Macrophages were initially described by Élie Metchnikoff in the late 19th century as phagocytic cells responsible for pathogen elimination, tissue development, and wound repair in many organisms ranging from invertebrates to vertebrates. Today, beyond the classical functions described by Metchnikoff, macrophages are well-recognized key regulators of both innate and adaptive immunity, as well as important mediators of systemic metabolism, hematopoiesis, vasculogenesis, apoptosis, malignancy, and reproduction. Work during the past decade has led to the appreciation that macrophages can assume diverse phenotypes, a finding that is perhaps not surprising given the numerous functions that these cells subserve. Although it is probably more accurate to view macrophages as a continuum of phenotypes with overlapping expression of cell surface markers, secreted cytokines and chemokines, and transcriptional regulators, the current model was developed for purposes of providing a simple framework for discussion. In this model, macrophages are classified into 2 major subtypes termed M1 (inflammatory or classically activated) macrophages and M2 (alternatively activated) macrophages.

In 1970, interferon (IFN)-γ was the first identified macrophage-activating factor. A soluble cytokine, IFN-γ is produced by activated CD4+ T helper (Th) 1 cells, CD8+ T cytotoxic 1 cells, and natural killer cells, and converts resting macrophages into potently activated cells with enhanced antigen-presenting capacity, increased synthesis of proinflammatory cytokines and toxic mediators, and augmented complement-mediated phagocytosis. This initial description of macrophage activation became known as classical activation or M1. Later, bacterial lipopolysaccharide (LPS) was found to promote the generation of M1 macrophages via the toll-like receptor 4 (TLR4) and autocrine production of IFN-β. Typical M1 markers include inducible nitric oxide synthase (iNOS), interleukin (IL)-1β, tumor necrosis factor (TNF)α, IL-12, and other typically proinflammatory products. Although M1 macrophages are essential for host defense, their functions can be usurped in disease states to promote wanton inflammation. For example, in the setting of metabolic disorders, such as obesity, various lipid products can activate M1 macrophages via TLR4. This low-level activation of macrophage contributes to the development of insulin resistance and glucose intolerance.

Th2 responses are the immunologic counterpart of the Th1 response, are mediated by CD4+ Th2 cells, which produce IL-4 and IL-13. Both IL-4 and IL-13 share ligand-binding receptor complexes IL4Rα and are believed to have overlapping downstream signaling. In contrast to IFN-γ, IL-4-mediated and IL-13-mediated immune responses are typically characterized by eosinophilia, basophilia, mastocytosis, enhanced B-cell class switching, and antibody production. Th2 responses are essential for the elimination of extracellular parasites, but also contribute to allergy, increased susceptibility to other pathogens, and complications of infection. In contrast to IFN-γ–mediated classical activation, IL-4 inhibits the respiratory burst and the production of IL-1β in macrophages. Thus, the concept of alternative activation or M2 emerged as an IL-4–mediated inflammatory response promoting recruited macrophages to acquire a unique repertoire of secreted cytokines and phagocytic receptor. Additional factors associated with alternative macrophage activation include glucocorticoids, IL-10, and immunoglobulin complexes, suggesting great

Key Words: alternative activation • classical activation • macrophage • M1 • M2
Signal Transducers and Activators of Transcription

IFN-γ was the first cytokine identified to induce M1 polarization.1 The seminal work of Darnell, Kerr, and Stark in the 1990s first described the Janus kinases (JAK)-signal transducers and activators of transcription (STAT) pathway of signal transduction through which IFNs acting on a cell surface receptor can induce the transcription of genes within the nucleus.18 Binding of IFN-γ to its receptor triggers JAK 1/2–mediated tyrosine phosphorylation and subsequent dimerization of STAT1 (Figure A).19 The STAT1 homodimer engages cis-elements known as γ-IFN–activated sites in the promoter region of target genes, including iNOS and IL-12.18 STAT1 activation additionally results in phosphorylation of a critical serine site on its C terminus required for maximal transcriptional function.20 Subsequent studies have demonstrated STAT1 and STAT2 activation in response to type I IFNs (α and -β) influencing macrophage polarization.21 Additional STAT isoforms, including STAT3 and STAT6, also modulate macrophage polarization.22

