REVIEW ARTICLE

Epigenetic Regulation of Macrophage Polarization

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Abstract

Increasing data shows that macrophages, the primary immune cells in the growing fetus/neonate, retain an innate immune memory of prior stimuli. This memory is rooted in epigenetic regulation of lineage- and tissue-specific transcription either to enhance the responses or to induce tolerance to repeated exposures to a stimulus. As we understand, epigenetics refers to the study of heritable information transmitted during cell divisions that can alter gene expression via inclusion of chemical tags but no changes in the DNA sequence. We now recognize the lineage-determining transcription factors as important mediators that can make the local chromatin more accessible to other factors; one example is the erythroblast transformation-specific gene PU.1 (purine-rich sequence binding protein 1). The PU.1 can upregulate the basal activation state of many promoters by increasing histone H3 lysine 4 trimethylation (H3K4me3). There are several other newly discovered regulators that perform similar regulatory roles. These mediators enhance macrophage differentiation into several phenotypes essential for host defense or tissue homeostasis in response to environmental stimuli. The two ends of this polarization spectrum include the classically-activated (M1) macrophages induced by interferon-γ and microbial products; and the alternatively-activated (M2) macrophages induced by the T-helper 2 cytokines interleukin (IL)-4 and IL-13. The M1 macrophages participate in host defense and clearing pathogens, whereas all the known subtypes of M2 cells promote resolution of inflammation and tissue repair. Maladaptive changes in macrophages can disrupt the normal sequence of immune/inflammatory responses and predispose to disease states. The review summarizes our current understanding of the involved mechanisms; this information can help understand the immune responses in neonates who are yet to develop mature neutrophil function or adaptive immunity and are largely dependent on mononuclear cells for immune defenses.

Keywords: Epigenetics, Hematopoiesis, Hematopoietic stem cells, Infant, Lineage-determining transcription factors, Macrophages, Macrophage polarization states, Monocytes, Neonate, Newborn.

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Key Points

- Epigenetics is the study of stable, heritable changes in gene expression that involve chemical modifications but not in the actual sequence of nucleotides. Some mechanisms include DNA methylation, interaction with noncoding RNAs, and posttranslational modification of histones.
- Tissue-resident macrophages are derived from the yolk sac progenitors and hepatic/bone marrow-derived monocytes. Emerging data show several subpopulations with varying effects; the differentiation of these subgroups likely involves epigenetic changes.
- Gene loci relevant for polarized macrophage phenotypes exist in repressed, poised, and active subgroups.
- The purine-rich sequence binding protein 1 (PU.1) plays a key role in the differentiation of macrophage lineages. Several downstream genetic and epigenetic mechanisms have been defined.
- Changes in macrophages can cause a broad spectrum of maladaptive immunity and inflammation that are causative factors of disease and, thus, represent key therapeutic targets.

Macrophage Development and Polarization

Origin of Macrophages

In the embryo, macrophages arise from multiple progenitors: (a) primary committed lineages independent of the transcription factor cellular factor Myb or the erythromyeloid progenitors in the yolk sac (YS); (b) those in the aorta-gonad-mesonephros zone;

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and (c) the hematopoietic stem cells in the liver and the bone marrow.[1–4] A late c-Myb-dependent wave generated in the fetal liver and the bone marrow produces multiple hematopoietic lineages, including monocytic intermediates throughout life; these include microglia in the brain, Kupffer cells in the liver, osteoclasts in the bones, and other tissue-resident macrophages and dendritic cells (DC).[5–7] During hematopoiesis, a multipotent stem cell again branches out to various tissues.[8] Unlike the monocyte-derived macrophages (MDMs), tissue macrophages originating from the YS have some self-regenerating properties.[9]

Resident macrophages in various tissues express specific genes following integration of transcriptional and epigenetic regulators. The MDMs are derived from circulating monocytes that migrate into tissues and express signal-specific genes during differentiation. Minor epigenetic modifications can happen with environmental changes such as those seen during infections; these cells can eliminate pathogens and restore tissue integrity.[10,11] In MDMs, epigenetic changes are coordinated by myeloid lineage- and tissue-specific transcription factors. There are many "topologically-associated domains (TADs)" where DNA looping can promote the interaction of genes with *cis*-acting regulatory elements; a protein ring consisting of cohesin can bind a DNA-binding protein called CTCF (CCCTC-binding factor) to establish TAD boundaries.[12] The circumscribed regions contain relatively stable chromatin loops, which can prevent/interrupt enhancer-promoter contacts.[13–17] Many embryonic cells contain TADs marked by repressive histone modifications such as histone H3 lysine 27 trimethylation (H3K27me3).[18–20] Some TADs correspond to nuclear lamina-associated domains.[21]

In various tissues, macrophages are enriched for DNA binding sites for local enhancers and recognition motifs for the corresponding transcription factors.[22] Lineage-determining transcription factors or pioneer factors/master regulators can open the local chromatin to merge with other factors.[23] One example is the ETS (erythroblast transformation specific; a conserved DNA-binding domain in specific proteins to bind to DNA) family member PU.1 (purine-rich sequence binding protein 1), which is associated with the basal activation state and trimethylation of histone H3 lysine 4 (H3K4me3) of many promoters. It occupies most macrophage enhancers to maintain H3K4me1 and activates many cell-specific enhancer-like elements.[24] The PU.1 also interacts with macrophage-specific enhancers and increases chromatin accessibility by binding the CCAAT/enhancer binding proteins (C/EBP), interferon regulatory factor (IRF), nuclear factor kappa B (NF-κB), and the activator protein-1 (AP-1).[25] It increases nucleosome remodeling and histone modifications to prime DNA, leading to differential activation of enhancers in response to H3K4 monomethylation at different places in the genome.[24] The binding of secondary signal-dependent transcription factors establishes tissue-specific enhancers and regulates gene expression.[26]

Monocyte-to-macrophage Transition under Stimulation

The MDMs are seen in the fetal/neonatal intestine in larger numbers in some tissues, such as the intestine and skin. In these organs, monocytes are viewed as an intermediate developmental stage between bone marrow precursors and tissue macrophages.[27] In addition, blood monocytes migrate to inflammatory tissues and differentiate into MDMs that can restore tissue integrity and eliminate the pathogen.

