD^+), which is produced from the amino acid tryptophan or via the NAD salvage pathway and is recycled from intracellular metabolic reactions, acts as a cofactor of not only enzymes involved in several major metabolic pathways but also the sirtuin family members for deacetylation of both histone and nonhistone proteins, such as α-tubulin and p65, respectively (194, 195). FAD, generated from the vitamin riboflavin by riboflavin kinase and FAD synthase (FADS), and α-ketoglutarate (α-KG), a key intermediate of the TCA cycle, act as cofactors of the two types of histone demethylases, lysine-specific demethylase and JMJD families, respectively, and DNA and RNA demethylases, like Tet family members (24, 196). Thus, the availability of these metabolites will determine the activity of the chromatin modifiers.

Signals eliciting or inhibiting the innate immune response can rewire metabolic pathways and change cellular levels of Krebs cycle–related cofactors of chromatin modifiers by modulating expression and/or activity of specific metabolic enzymes, thus concurrently reprogramming the epiregulome. During macrophage polarization, alternative macrophage activation promotes production of α-KG via glutaminolysis, and increased α-KG promotes demethylation of H3K27me3 by Jmjd3 at promoters of M2-associated genes, elevating transcription of these genes during IL-4-induced M2 polarization (197). Viral infection–induced cytoplasmic lncRNA-ACOD1 can directly bind GOT2 and enhance its activity for α-KG production to promote virus replication via an interferon-independent pathway (198). The Akt-mTORC1 axis increases the acetyl-CoA level by enhancing both phosphorylation and protein levels of ATP-citrate lyase (Acly), which catalyzes production of acetyl-CoA from citrate, and contributing to M2 activation (199). Early LPS-stimulated M1 activation can also promote glycolytic flux and the TCA cycle for citrate production and thus increase the activity of Acly, through MyD88 and TRIF signaling, increasing the level of acetyl-CoA (200). Increased acetyl-CoA can promote histone acetylation of promoters of a subset of genes regulating cellular proliferation and chemokine production during M2 activation or of promoters of a subset of SRGs during M1 activation (199, 200). During LPS stimulation, increased offshoots of glycolysis, the pentose phosphate pathway and serine synthesis pathway, also lead to an increased SAM level because ATP is provided to the methionine cycle, enhancing the transcription-elongation-associated histone mark H3K36me3 at gene bodies of proinflammatory genes, especially *Il1b*, by increasing the activity of Setd2 (201). Mitochondrial glycerol 3-phosphate dehydrogenase 2, expression of which is increased by LPS stimulation, can either support the production of acetyl-CoA for histone acetylation during acute exposure of the macrophage to LPS or inhibit production of acetyl-CoA during LPS tolerance by mediating glycerol phosphate shuttle–related forward or reverse electron flow through the electron transport chain for glucose oxidation (202).

Metabolites produced in the cytoplasm should be transported into the nucleus for chromatin modification. These processes may also be regulated during the innate immune response so as to modulate the availability of the material. Moreover, in addition to Acly, the pyruvate dehydrogenase complex can be transported to the nucleus from mitochondria to mediate the generation of acetyl-CoA from pyruvate there and to regulate histone acetylation in response to environmental signals (203). This process may also contribute to the elevated histone acetylation for induction of innate immune effectors. Whether there are metabolic pathways in the nucleus, the same as or different from those in the mitochondria or cytoplasm, to provide cofactors for rapid epigenetic reprogramming in the innate immune response is yet to be investigated.

Recently, lactate was identified as a new substrate for histone modification: Increased production of lactate by glycolysis in macrophages during hypoxia and bacterial challenges leads to increased histone lactylation, which promotes transcription of homeostatic genes, including *Arg1*. The enzyme catalyzing this modification needs to be further identified (204). New modifications of chromatin components based on innate immune response–mediated metabolic rewiring are yet to be explored.

6.2. Indirect Regulation of Chromatin Modifications by Metabolites

During activation of innate cells, reprogrammed metabolic pathways, especially glycolysis, lead to significant fluctuation of levels of special metabolites, such as succinate and itaconate, that act as proinflammatory or anti-inflammatory signals in macrophages (reviewed in 13). With regard to transcription regulation of the innate immune response, several studies have found that these metabolites can modulate protein levels of transcription factors. Succinate stabilizes hypoxia-inducible factor 1α (HIF-1α) by inhibiting prolyl hydroxylase–mediated degradation in a mitochondrial reactive oxygen species–dependent manner (205, 206), while itaconate can inhibit inflammation not only by directly alkylating cysteine residues in KEAP to stabilize NRF2, an anti-inflammation transcription factor, but also by inhibiting translation of IκBζ, an LPS-induced coactivator of proinflammatory cytokines, in an ATF3-dependent manner, probably through its electrophilic properties (207, 208).