STATs are involved in LPS-mediated M1 polarization via activation of TLR4.23 TLR4 mainly promotes nuclear factor κB (NFκB)–mediated transcription of target inflammatory genes as discussed in further detail below. In response to LPS, autocrine production of IFN-β activates the type 1 IFN receptor triggering STAT1 and STAT2 phosphorylation. The STAT1-STAT2 heterodimer recruits IFN-recognition factor (IRF) 9 as part of the IFN-stimulated gene factor 3 complex to bind cis-elements known as IFN-stimulated response elements.18,24–27 More recently, Lawrence et al28 described the interaction of STAT1 and IRF5 to induce M1 polarization. The crucial role of STAT1 in M1 polarization has been further studied in the setting of STAT1 deficiency. Additionally, mice bearing serine mutation of STAT1 (Stat1S727A) are severely impaired in clearance of intracellular pathogens and demonstrate increased mortality on infection with Listeria monocytogenes.29 STAT1-deficient macrophages clearly show that gene expression induced by both type I IFNs (α- and -β) and type II IFN (γ) is dependent on STAT1. Importantly, impaired STAT1 signaling abrogates the expression of IFN-β to further repress the M1 phenotype.20

The role of STAT2 in macrophages has also been studied using STAT2-deficient mice.21 STAT2-null macrophages lose type I IFN paracrine/autocrine responsiveness, rendering these mice remarkably sensitive to viral infection.21 Type I IFN–induced STAT1 homodimerization in the absence of STAT2 promotes γ-IFN–activated site-dependent gene expression, such as major histocompatibility complex (MHC) I and IFR1. Interestingly, MHC II expression is also potently induced in response to IFNα in these mice because of loss of suppressor of cytokine signaling (SOCS) 1–mediated negative feedback inhibition of the type I IFN response. SOCS1 impairs the interaction of the JAK catalytic domain with STAT2, thereby halting signal propagation.22 Therefore, STAT2 is necessary for IFNα-mediated SOCS1 expression to prevent unchecked MHC II expression.29

STAT3 is the key transcription regulator of IL-10, a major anti-inflammatory mediator.30 IL-10 binds the IL-10 receptor complex, resulting in JAK1-mediated activation of STAT3 and repression of proinflammatory cytokines, including TNFα, IL-1β, IL-12, and IFNγ. IL-10 has also been shown to be an important mediator of inflammation resolution. Murine studies of STAT3 knockout in macrophages highlight the anti-inflammatory role of STAT3; these mice have impaired bactericidal activity and develop enhanced production of IL-12, IL-6, TNFα, IL-1β, IFNγ, as well as IL-10 in response to LPS.31 Furthermore, IL-10 derived from regulatory T-cells induces STAT3 activation and drives M2 polarization in mice with severe combined immunodeficiency, which results in suppression of inflammatory cytokine expression.30 Alternatively, STAT3 activation by IL-6 and INFβ promotes a proinflammatory phenotype. A negative regulator of STAT3 activity, SOCS3 is induced by inflammatory products, including TNFα and IL-1β, and can either inhibit JAK kinase activity or promote ubiquitin-mediated degradation of the transcription factor complex. Interestingly, SOCS3 does not inhibit IL-10–mediated STAT3 activity, thus perpetuating the anti-inflammatory milieu.22,23 The mechanisms underlying the dichotomous role of STAT3 in macrophage activation is not completely understood and may be context dependent. Further investigation is needed.