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Initial Inflammatory Activation of MDMs

Toll-like receptor (TLR) ligands, such as LPS, and Th1 cytokines, including IFN-γ, elicit M1 activation alone or in combination and can affect epigenetic processes and lead to epigenetic modifications.[28] TLR4 and analogous receptors are key sensors in the M1 response that triggers inflammation through mitogen-activated protein kinase (MAPK), NF-κB, and IRF gene networks, which have downstream genes that encode inflammatory cytokines, such as CXCL10, IL-1β, IL-6, IL-12, p40, and tumor necrosis factor (TNF).

Epigenetic Regulation of Macrophage Polarization

Macrophages show several polarized phenotypes. Increasing information suggests that these are largely rooted in epigenetic differences, which explains the plasticity in transition between cellular programs.[2] This polarization involves exposure to microbial flora, host cytokines, and other environmental cues; which alter the interaction among transcription factors, DNA, and downstream signaling pathways.[29–32] The determinants of the epigenetic landscape include DNA methylation, three-dimensional changes in chromatin, and proteins bound to gene regulatory promoters and enhancers; these changes regulate gene expression and functional outcomes.[33] The differences in the chromatin structure can alter its accessibility and consequent genomic localization of "signaling transcription factors," such as NF-κB and the STATs (signal transducer and activator of transcription).[34,35] These structural changes can get remodeled following further exposure to polarizing stimuli, and thereby recalibrate the responses to successive stimuli.

Polarizing Factors and Macrophage Phenotypes

Macrophage polarization states (Table 1) are defined by the inducing stimulus and by the ensuing patterns of gene expression, which determine function.[36–39] A variety of activation states are being defined (Fig. 1). Infection or tissue injury activates macrophage host defense functions like microbial killing and production of cytokines and chemokines.[36–39] This is one end of the activation spectrum, labeled as the classical activation (also termed M1) and is induced by interferon (IFN)-γ and microbial products such as TLR ligands. An alternative activation state (M2) is induced by the T-helper (Th)2 cytokines interleukin (IL)-4 and IL-13.[36,37,40]

The M1 or classically-activated macrophages, are proinflammatory cells. These are characterized by efficient antigen presentation, high bactericidal activity, and the production of proinflammatory cytokines and reactive oxygen and nitrogen species.[41]

The M2 or alternatively-activated macrophages, have immunoregulatory functions. These cells express anti-inflammatory cytokines and are not as effective as M1 cells in the production of proinflammatory cytokines and antigen presentation. These cells are primarily regulatory, and promote tissue remodeling, wound healing, angiogenesis, antihelminth responses, and possibly, atopy. In premature and term neonates, macrophages show relatively

Table 1: Differences between M1 and M2 polarization states

M1	*M2*
M1-like macrophages (induced by IFNs, granulocyte macrophage colony-stimulating factor (GM-CSF), lipopolysaccharide (LPS), and other microbial products) are potent microbial killers, generate inflammatory cytokines, but may lead to toxicity and collateral tissue damage.	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 aspects of M1 macrophages are high expression of major M1 effector molecules like TNF, IL-1 and IL-12, antimicrobial molecules, reactive oxygen/nitrogen intermediates, and IFN-induced genes-Th1-recruiting chemokines CXCL9 (C-X-C motif chemokine ligand 9) and CXCL10 (C-X-C motif chemokine ligand 10).	Core aspects of M2 macrophages are expression of scavenger receptors, growth factors (heparin binding epidermal growth factor and insulin-like growth factor), Th2 chemokines (CCL18 and CCL22), and suppressors of inflammation and immunity like IL-10 and indoleamine 2,3-dioxygenase.[39] Macrophage colony-stimulating factor (M-CSF) shows a predisposition to an M2 phenotype, resulting in suppression of inflammatory activation and inappropriate responses to innocuous stressors.[44,45]