Metabolites may also indirectly regulate chromatin modification. Using myeloid cell–specific deletion of ornithine decarboxylase, the rate-limiting enzyme in polyamine metabolism, as a model, researchers found that putrescine, the level of which decreased in macrophages during *Helicobacter pylori* infection, inhibited M1 macrophage responses by affecting levels of both positive and negative histone modifications by means of an unknown mechanism (209). As described in Section 5.3, fumarate may inhibit the activities of KDM5 family histone demethylases (187), and succinate and fumarate were also reported to broadly inhibit the activity of α-KG-dependent dioxygenases, probably by acting as competitors of α-KG (210). Metabolites derived from commensal microbes can also mediate epigenetic regulation in intestine-localized cells. The short-chain fatty acid n-butyrate, a metabolite secreted by commensal bacteria, can inhibit the induction of proinflammatory cytokines in an LPS-stimulated macrophage, like the commercial inhibitor of HDAC, sodium butyrate (211). Furthermore, butyrate treatment endowed monocyte-derived macrophages with enhanced antimicrobial activity, probably by inhibiting HDAC3 (212). Butyrate can also promote histone crotonylation by inhibiting class I HDACs (213). *Staphylococcus aureus* biofilm–derived lactate can increase transcription of *Il10* in macrophages, probably by inhibiting HDAC11 (214). On the other hand, commensal microbe–derived inositol phosphate can enhance the activity of HDAC3 (215). These findings imply that metabolic communication between the pathogen and host may also mediate epigenetic regulation in innate cells.

From a metabolic view, how metabolites mediate epigenetic regulation is still elusive, especially for indirect regulation. Potential mechanisms include 2-oxoglutarate–dependent dioxygenases, which include chromatin modifiers that sense nuclear oxygen, being regulated by oxidative phosphorylation (216), and reactive oxygen species may also mediate posttranslational regulation of transcription factors and chromatin modifiers by regulating cysteine redox (217). From an epigenetic view, according to these studies, fluctuation of the levels of cofactors and metabolites selectively affects specific chromatin modifications at specific gene loci. This implies that different subsets of genes are regulated by different chromatin modifiers, even for the same type of modification, and different chromatin modifiers may have different affinities for the same metabolite and thus have different sensitivity to the fluctuation in the concentration of the metabolites.

Furthermore, the status of the local chromatin and its associated epigenetic regulators in situ may also determine which metabolite can establish the epiregulome in the context of microenvironmental signal–mediated metabolic rewiring. On the other hand, training signals, mRNA m6A, or lncRNAs like lnc-Dpf3 can modulate metabolic pathways by mediating the regulation of gene expression for metabolic enzymes in macrophages and DCs (166, 218, 219). The reprogrammed epiregulome modulated during training, initiating, and resolving of the innate immune response can also regulate the expression of specific enzymes in metabolic pathways and then mediate the metabolic rewiring.

7. CONCLUSIONS AND PERSPECTIVES

From innate cell development and subset specification, initiation, and resolution of the innate immune response and inflammation to the establishment of innate memory, epiregulomes reprogrammed by innate and niche signals endow innate immune cells with the ability to respond rapidly to danger signals from pathogens and stressful stimuli. Conclusively, a variety of in-depth studies have focused on two aspects: how the epiregulomes are reprogrammed and how the reprogrammed epiregulomes in turn establish function-specific gene expression patterns in innate immunity. However, from an epigenetic view, the mechanisms underlying the two aspects need to be further investigated (see the section titled Future Issues). Moreover, selectivity is still the most puzzling aspect of epigenetic regulation. For example, why does a special signal induce one subset of NF-κB target genes but not another? Why does one epigenetic mechanism regulate a particular subset of genes whereas another epigenetic mechanism regulates a different one? And what is the immunological significance of gene-selective epigenetic regulation—for example, why are PRGs and SRGs regulated differently at the chromatin level? For trained immunity, epigenetic memory may determine the memory phenotype. Therefore, clarifying the mechanisms for long-term maintenance of the epiregulome will help to identify the master regulators of trained immunity. For example, why are increased acetylation and methylation of histones erased during inflammation resolution but not during trained immunity? When studying trained immunity, we should also consider that different epiregulomes of tissue-specific subsets of innate cells, especially the tissue-specific macrophages, may lead to different phenotypic and functional reprogramming in response to the same stimulants. Metabolic rewiring may be a key mechanism for epiregulome reprogramming. However, mechanisms for regulating specific metabolic pathways by means of innate immune signals are still elusive. They are probably dependent on epigenetic regulation of gene expression of special metabolic enzymes. Moreover, systematically charting the dynamic epiregulomes of innate cells, especially in vivo during the innate immune response using high-throughput techniques and even at single-cell and single-molecule levels, will help in deciphering the epigenetic codes of innate immunity.

FUTURE ISSUES

1. Lineage-determining chromatin modifiers need to be explored during development, specification, and mutual transition of subsets of innate immune cells in a niche signal–specific manner.
2. Epiregulomes need to be established even at the single-cell level to identify new subtypes of innate cells and their origins and to reveal their functional differences in naive and infection-specific niches.

3. Innate memory–determining chromatin modifiers, the inhibitors of which can modulate or even abrogate tolerance or trained immunity by reversing the trained epiregulome, need to be identified.
4. Further research is needed to understand the mechanisms that allow the epiregulome of trained immunity to be maintained for a long time and to be inherited during differentiation of progenitor cells.
5. Nucleus-localized metabolites regulated by innate signals, which directly modulate cofactor levels of chromatin modifiers, need to be screened.
6. Epigenetic mechanisms for regulating metabolic rewiring during training, initiation, and resolution of the innate immune response need to be illustrated, especially the cross talk between mitochondria and the nucleus.
7. Researchers will need to dissect the transcription regulatory mechanisms of the mitochondrial genome for metabolic rewiring during the innate immune response.
8. A new dimension of innate immunity–specific epiregulomes, including chromatin condensates and organization, nuclear granules, and newly identified chromatin modifications, needs to be expanded.
9. The functions of nuclear localized RNA-binding proteins and the noncoding RNAs or mRNAs they bind in a structure-dependent manner are yet to be revealed.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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