STAT6 is the key transcription factor in IL-4 and IL-13–mediated M2 polarization. The IL-4 and IL-13 receptors share a key signal transducer, IL-4 receptor-α (IL4Rα; Figure B).30,31 Ligand binding triggers tyrosine phosphorylation on the IL4Rα cytoplasmic tail to facilitate recruitment and subsequent tyrosine phosphorylation of STAT6 by JAK1/JAK3 or JAK1/Tyk2 in the case of IL-4 or IL-13 binding, respectively. Subsequent homodimerization of STAT6 results in...
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- AP-1 indicates activated protein-1; Arg1, arginase-1; C/EBPβ, CCAAT/enhancer-binding proteins; GR, glucocorticoid receptor; HIF, hypoxia inducible factor; HRE, hormone response element; IFN, interferon; IL, interleukin; iNOS, inducible NO synthase; IRF, interferon-recognition factor; Jmdj3, Junmji domain containing-3; KLF, krüppel-like factor; miR, microRNA; NCoR, nuclear receptor corepressor; NFκB, nuclear factor κB; NO, nitric oxide; PPAR, peroxisome proliferator–activated receptor; SMRT, silencing mediator of retinoic acid and thyroid hormone receptor; STAT, signal transducers and activators of transcription; TLR, toll-like receptor; and TNF, tumor necrosis factor.
recruitment of IRF4 and activation of target gene promoters to initiate transcription. Similar to most other STAT proteins, STAT6 recognizes γ-IFN–activated sites. Normally, γ-IFN–activated sites consist of a palindromic sequence separated by a 3-bp spacer (TTCCNNNGAA; N3 site). STAT6 instead displays greater preference for sites with 4-bp spacers (TTCCNNNGAA; N4 site). Many of the genes associated with mouse M2 macrophages are regulated by STAT6, including Arg1, macrophage mannose receptor 1 (Mrc1; also known as Cd206), resistin-like-α (Retnla; also known as Fizz1), and chitinase 3-like 3 (also known as Ym1). Moreover, STAT6 is indispensable for IL-4–mediated inhibition of many inflammatory genes. STAT6-deficient mice lose IL-4–mediated suppression of the IFNγ-STAT1 pathway, resulting in augmented chemokine (monokine of IFNγ) production. Additionally, STAT6-deficient mice lose the IL-4–induced Th2 response, illustrating the essential role of STAT6 in IL-4 signaling in vivo. Similarly, a murine model of myeloid cell–specific IL4Rα knockout lacks M2 polarization in response to helminth infection and models of Th2 cell–mediated inflammation.

Additionally, endogenous inhibitors of STAT proteins, SOCS proteins inhibit the JAK-STAT pathway by negative feedback inhibition of cytokine signaling. Recent work highlights the importance of SOCS2 and SOCS3 proteins in macrophage polarization. Spence et al demonstrate that these SOCS proteins reciprocally regulate cytokine expression and macrophage polarization in response to endotoxin stimuli. LPS-stimulated SOC2-deficient mice elaborated significant levels of IL-6 and TNFα with markedly reduced expression of IL-10, whereas the opposite effect was seen in SOCS3-deficient macrophages (elevated IL-10 and minimal IL-6 and TNFα expression). Flow cytometric assessment of macrophages from these mice demonstrated a bias toward M1-macrophage polarization in SOCS2-deficient mice.
whereas SOCS3-deficient macrophages express surface markers associated with M2-macrophage polarization. Additionally, stimulation of these macrophages does not alter their phenotype. Mechanistically, macrophages of SOCS2-deficient mice have enhanced STAT1 phosphorylation in response to LPS stimuli, whereas SOCS3-deficient macrophages exhibit constitutive phosphorylation of STAT6, further enhanced by IL-4 and IL-13 stimulation. Activation of the STAT proteins in the absence of SOCS was associated with enhanced promoter binding and activation of typical macrophage markers; SOCS2 macrophage-deficiency was associated with enhanced STAT1 activation and increased binding to Nos2 and Tnfa; SOCS3 macrophage deficiency was associated with enhanced STAT6 activity and attenuated binding to Arg1 an CCL17 promoters.22