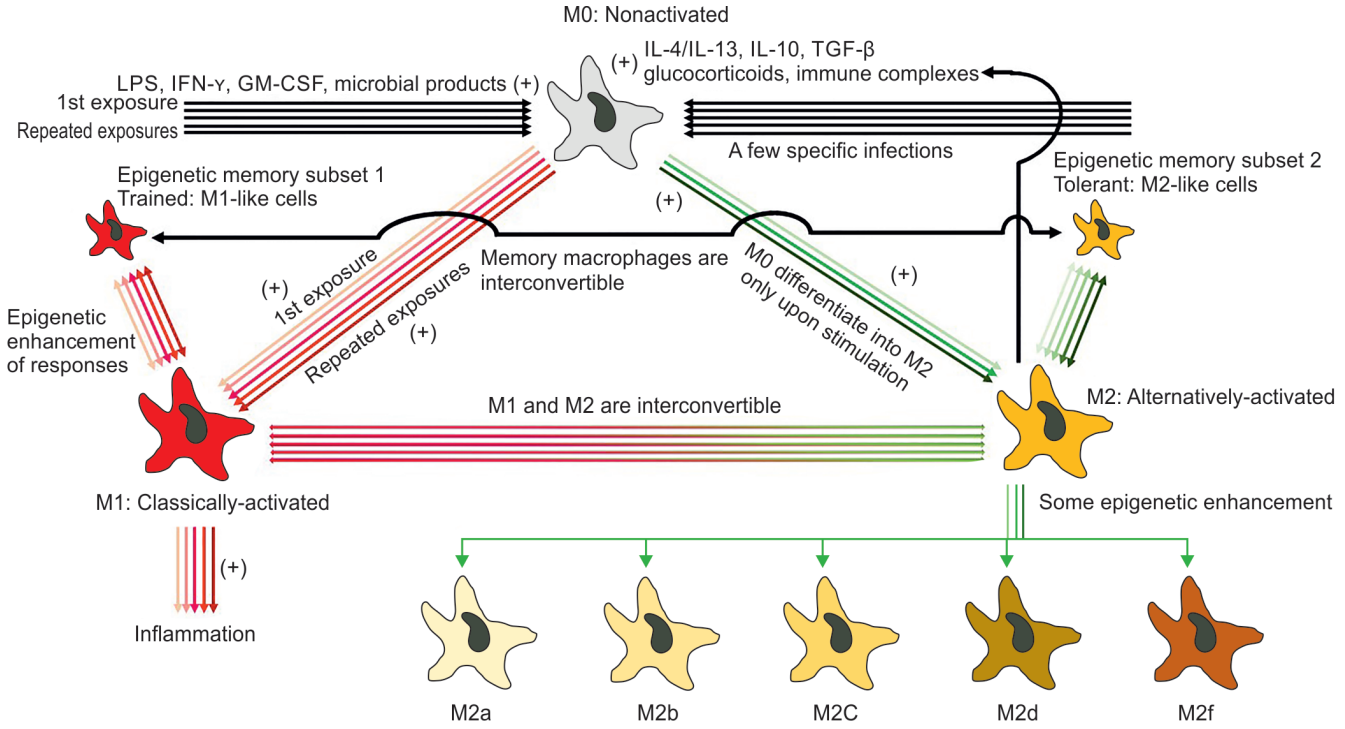

Fig. 1: Differentiation of MDMs. The neonatal immune system shows an inflammatory bias as compared with adults, with a predominance of the M1 lineage and a relative immaturity of anti-inflammatory systems. Repeated stimulation with stimuli such as TLR agonists leads to epigenetic enhancement and progressive amplification of the innate immune/inflammatory responses. The figure shows macrophages differentiating in the categories M0 (gray), M1 (deep red), and M2 (amber). The deepening of shades in the arrows in M0 to M1, M1 to memory M1, and the inflammatory responses shows progressive amplification of these responses with multiple exposures. Single- vs double-headed arrows show unilateral or reversible responses. Fewer arrows in the M0 to M2 differentiation (green) shows that such differentiation is relatively limited in infants. The smaller size of the memory macrophages is a graphic representation of the smaller number in these cohorts, and not a morphological difference. M with added letters show the categories of macrophages. [Some components of the figure were adapted with permission from *Maheshwari A. Innate immune memory in macrophages. Newborn 2023;2(1):60–79*]

strong M1 profile, which predisposes them to conditions such as necrotizing enterocolitis.[42,43]

Subtypes of M2 Macrophages[4]

M2 macrophages are a relatively heterogeneous group, comprising of five subcategories with distinct inflammatory functions and physiological roles (M2a, M2b, M2c, M2d, and M2f (Fig. 1 and Table 2).[4] M2a are activated by the cytokines IL-4 and IL-13, and regulate the expression of platelet-derived growth factor-BB and TGF-β. These cells support pericyte and smooth muscle differentiation. Upon activation by immune complexes, IL-1β, pathogen-associated molecular patterns (PAMPs), and TLR ligands, these cells express inflammatory cytokines (IL-1, IL-6, and TNF), and anti-inflammatory IL-10.[46–48] They are involved in altered regulation of the PI3K/Akt/ FoxO3a pathway.[49] M2c macrophages are activated by IL-10, TGF-β, and glucocorticoids.[50] They express MMPs and IL-10, TGF-β, and pentraxin-3.[50,51] They are involved in vascular remodelling.[52] M2d macrophages are activated by TLR agonists and adenosine A2A receptor agonists.[53,54] They suppress inflammatory responses.[55] They regulate the expression of IL-10

Table 2: Macrophage subpopulations

Macrophage subpopulation	*Activation*	*Function*	*Biological processes*
M0 (naïve, unstimulated macrophages)			
M1 (classically activated, inflammatory macrophages)			
	• Activated by LPS and IFN-γ. • Macrophage-produced inducible nitric oxide synthase.[52] • Macrophage-produced IL-12, IL-18, and IL-2.[59] • GM-CSF.	• Proinflammatory, antimicrobial. • Regulate angiogenesis.[52,60,61]	• Activate Tie-signaling.[58] • Promote endothelial cell chemotaxis, and cell migration in angiogenesis.[62]
Innate immune memory (IIM) macrophages			
Trained (M1-like)	• Epigenetic reprogramming, especially histone modification.[63] • After stimulation, H3K4me1 levels and the binding of TFs increase at latent enhancers. • Increased H3K4me3 at promoters of innate immunity genes.	• Memory of previous infections, which can rapidly recruit and activate innate immune cells.[10] • Rapid induction of inflammatory mediators upon secondary infections with pathogenic bacteria and *Candida* spp.[64]	• Host defense. Particularly important in neonates and young infants before adaptive immunity becomes functionally adequate.[65]
Tolerized (M2-like)	• Including nucleosome remodeling, the reduced recruitment of transcription factors and chromatin remodeling complexes, and histone modification. • NF-κB-associated inhibitory mechanisms.	• Memory of previous infections; can suppress unduly severe inflammatory responses.[66]	• Host protection. May protect young infants, who are still developing adaptive responses, from severe tissue damage.[66]
M2 (alternatively-activated/immunoregulatory, anti-inflammatory, prohealing macrophages)			
M2a	• Cytokines, IL-4, and IL-13.[39]	• Regulate the expression of platelet-derived growth factor-BB and TGF-β.[52]	• Support pericyte and smooth muscle cell differentiation.[58]
M2b	• Immune complexes, IL-1β, and molecules with PAMs. • Immune complexes and TLR ligands.[56]	• Express inflammatory cytokines (IL-1, IL-6, and TNF), and anti-inflammatory IL-10.[48]	Altered regulation of the PI3K/Akt/FoxO3a pathway.[49]
M2c	• IL-10, TGF-β, and glucocorticoids.[50]	• Express MMPs. • Express IL-10, TGF-β, and pentraxin-3.[51]	• Vascular remodeling.[52]
M2d	• TLR agonists. • adenosine A2A receptor agonists.[54]	• Suppress inflammatory responses.[55]	• Regulate the expression of IL-10 and VEGF.[56]
M2f	• Phagocytosis of apoptotic cells.[57] • Upregulate TGF-β_1.[58]	• Express anti-inflammatory mediators.[58]	• Regulate vascular permeability.[58]