Nuclear Factor κB

NFκB orchestrates the expression of many inflammatory genes in response to various physiological and environmental stimuli.37 Under basal conditions, NFκB is maintained in inactive form by 1kB. In response to inflammatory stimuli, 1kB phosphorylation liberates NFκB whose exposed nuclear localization sequence directs the transcription factor to the nucleus for target gene expression.37 LPS, a stimulus of NFκB signaling, binds LPS-binding protein to deliver the LPS ligand to the high-affinity receptor, CD14.38 TLR4 in conjunction with the small extracellular protein MD2 interacts with the CD14–LPS complex to activate intracellular signaling via NFκB.23 TLR4 signaling proceeds through myeloid differentiation primary response gene 88 (MyD88)-dependent and MyD88-independent (TRIF-dependent) pathways. Based on studies using MyD88-deficient macrophages, the MyD88-dependent pathway was shown to be responsible for proinflammatory cytokine expression, whereas the MyD88-independent pathway mediates the induction of type I IFNs and IFN-inducible genes. Both MyD88-dependent and MyD88-independent pathways activate 1kB kinase (IKK) to phosphorylate 1kB and liberate NFκB. Additionally, LPS promotes the feed-forward signaling of NFκB by inducing autocrine cytokines, IL-1β and TNF-α, to sustain NFκB activity (Figure A).23

In addition to recognizing bacterial wall components, such as LPS derived from Gram-negative bacteria, TLRs likely recognize endogenous pathogenic stimuli that promote tissue injury and inflammatory disease. For example, the abnormal accumulation of proatherogenic low-density lipoproteins, saturated fatty acids, and amyloid precursors can activate TLR4 and TLR2 and subsequently NFκB. Such aberrant induction of inflammatory pathways is associated with the development of atherosclerosis, insulin resistance, and Alzheimer disease.6

In addition to activation of IKKs, LPS also activates mitogen-activated protein kinases, which ultimately triggers activation of another transcription factor activated protein-1 (AP-1). Moreover, mitogen-activated protein kinase–independent IKKe-mediated phosphorylation of AP-1 has been reported to be dependent on p65, a key member of NFκB.39 The cross-talk between NFκB and AP-1 signaling pathways will be discussed in the corepressor section. Thus, NFκB signaling plays an essential role in LPS-induced macrophage M1 polarization. Other pathogens, like Gram-positive bacteria, virus, also activate IKK–NFκB cascades through MyD88 or TRIF/TIRAP pathways. Many M1 genes have xB sites in their promoter region, including iNOS, monocyte chemoattractant protein-1, (CCL2), cyclooxygenase-2, and regulated on activation, normal T expressed and secreted (CCL5) among others.39

A central regulator of inflammation and immunity, the NFκB system is equipped with several negative modulators. The NFκB family consists of 5 members: NFκB1 (p105/p50), NFκB2 (p100/p52), RelA (p65), RelB, and c-Rel, whose homo- and heterodimerization are associated with differential regulation of target genes. The p50 and p52 homodimers function as repressors because of their lack of a transcription activation domain, present in RelA, RelB, v-Rel, and c-Rel.40 In vivo murine studies reveal p50 NFκB-deficiency results in exacerbation of M1-driven inflammation after endotoxin challenge and impaired ability to mount adequate M2-driven immunity during parasitic challenge.40 Peripheral monocyte/macrophages in patients who are septic exhibit accumulation of p50 NFκB-homodimers and is thought to mediate LPS tolerance.41 Additionally, significant levels of p50 NFκB-homodimers found in M2-like tumor-associated macrophages of murine fibrosarcomas suggest that these transcriptional repressors orchestrate the M2 phenotype and repress expression of M1 cytokines.42 Studies from p50-deficient mice revealed that p50 NFκB–deficient macrophages challenged with LPS exhibit enhanced mRNA expression and protein secretion of IFN-β, elevated STAT1 phosphorylation, and augmented induction of inflammatory genes (eg, iNOS). In vitro, macrophage-specific p50-deficiency revealed impaired Pol II recruitment to M2-associated gene promoters, including Arg1 and CCL17, whereas enhanced recruitment to M1-gene promoters resulted in increased expression of iNOS, IFN-β, and TNF-α after LPS challenge.40 In summary, NFκB signaling is a key transcriptional regulator of both M1 and M2 polarization.

Activated Protein-1

Another major proinflammatory pathway within macrophages involves the JNK/AP1 pathway whose proinflammatory targets overlap those of the NFκB pathway.43 AP1 is a group of basic leucine zipper transcription factors, including the Fos and Jun families of transcription factors.37 High-affinity AP1 isoforms predominately exist as c-Fos/c-Jun heterodimers, whereas c-Fos homodimers have less affinity for AP1 sites. Inflammatory stimuli lead to phosphorylation and subsequent activation of JNK. Active JNK phosphorylates the N terminus of c-Jun on target genes leading to c-Jun/c-Fos heterodimerization and ultimate transactivation of proinflammatory genes. TNFα produced on TLR4 stimulation in classically activated macrophages promotes a positive-feedback loop to further stimulate AP1 activity via JNK activation (Figure A).43 In M1 macrophages, there is much overlap between the NFκB and AP1 pathways, including activation by the same extracellular stimuli, mitogen-activated protein kinase pathway–induced activation of JNK and IkB and overlap in downstream gene activation, suggesting cooperative transcription factor activity.44 In fact, accessory proteins can promote the activity of both pathways.

IFN Regulatory Factors

There are 9 IRFs in mammals (IRF1-9) implicated in IFN production, regulation of cell growth, and IFN-induced gene
expression. IRFs were originally described as regulators of type I IFN expression and signaling. However, it is now well established that they have additional important functions, including the regulation of macrophage activation.

The role of IRFs in M1 polarization has been well studied. IRF9 is the component of the tertiary complex IFN-stimulated gene factor 3 that is formed in type I IFN–treated cells to induce transcription. Stimulation of TLR4 by LPS, or TLR3 by dsRNA, also activates IRF3 through the MyD88-independent pathway and induces transcription of IFN-β gene to form the type I IFN loop for optimal M1 activation of macrophage (Figure A). IRF5 has been shown to be required for optimal expression of IL-12 and proinflammatory cytokines, a hallmark of M1-macrophage polarization.

Recently, IRF4 was shown to specifically regulate M2-macrophage polarization in response to hemointhic infection as well as exposure to a fungal and parasitic cell wall component, chitin. This pathway involves Jumonji domain containing-3 (Jmjd3), a histone 3 Lysine 27 demethylase (H3K27) whose absence completely abrogates M2 polarization in mice exposed to chitin or parasitic infection, demonstrating a role for the epigenetic modulator in M2 macrophages. Jmjd3 was found to regulate expression of the transcription factor IRF4, which in turn is required for M2 polarization of macrophages in vitro and in vivo. Chartouni et al also demonstrate a role for IRF4 in alternatively activated macrophages using cytokine stimulation with IL-4. STAT6-mediated signaling by IL-4 results in enhanced expression of IRF4. IRF4-deficient macrophages likewise stimulated show diminished expression of genes associated with the M2 phenotype, including Il1rn. Il1rn encodes IL-1R antagonist, whose expression in classically and alternatively differentiated macrophages is reduced and augmented, respectively.

Hypoxia Inducible Factors

Hypoxia is a characteristic feature of the tissue microenvironment during bacterial infection and tumor growth. The effectiveness of macrophages in innate defense reflects their capacity to function in low-oxygen environments. Therefore, it is not surprising that hypoxia inducible factors (HIF) play important roles in macrophage polarization. Two isoforms of HIFα, HIF-1α and HIF-2α, are differentially activated under hypoxia, HIF-1α acts and HIF-2α limits nitric oxide production by induction of Arg1, an enzyme competing for ω-arginine substrate availability. Via Arg1, HIF-2α induces ornithine and polyamine or urea production. Thus, HIF-1α and HIF-2α act antagonistically in terms of M1/M2 polarization by inducing iNOS and Arg1 expression.

**Peroxisome Proliferator–Activated Receptors**

The peroxisome proliferator–activated receptor-γ (PPAR-γ) is a member of the nuclear receptor superfamily of ligand-dependent transcription factors predominantly expressed in adipose tissue, adrenal glands, and the spleen whose activity inhibits M1 polarization. The pioneering work of Christopher K. Glass’ laboratory demonstrated that PPAR-γ activation inhibits 3 important classes of transcription factors operative during macrophage polarization: STAT, NFXb, and AP-1. The ligand-dependent inhibitory effects of PPAR-γ on inflammatory gene transcription is attributed to nuclear receptor co-repressor-corepressor complex stabilization on the promoter of target genes, which blocks NFXb-mediated gene transactivation, discussed in further detail below.