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and vascular endothelial growth factor (VEGF).[56] M2f macrophages are activated by phagocytosis of apoptotic cells and upregulate TGF-β1.[57,58] They express anti-inflammatory mediators and regulate vascular permeability.[58]

Macrophage Polarization States

Epigenetic changes are paramount to initial inflammatory activation (M1) by TLRs, induction of an IFN response by the microbiome, or LPS; transition from an M1 to a tolerant/M2-like phenotype or to a tolerant DC phenotype after TLR or TNF stimulation; inhibition by IL-10; M2 polarization by M-CSF and IL-4; and/or polarization toward the osteoclast pathway by RANKL (receptor activator of NF-κB ligand).[67–87] Epigenetic transcriptional memory bestows the molecular foundation of polarizing signals into a coherent phenotype, and reprogramming for specific responses to environmental stressors.

The pattern of histone marks behaves like a cryptic message to be "read" by additional chromatin regulators and transcriptional coactivators/corepressors (Co-R) to determine the rates of transcription initiation and elongation. There is an equipoise of positive and negative histone marks at gene promoters and distal regulatory elements (enhancers) which synchronizes transcription rates.

The gene loci relevant for polarized macrophage phenotypes exist in four states (details in Table 3).[30–33]

- Repressed: Closed chromatin conformation shows negative marks on histones; refractory to induction of transcription by stimuli.
- Poised: Both partially open and partially closed chromatin regions. Open regions show activating histone marks and a prebound RNA polymerase II near the transcription start site. Partially closed regions show repressive histone marks and Co-R complexes.
- Active: Open chromatin shows active histone marks and ongoing transcription.
- Deactivated: Nucleosome remodeling with decreased histone acetylation and inactive inflammatory genes.

Table 3: Epigenetic regulation of inflammatory cytokine gene regulation in macrophages

Repressed	In cells that do not express inflammatory cytokines, corresponding gene loci exhibit inaccessible chromatin, occupancy by transcriptional repressors and Co-R, and negative histone marks.
Poised	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. During macrophage differentiation, pioneer factors (PU.1) bind to cytokine gene promoters and enhancers to facilitate the opening of chromatin by nucleosome remodeling, histone acetylation, and promotion of positive methyl marks (H3K4me3 at promoters and H3K4me1 at enhancers). Activating histone marks [H3K4me3, H3K9/14ac (histone H3 acetylated at lysines 9/14)], a chromatin configuration that is at least partially-open, and in some genes, a prebound RNA polymerase II (pol II) located near the transcription start site. Repressive histone marks such as H3K9me3 and H3K27me3, Co-R complexes, and partially closed chromatin (requiring positive histone marks and nucleosome remodeling for transcription factor binding) restrict transcription.
Active	Enhancers of active genes are characterized by occupancy by p300, H3K27-Ac (acetylation of the lysine residue at N-terminal position 27 of the histone H3 protein), and low levels of transcription of noncoding enhancer RNA. Stimulation of macrophages by TLR ligands releases Co-R, increases histone acetylation, and leads to nucleosome remodeling by Brahma-related gene (Brg)-1 and recruitment of signaling transcription factors such as NF-κB. These changes increase active transcription through the recruitment of general transcription factors and RNA polymerase II.
Deactivated	Inflammatory genes are deactivated by occupancy by transcriptional repressors, decreases in histone acetylation by histone deacetylases (HDACs), and nucleosome remodeling by the nucleosome remodeling and deacetylation (NURD) complex that also contains HDACs.

M1 Macrophage Activation

The neonatal immune system has a strong proinflammatory bias.[88–91] The TLR signaling is particularly active with enhanced activity of MAPKs, NF-κB, and the IRFs. These pathways also induce inflammatory cytokines such as TNF, IL-1β, IL-6, IL12, p40, and chemokine CXC ligand 10 that are consistently seen in acute M1 responses.[30,31,33]

In the absence of TLR signaling, inflammatory cytokine gene transcription is restrained ("poised" state) by gene-specific repressive mechanisms. Gene loci inhibited by repressors such as B-cell leukemia-6 and nuclear receptors that engage Co-R complexes (HDACs and histone demethylases) help restrain positive histone marks.[32,74] Inflammatory gene loci also contain negative histone marks H3K9me3, H3K27me3, and H4K20me3; and possibly alter nucleosome positioning and chromatin accessibility of genes such as IL-12b.[67,69,73,92–95] The TLR stimulation can "release" these epigenetic "brakes" via reduced binding of B-cell lymphoma protein 6 and Co-R from gene loci and concomitant activation of demethylases such as Jumonji domain-containing protein (JMJ)-D3, JMJD2d, lysine demethylase 1B, and plant homeodomain finger 2 that erase the negative histone marks H3K27me3, H3K9me3, and H4K20me3.[67,73,93,94]