PPAR-γ is constitutively expressed at low level in macrophages, but its expression can also be induced by IL-4 and IL-13, indicating a potential role for this NF in M2 polarization in the setting of Th2 cell responses. Interestingly, a recent study has shown a crucial role for STAT6 as a cofactor in PPAR-γ-mediated gene regulation in vitro; therefore, crosstalk between PPAR-γ and the IL-4–STAT6 axis might coordinate regulate the M2 phenotype (Figure B). PPAR-γ–deficient macrophages are resistant to M2 polarization, and mice lacking PPAR-γ develop obesity and insulin resistance when challenged with a high-fat diet. The metabolic phenotype is attributed to altered glucose metabolism in muscle and inflammation in adipose tissue, demonstrating a protective role for alternatively activated macrophages in obesity-associated metabolic disease. Although the identities of the endogenous ligands for PPAR-γ are still not clear, it should be noted that synthetic PPAR-γ ligands have been shown to have therapeutic applications in diseases in which activated macrophages play prominent pathogenic roles, such as diabetes mellitus and atherosclerosis.

PPAR-δ activity similarly induces a protective M2 phenotype in metabolic tissues, and its expression is also induced by the IL-4–STAT6 pathway. Similar to PPAR-γ deficiency, PPAR-δ deficiency results in inhibition of M2 differentiation and development of obesity, insulin resistance, and hepatosteatosis. Therefore, the protective role of the PPAR family of transcription factors is evident in models of inflammatory models of metabolic disease.

**Glucocorticoid Receptors**

Glucocorticoids (GC) are well-known regulators of gene transcription that interact with homodimeric nuclear receptors at hormone response elements near target genes. GCs are released from adrenal glands in response to diverse stressors, including infection, pain, starvation, and trauma. Under physiological conditions, GCs circulate in circadian fashion and are necessary for maintenance of homeostatic function. The M2c subpopulation of M2 macrophages develops in response to GC
stimulation and is involved in phagocytosis of apoptotic cells necessary for wound healing and resolution of inflammation.58 GC-treated human macrophages limit tissue damage by augmenting phagocytosis of proinflammatory particles, including cellular debris and microbial products. Affymetric analysis of 4 human donors demonstrated significant upregulation of anti-inflammatory genes (IL-4, IL-10) and downregulation of pro-inflammatory genes (IL-1, IL-6, and TNFα) in macrophages in response to GC treatment.59 Among these genes, CD163 and IL-1RII were upregulated, whose function includes clearance of proinflammatory hemoglobin–haptoglobin complexes and inactivation of IL-1, respectively.59

Furthermore, activation of glucocorticoid receptors (GRs) impairs TLR4 signaling via inhibiting the function of both AP1 and NFκB to ultimately suppress inflammatory signals. In macrophages, GRs alter activation of mitogen-activated protein kinase and mitogen-activated protein kinases in the activation of AP1 proteins and additional transcription factors. Glucocorticoid activation of nuclear receptors alters the interaction of AP1 and NFκB proteins with coactivator complexes requisite for initiation of transcription. In the case of NFκB, activated GR binds its p65 subunit impairing formation of the NFκB–IRF3 complex.60 Similarly, TLR9-mediated activation of IFN-stimulated response elements-containing genes, as in the setting of viral infection, is sensitive to GR transrepression. Corticosteroid use in patients with herpes simplex virus infection is associated with exacerbation of disease course and is therefore contraindicated.60 The GR also inhibits the potential for rapid gene induction by impeding positive transcription elongation factor b complex formation with p65, whose interaction promotes the activity of RNA polymerase II and NFκB-mediated gene transcription.61