Induction of a subset of genes that includes IL-6 and IL-12b requires nucleosome remodeling by the ATP-dependent complex BAF (barrier-to-autointegration factor; also termed as the SWItch/Sucrose Non-Fermentable (SWI/SNF), an ATP-dependent chromatin remodeling complex).[68,69] This facilitates recruitment of signaling transcription factors such as NF-κB, an increase in positive histone marks such as H3S10p (histone H3 serine 10 phosphorylation), H4-Ac (histone H4 acetylation), and H3K4me3, and release of paused pol II to promote transcription elongation. Enhancers are also activated, as shown by recruitment of the histone acetyltransferase (HAT) p300, increased histone acetylation, binding of signaling transcription factors, and transcription of enhancer RNA.[24,71,75,96–98]

An inhibitor of bromodomain and extraterminal domain proteins (iBET) disturbs engagement of BET proteins and acetylated histones to block expression of TLR4-induced genes. It has shown efficacy in mouse models of endotoxin toxicity and sepsis.[99] The iBET and related compounds JQ1 and iBET151 suppress the expression of the Myelocytomatosis Viral Oncogene Homolog (Myc) gene.[100] Inhibitors of LSD1, JMJD3, ultrathorax (UTX) histone demethylases, and HDAC inhibitors can also suppress inflammation.[101] Targeting chromatic regulators could also help in gene- and patient-specific therapy. TLR-induced expression of core M1 inflammatory cytokines is transient, and gene expression is subsequently repressed to near-baseline levels. Nuclear receptors, TLR-induced transcriptional repressors ATF3 (activating transcription factor 3), and hairy and enhancer of split (Hes)-1, feedback inhibitors induced by IL-10, and the p50 NF-κB subunit can recruit Co-R complexes that contain HDACs and histone demethylases and decrease gene expression.[32,83,102–106]

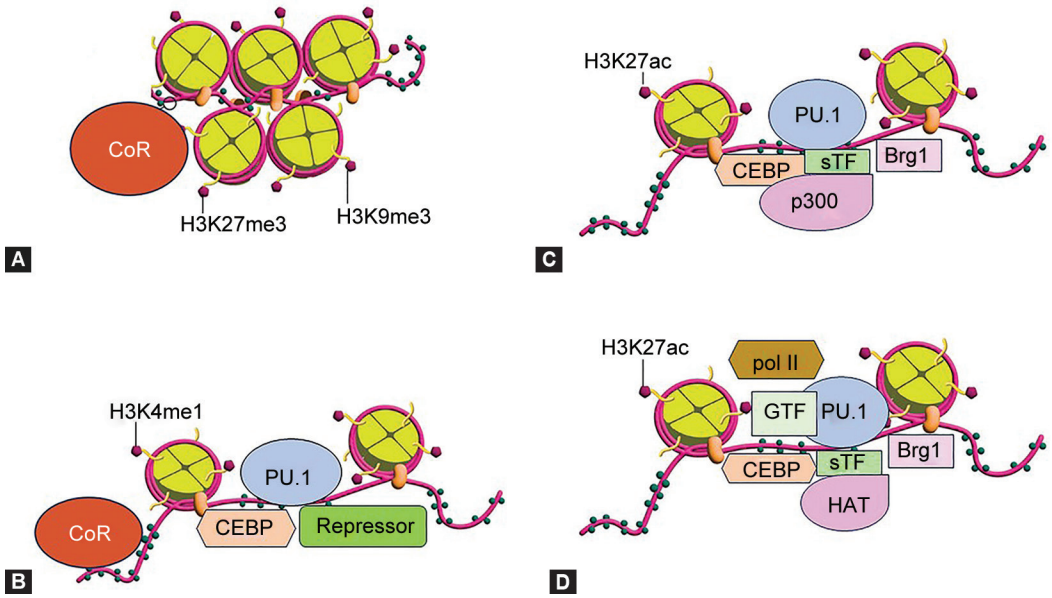
Priming of the M1 State

Interferons prime macrophages for intensified and protracted expression of inflammatory cytokine genes on encountering PAMPs. 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.[107]

Several TLR ligands and TNF induce an autocrine IFN-β–Jak–STAT loop that is an important component of M1 activation.[38,108] HDAC3 plays a key negative role in this process; diminished histone acetylation may be an indirect effect, where repression of genes such as Ptgs1 (prostaglandin-endoperoxide synthase 1) can promote LPS-mediated inflammation.[77] Interferon-γ is the most potent M1-activating cytokine and acts as an enhancer of a TLR-induced inflammatory activation state. Interferon-γ and STAT1 are associated with nucleosome remodeling and opening of chromatin and may prime formation of new enhancers that augment gene expression.[109,110] The HDAC inhibitors can possibly be useful for clinical translation; these suppress induction of various inflammatory and IFN target genes.[111]

M1 to M2 Transition

Acute activation of macrophages by TLR ligands or TNF is transient and is followed by a state of tolerance.[112] Tolerant macrophages exhibit a selective defect in the induction of a subset of genes, including inflammatory cytokine genes, decreased chromatin



Figs 2A to D: Epigenetic regulation of inflammatory cytokine gene loci in macrophages. (A) Enhancer in repressed state; (B) Enhancer in poised state; (C) Enhancer in active state; and (D) Promoter in active state. The PU.1 is a transcription factor that binds the purine-rich PU-box sequence seen near promoters; CoR is a cold-responsive gene; the notations H3K27 indicate methylation of histone H3 protein at the specified lysine (K); CEBP is a member of the CCAAT/enhancer binding protein family of transcription factors; sTF-1 is a putative insulin gene transcription factor; Brg1 is a Brahma-related gene 1-encoded adenosine-5′-triphosphate (ATP)-dependent catalytical subunit of the switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling complexes; p300 is a transcription coactivator that mediates histone 3 lysine 27 acetylation; pol II is a nuclear RNA polymerase; GTF is a glucosyltransferase; and HAT is the histone acetyltransferase 1

accessibility, and lesser recruitment of transcription factors such as p65. Decreased ease of chromatin access can be attributed to decreased TLR-induced recruitment of Brg1-containing nucleosome remodeling complexes, and complex changes in histone acetylation and methylation. Nontolerized genes maintain an open chromatin state. Interferon-γ prevents tolerance by preserving expression of the receptor-interacting protein 140 (RIP140) coactivator and promoting TLR-induced chromatin accessibility upon secondary TLR challenge.[110,113]

A key mediator of the alternative activation of macrophages is the histone demethylase JMJD3, which promotes the expression of the transcription factor IRF4; it removes the negative H3K27me3 marks at the Irf4 locus to achieve these results.[86] In growing infants, JMJD3 might play an important role in increasing nuclear factor of activated T-cells c1 expression and consequently, RANKL-induced differentiation down the alternative osteoclast pathway.[87] The HDAC3 regulates IL-4-induced M2 polarization by deacetylating enhancers of IL-4-induced M2 genes.[85] Histone methylation and acetylation are paramount to M2 polarization.

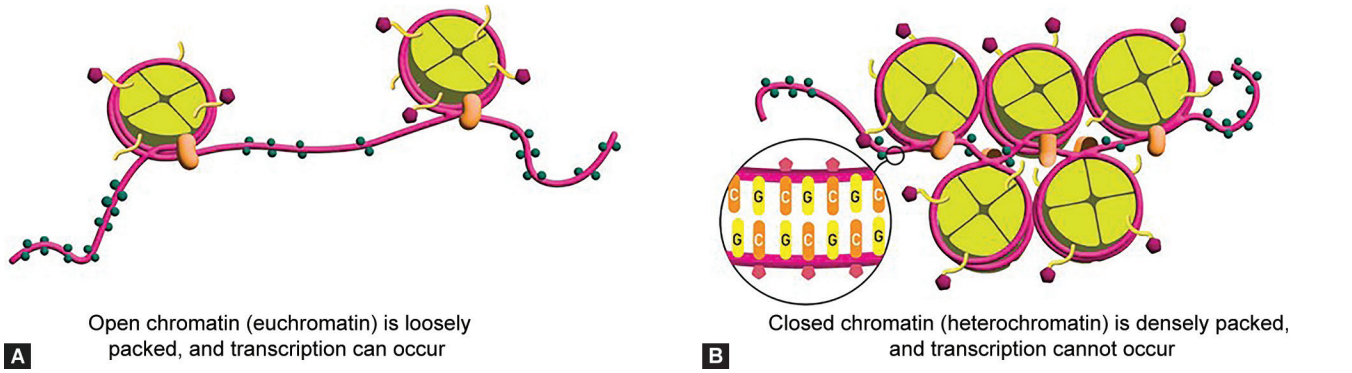
Maturing Macrophages Can Alter Tissue Homeostasis

Tissue-resident macrophages include those derived from the YS progenitors and fetal/adult MDMs. Despite different ontogeny, these cells show intrinsic anti-inflammatory and immunosuppressive functions. Tissue macrophages are primed to respond rapidly to subsequent challenges, maintaining low levels of constitutive IFN-β and downstream JAK–STAT signaling.[107] Commensal microbiota play an important role in IFN expression.[76,114] PU.1 is indispensable to macrophage development in most of the tissues. The enhancer of Spi1, which controls the expression of PU.1, has H3K4me2 and H3K27ac marks in all macrophage populations (Figs 2 and 3).[115]

Microglia are resident macrophages in the brain and spinal cord that are entirely derived from the YS during embryogenesis, as seen in the privacy behind the blood–brain barrier. The Spalt-like transcription factor-1 is a microglia-specific transcription factor regulated by enhancers which are open and active only in these cells.[116] It controls transcriptional regulation that maintains microglial identity and physiological properties as a critical factor for homeostasis in the brain.[117]

The intestine contains the largest pool of macrophages among the tissues.[118] Embryonic precursors seed the intestinal mucosa and multiply substantially in the neonatal period.[119] Bone marrow-derived monocytes begin to replace the original macrophages around the time of weaning, and these differentiate locally into mature tissue macrophage populations in the lamina propria to help promote gut homeostasis. Intestinal macrophages are enriched for RUNX (Runt-related transcription factor) family motifs; RUNX3 is highly expressed in these cells.[116] The gut microbiome is an important regulator of the macrophage populations in the intestine (Fig. 1).

Alveolar macrophages differentiate from fetal liver monocytes depending on the colony-stimulating factor 2 (CSF2; GM-CSF) through the induction of peroxisome proliferator-activated receptor-γ.[120] Embryonic macrophages and fetal monocytes colonize the developing lung.[121] These macrophages can be seen in the saccular stage of lung development, when fetal monocytes, not embryo-derived macrophages, are the main precursors of alveolar macrophages. Transcriptional inhibitors BTB Domain And CNC Homolog 2 (Bach2) and also Bach1 promote alveolar maturation.[122] The long noncoding RNA (lncRNA) MEG3-4 (maternally expressed gene 3–4) is a tissue-specific regulator of inflammatory responses in alveolar macrophages during bacterial infections through the transcriptional regulation of immune response genes.[123]



Figs 3A and B: Chromatin condensation state affects gene expression. (A) Euchromatin state: stimulation with a pathogen/danger signal demethylates DNA, decondenses chromatin, and makes these genes accessible for transcription; (B) Heterochromatin state: chromatin housing the immune response genes in naïve macrophages is highly condensed and inaccessible due to high DNA methylation and is either not transcribed at all or at very low levels. [Some components of the figure were adapted with permission from *Singh S, Frydrysiak-Brzozowska A, Ayad AEB, et al. A primer on epigenetic changes: The more we know, the more we find in fetuses and infants. Newborn 2024;3(3):219–232]*