Coregulation of GR via cooperation with GR interacting protein 1 results in differential regulation of downstream targets.62 GR interacting protein 1 cooperates with activated GR and is recruited to p65-bound promoter regions of pro-inflammatory genes. A murine model of GR interacting protein 1–deficient macrophages exhibits impaired repression of NFκB target genes. Furthermore, systemic challenge with LPS resulted in clinical signs of shock associated with augmented expression of c-fms, Csf1r, is a receptor tyrosine kinase responsible for the differentiation and maturation of macrophages. In vitro studies of Shi and Jepson demonstrated that silencing mediator of retinoic acid and thyroid hormone receptor knockdown augmented expression of c-fms, whereas overexpression of FOXP1 inhibited monocyte differentiation. Silencing mediator of retinoic acid and thyroid hormone receptor is recruited to the promoter region of c-fms by FOXP1 to form the corepressor complex.67,68

Krüppel-Like Factors
Krüppel-like factors (KLFs) are a subfamily of the zinc-finger class of DNA-binding transcriptional regulators. Members of this gene family have been shown to play important roles in a diverse array of cellular processes, including macrophage polarization. To date, 2 KLFs, KLF2 and KLF4, have been shown to regulate macrophage function.50,69 KLF2 and KLF4 inhibit cytokine-mediated activation of macrophages by impairing NFκB activity. Overexpression of either factor impairs M1-gene expression via impaired recruitment of the NFκB transcriptional coactivator complex PCAF/p300 to the target gene promoter. Conversely, macrophages deficient in KLFs exhibit enhanced M1 polarization in response to LPS challenge as evidenced by augmented expression of inflammatory genes and enhanced bactericidal activity. In vivo, myeloid-specific KLF2-deficient mice are sensitive to LPS-induced sepsis and develop a robust inflammatory response and likewise demonstrate enhanced pathogen clearance in models of bacterial peritonitis.60 Lingrel et al70 studied the role of KLF2 in macrophages in atherosclerosis and found that myeloid-specific KLF2 null mice on a low-density lipoprotein-receptor–null background developed significant
high-fat diet–induced atherosclerosis, demonstrating a role for KLF2 role in chronic inflammatory disease states. Myeloid-specific KLF4-deficient mice are prone to obesity, diabetes mellitus, and atherosclerosis. The parallel effects of KLF2 and KLF4 on M1 function are not preserved in the context of M2 differentiation. IL-4–stimulated macrophages show significant increases in KLF4 expression. Macrophages deficient in KLF4 exhibit impaired expression of M2 markers in the presence of IL-4 or IL-13. In contrast, KLF2-deficient macrophages did not reveal significant defects in M2 marker expression. KLF4-deficient macrophages have heightened inflammatory gene expression and bactericidal activity, and mice bearing myeloid-specific deletion of KLF4 develop an altered metabolic phenotype characterized by glucose intolerance and insulin resistance. These observations of our laboratory strongly suggest that KLF4 is an important transcription factor requisite for M2 polarization (Figure B). Indeed, overexpression of KLF4 in RAW264.7 macrophages enhanced IL-4–induced M2 gene expression. Mechanistically, IL-4 induces STAT6 phosphorylation to promote KLF4 gene expression. KLF4 in turn cooperates with STAT6 to promote an M2 gene profile. Similar to the STAT1 and STAT6, KLF4 serves as a tipping point in M1 versus M2 polarization; lack of KLF4 facilitates M1 polarization and impairs M2 polarization.

**MicroRNA and CCAAT/Enhancer-Binding Proteins**

MicroRNAs (miRs) are a family of regulatory RNA molecules, similar to endogenous mediators of RNA interference that modulate many physiological processes, including apoptosis, differentiation, and activation of immune cells. These highly conserved small noncoding RNAs, ≈22 nucleotides in length, bind complementary base pairs within target mRNAs, resulting in target degradation or inhibition of translation. Recently, 3 miRs, miR-124, miR-155, and miR-223, have gained attention for their role in macrophage polarization and human disease.