Splenic macrophages begin to appear in growing infants. The mature spleen in growing infants shows three typical zones—the marginal zone (MZ), red pulp, and white pulp, and each contains specific macrophage subpopulations.[124] The MZ macrophages that differentiate under the influence of the expressing nuclear receptor Liver X receptor alpha remove apoptotic cells.[125,126] These macrophages express transcription factor Spi-C, a PU.1-related transcription factor.[127]

The abdominal cavity shows at least two distinct macrophage subtypes.[128] Large peritoneal macrophages contribute to most of the peritoneal cavity macrophages, but these are rapidly replaced by smaller macrophages after stimulation. This new population of macrophages efficiently removes apoptotic cells. The localization and polarization of peritoneal macrophages is regulated by the retinoic acid-induced GATA-binding factor 6, a specific transcription factor that alters the transcriptional and epigenetic properties of these cells.[129] A key downstream regulator is the retinoic acid receptor beta (Rarb) gene, which promotes H3K27ac specifically in peritoneal macrophages.[26] The Rarb gene enhancer is poised in other tissues but remains active in peritoneal macrophages because of locally produced retinoic acid.[130]

Epigenetic Modifications in Macrophages during Inflammation

Changes in macrophages can cause a broad spectrum of maladaptive immunity and inflammation that are causative factors of disease and thus represent key therapeutic targets.[131] Immune response associated with sepsis is greatly impacted by macrophage epigenetic landscape. For instance, HDAC6 inhibitors decrease proinflammatory mediators, inhibit macrophage apoptosis, and promote bacterial clearance.[132] The expression of several microRNAs (miRNAs) is altered in neonatal sepsis and could be possible therapeutic targets. For instance, miRNA-Let7A (let-7a) is decreased in sepsis due to gram-negative bacilli; it regulates the TLR4-mediated sepsis-induced inflammatory response.[133] The lncRNA Nuclear Enriched Abundant Transcript 1 is upregulated in sepsis. Decreased macrophage levels of microRNA-141 also increased the inflammation.[3]

Macrophage functions are altered in hypoxic–ischemic encephalopathy, bronchopulmonary dysplasia, necrotizing enterocolitis, retinopathy of prematurity, and renal failure. Macrophage epigenetics can show prognostic markers and might provide useful immunomodulatory treatments for neonates, and even adults.[3,134,135]

IIM in Macrophages

The IIM of macrophages impacts immune responses to ensuing stimuli. It is classified as tolerance and training. It is also seen in in tissue-resident mononuclear phagocytes such as microglia.[136] In trained immunity, the first stimulation induces long-lasting histone marks. Later hits alter macrophage properties with H3K27ac and H3K4me3 and induce tolerance.

Tolerance

Acute inflammatory TLR activation of macrophages is a transient, tightly-regulated process.[112] The activation state is transient and is followed by tolerance, indicating that this is a self-limited response to stimuli despite sustained action of the agonist.[4] The TLR-induced responses include selective and transient silencing of proinflammatory genes and the priming of the second-class genes of M2 activation.[81] Following the first LPS exposure, anti-inflammatory genes are altered in the second stimulation, thereby increasing the efficiency of innate host defense. This LPS tolerance is mainly controlled by epigenetic regulation, including nucleosome remodeling, reduced recruitment of transcription factors and chromatin remodeling complexes, and histone modifications.[137] This process involves silencing of proinflammatory and priming of anti-inflammatory genes in tolerant macrophages through differential alteration of chromatin at promoters of pro- and anti-inflammatory genes. The NF-κB-associated inhibitory mechanisms can lead to tolerance by recruitment of the NCOR-HDAC3-P50 repressive complex into targeted genes.[107] It can also recruit histone methyltransferase G9a to promoters to induce H3K9 methylation and binding of the heterochromatin protein 1, leading to epigenetic silencing.[138]

Noncoding RNAs, especially miRNAs, can regulate macrophage tolerization. The miRNA-146a induces TLR signaling tolerance following a primary stimulus with Myeloid differentiation primary response 88-dependent TLR pathways.[139] The miRNA-221 and miRNA-222 regulate BRG1 and, consequently, functional reprogramming of macrophages during LPS tolerization.[140]

Trained Immunity

The traditional view that only the adaptive immune system can build immunological memory no longer holds true at present. In

organisms lacking adaptive immunity, such as invertebrates, the innate immune system can mount long-term memory for resistance to reinfection.[141]

Microglia develop a "trained" IIM after repeated exposures to infectious stimuli.[142] This epigenetic reprogramming is marked by higher H3K4me1 levels and TF binding on the promoters of "latent" enhancers, which revert to baseline after cessation of stimulation.[143] The progressively stronger cellular responses with repeated stimulation have been further linked with increased H3K4me3 on promoters of canonical mediators, such as the TLR-activated adaptor Myd88 and downstream cytokines, TNF, IL-6, and IL-18.[63]

Trained immune genes interact with lncRNAs through βG and its receptor dectin-1. The upstream master lncRNA of inflammatory chemokine loci form chromosomal contacts at the promoters of the key chemoattractants such as ELR$^+$ CXC-ligand (CXCL) chemokines – IL-8/CXCL8, CXCL1, CXCL2, and CXCL3, and *cis*-directs the protein complex of WD repeat-containing protein 5 (WDR5) and mixed lineage leukemia 1 (MLL1), facilitating H3K4me3 epigenetic priming before transcriptional activation.[144–146]

Innate immunity training plays a vital part in disease prevention. Bacillus Calmette–Guérin (BCG) can be used to train macrophages *ex vivo* and thereby provide cross-protection.[147] The ability of macrophages to transmit memory phenotypes to offspring and provide sustained protection remains unclear, mainly due to the short lifespan of macrophages. Long-term IIM is vital to developing a robust immunity.

Epigenetic Mechanisms of IIM in Macrophages

Emerging evidence shows macrophages show and mature with epigenetic memory of recent inflammatory exposures. In one study, an epigenetics compound library was screened that affects trained immunity or LPS tolerance in macrophages using TNF as a readout.[148] The investigators tested 181 compounds, where 1 showed suppressive effects and 2 promoted β-glucan (βG)-trained TNF production. In contrast, inhibitors of Aurora kinase, histone methyltransferase, histone demethylase, HDAC, and DNA methyltransferase suppressed LPS tolerance. Several proteins previously unknown to be involved in IIM, such as O^6-methylguanine-DNA methyltransferase, Aurora kinase, lysine-specific histone demethylase 1 (LSD1), and protein arginine methyltransferase 5 were revealed. Protein network analysis revealed that the trained immunity targets are linked via Trp53, while LPS tolerance targets form three clusters of histone-modifying enzymes, cell division, and base-excision repair. In trained immunity, the SETD7 [Su(var)3-9, Enhancer-of-zeste and Trithorax (SET) domain-containing 7, histone lysine methyltransferase] was identified, and its expression was increased during βG treatment. The LPS priming increased LSD1 expression, whereas siRNA-mediated reduction resulted in increased expression of IL-1β in LPS tolerance. This study confirmed the importance of epigenetic modifications in IIM and provided potential novel targets for intervention.

In *Candida* infections, macrophage memory is trained by βG-induced epigenetic changes such as enhanced trimethylation of histone H3 at lysine 4 (H3K4me3).[149,150] This H3K4me3 marker is located on the promoter regions of IL-6, TNF, and IL-1β, and has been associated with increased expression of these cytokines, thereby enhancing the response to secondary stimulation in infections.[150–152] H3K4me3 is also upregulated following *in vitro* stimulation of human monocytes trained by oxidized low-density lipoprotein. These cells show TLR-2 and TLR-4 activation with higher expression of IL-6, IL-8, IL-18, and TNF, monocyte chemoattractant protein 1/CC-ligand 2, and matrix metalloproteinases.[153]

Kleinnijenhuis et al. also reported that the protective effect of monocytes against secondary reinfection after primary BCG vaccination in healthy volunteers was mediated by H3K4me3, accompanied by the production of the inflammatory cytokines IFN-γ, TNF, and IL-1β increasing several-fold in response to infections by nonspecific bacterial and fungal pathogens.[152] Moreover, the inhibition of histone methyltransferase was found to be associated with a significant reduction in BCG-induced macrophage memory.

A stable differential DNA methylation pattern was observed in peripheral blood mononuclear cells (PBMCs) isolated from BCG-vaccinated individuals compared with counterparts without BCG vaccination. Gene ontology analysis revealed that promoters with altered DNA methylation patterns were strongly enriched among immune-related genes, thereby enhancing the resistance of macrophages to mycobacteria.[154,155] In another study, a global methylation analysis of PBMCs showed more than 1,000 differentially methylated regions (DMRs) between tuberculosis patients and healthy controls. After completion of treatment, the number of DMRs increased to nearly 4,000; most of these genes were associated with the autophagy-related pathways.[156] When alveolar macrophages were infected with *Mycobacterium tuberculosis*, a distinct DNA methylation profile enriched in the pentose phosphate pathway, T-cell migration, and IFN-γ production pathways was seen.[157]

Macrophage Epigenomics

Transcriptomes and the epigenomes of three populations of macrophages derived from healthy human monocytes *in vitro* were analyzed. These cells were maintained in culture in 10% pooled human serum for 6 days. In the first 24 hours, the monocytes were maintained as control or were exposed to βG or LPS. On the 6th day, βG-treated cells were consistently proinflammatory with enhanced efficiency in phagocytosis.[158] Hence, these were viewed as "trained."[159] In contrast, LPS-exposed MDMs showed downregulation of IL-6 and TNF expression and were labeled as "tolerant."[63] In both instances, these 6th day macrophages showed some functional memory of the stimuli.

In human studies, a part of the macrophage genome begins to show nucleosome-borne epigenomic dynamics following exposure to PAM patterns. The three major macrophage subtypes (M0, M1, and M2) show consistent differences in the transcriptome (Fig. 1). Controls, trained, and tolerant macrophages display transcriptomic differences at all levels, including interactions with extracellular matrix, signal receptors, metabolite transporters, organelle and cytoplasm constituents and signaling pathways, RNA processing, and transcription factors. About 200 TFs, 100 kinases, and 20 epigenetic enzymes were differentially expressed following monocyte-to-macrophage differentiation.[147] For instance, monocytes uniquely express lysine (K)-specific demethylase 6B (KDM6B; also called JMJD3). The E-box binding C2H2 (cysteine-2/histidine-2) zinc finger TF Snail family transcriptional repressor 1 (SNAI1) of monocytes is largely replaced by its SNAI3 paralog in macrophages. The NF-κB regulator IRAK3 (IL-1 receptor-associated kinase 3) is epigenetically upregulated in LPS-paralyzed macrophages; the G-coupled receptors such as the Adora receptors and many cAMP (cyclic adenosine monophosphate) signal transduction factors are expressed differentially in control, βG-trained, and trained macrophages.[147]

Conclusion

Epigenetic changes are an important mechanism by which macrophages differentiate into various functional subgroups. The evidence for plasticity between M1 and M2, and within the M2 subgroups supports this construct.[160] However, M1 macrophages do not show consistent reversibility in responses following IL-4 treatment.[161] Trained cells show considerable remodeling in metabolic pathways, as seen with increased glycolytic capacity, in some steps in the tricarboxylic acid cycle, and in cholesterol metabolizing enzymes.[147] These metabolic changes likely affect chromatin modifications through altered availability of the writers and erasers of histone modifications.[162–165] Macrophages are particularly important in the immune profile of neonates, and hence, further work is needed to understand the molecular mechanisms of activation of various subcategories of these cells.[166–169]

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