Brain-specific miR-124, involved in microglial differentiation, functions as an inhibitor of macrophage activation. A murine model of multiple sclerosis suggests that mir-124 promotes macrophage quiescence and suppression of experimental autoimmune encephalomyelitis. Overexpression of miR-124 resulted in attenuated expression of M1–associated cytokines and cellular markers, including TNFα, iNOS, and MHC class II. Moreover, expression of M2–associated cytokines, including Arg1, transforming growth factor-β1, and found in inflammatory zone-1, was upregulated and associated with reduced activation of myelin-specific T-cells and significant suppression of disease. Mechanistically, miR-124 binds 3 putative binding sites within the 3′untranslated region of a monocytic transcription factor, CCAAT/enhancer-binding protein-α (C/EBPα), thereby inhibiting mRNA translation. Conditional murine Cebpa knockout studies revealed that miR-124 indirectly regulates PU.1 expression by impairing C/EBPα-mediated transcription; additionally, CD11b, MHC class II, and CD86 were downregulated. C/EBPβ is expressed in tumor-associated macrophages and promotes an M2-like phenotype believed to support tumor growth by impairing a cytotoxic immune response toward tumor cells. In human solid tumors, higher C/EBPβ expression is associated with attenuated miR-155 expression, whose binding to the 3′UTR of C/EBPβ mRNA inhibits CEBPβ protein expression. In contrast, miR-155 expression, induced by LPS, limits C/EBPβ expression, thereby promoting the M1 phenotype. Furthermore, targeted mutagenesis of the murine C/EBPβ promoter illustrates the requirement of cAMP responsive element–binding protein binding for LPS and IFNγ induction of CEBPβ expression. Impaired C/EBPβ expression was associated with M1 polarization and induction of TNFα, IL-1, IL-6, and IL-12, whereas expression of M2 cytokines, including IL-12 and Arg1, was suppressed. Physiologically, the anti-inflammatory role of C/EBPβ apparent in cardiotoxin-induced muscle injury illustrates that lack of the transcription factor results in failure of injury resolution and ultimate scar formation.

Murine models of miR-223 deficiency have suggested a role for this miR in chronic inflammatory disease, including rheumatoid arthritis and type 2 diabetes mellitus. Macrophage-specific deletion of mir-223 results in M1 polarization and delayed M2 polarization after LPS and IL-4 stimulation, respectively. Mice bearing mir-223–deficient macrophages on high-fat diet exhibit enhanced adipose tissue infiltration of M1-polarized macrophages associated with augmented inflammatory responses and insulin intolerance. The mechanism underlying these observations is unclear at this time, however inverse expression of a novel target of miR-223, Pknox1, suggests this gene may be involved in mir-223–mediated macrophage polarization.

**Closing Remarks**

Among the most important selection pressures that have shaped human evolution is the ability to combat infection. Consequently, humans have evolved a robust, perhaps slightly hyper-responsive immune system to defend against infectious organisms. Humans have developed in an environment outnumbered by the plastic genotypes of bacteria, viruses, and parasites that constantly challenge the integrity of our immune system. As the central component of the innate immune system, the macrophage bears tremendous responsibility as a first-responder to be nimble and malleable in challenging invading pathogens seeking to undermine our successful existence. Ironically, humans have themselves added to this challenge. Numerous advances in agriculture, industry, healthcare, and technology have culminated in a man-made world that is increasingly characterized by nutritional surplus, sedentary lifestyle, and advanced age. Intriguingly, the macrophage exquisitely designed to recognize the pathogen-associated molecular patterns of prokaryotes similarly identifies the saturated fatty acids in our take-out dinners as pathogenic, leading to a low-grade state of activation. Furthermore, lack of exercise and advanced age confer changes to the cellular milieu that further augment myeloid activation. Akin to Caesar’s famous last words Et tu Brute?, our biological shield turns against us. The eventual consequence is a chronic smoldering level of inflammation that likely contributes to the development and progression of metabolic, cardiovascular, autoimmune, and oncological diseases. Epidemiologists forewarn that future generations will not outlive the lifespan of their predecessors.
Thus, a greater understanding of the molecular mechanisms governing macrophage biology may provide novel targets that can be exploited for therapeutic gain. Such efforts, coupled with rigorous preventive strategies that improve diet and enhance activity, are likely to be most impacting in thwarting numerous illnesses that afflict modern societies.

Disclosures

None.

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