Control of Macrophage Activation and Function by PPARs

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Abstract: Macrophages, a key component of the innate defense against pathogens, participate in the initiation and resolution of inflammation, and in the maintenance of tissues. These diverse and at times antithetical functions of macrophages are executed via distinct activation states, ranging from classical to alternative to deactivation. Because the dysregulation of macrophage activation is pathogenically linked to various metabolic, inflammatory and immune disorders, regulatory proteins controlling macrophage activation have emerged as important new therapeutic targets. Here, the mechanisms by which peroxisome proliferator-activated receptors (PPARs) transcriptionally regulate macrophage activation in health and disease states, including obesity, insulin resistance and cardiovascular disease, are reviewed. (Circ Res. 2010;106:1559-1569.)

Key Words: inflammation ■ insulin resistance ■ atherosclerosis ■ diabetes ■ PPARs ■ alternatively activated macrophages
storage, mobilization, activation and oxidation of fatty acids. In general, genetic disruption of PPARs does not significantly alter the basal expression of genes, but rather impairs their inducibility in response to endogenous or synthetic ligands. Because the volume of ligand binding cavity of PPARs is substantially larger than those of the classical steroid receptors, a diverse group of chemical ligands can activate PPARs. Whereas native and modified fatty acids serve as endogenous ligands for the PPARs, fibric acids and thiazolidinediones (TZDs) are the pharmacological activators of PPARα and -γ, and are used to treat hypertriglyceridemia and type 2 diabetes, respectively.

Lastly, binding of endogenous or synthetic ligands induces a conformation switch in the receptors, leading to dissociation of corepressor proteins and recruitment of coactivator proteins to enhance the transcription of target genes.

The generation of global and tissue-specific PPAR knock-out mice, along with the availability of potent synthetic PPAR agonists, has allowed various investigators to elucidate the critical functions of these receptors in glucose, cholesterol, and fatty acid metabolism. Because these findings have been covered in recent reviews, they will not be discussed in detail here. Rather, this review will focus on the transcriptional mechanisms by which PPARs regulate macrophage activation and the functional importance of macrophage activation in inflammatory and metabolic diseases, such as obesity, insulin resistance, and cardiovascular disease. The importance of macrophage heterogeneity and activation will be discussed first, followed by a brief historical account of the involvement of PPARs in macrophage cholesterol metabolism and vascular disease, and then ending with a discussion of how PPARs regulate macrophage activation in health and disease states.

Specialization of Macrophage Function in Tissues

Macrophages, the sentinels of innate immunity, take residence in nearly every tissue and display marked heterogeneity in their cell surface markers, location, and function. These long-lived resident tissue macrophages perform a variety of functions, including host defense, clearance of cellular debris, remodeling of tissues, and regulation of the inflammatory response (Figure 1). Although circulating monocytes are known to give rise to resident tissue macrophages, the regulatory pathways that direct the specification of macrophages into distinct functional subsets are largely unknown. However, for 2 types of tissue macrophages, the osteoclasts and the splenic red pulp macrophages, signaling and transcriptional pathways that specify their identity in tissues have been elucidated.

In the case of osteoclasts, RANK ligand (receptor activator of nuclear factor κB) and M-CSF (macrophage colony-stimulating factor)
Genetic disruption of M-CSF or RANK ligand impairs osteoclastogenesis, resulting in impaired bone resorption and dense osteopetrotic bones. Interestingly, recent studies have demonstrated a critical role for PPARγ in differentiation of hematopoietic progenitor cells into osteoclasts. In agreement with this observation, activation of PPARγ by TZDs causes osteoporosis in mice and humans, whereas its genetic deletion in hematopoietic cells results in osteopetrosis.

Splenoc red pulp macrophages represent another example of highly specialized tissue macrophages whose sole function is to recycle senescent red blood cells. In this case, Spi-C, a PU.1-related transcription factor, controls the development of red pulp macrophages. Deletion of Spi-C in mice leads to selective loss of red pulp macrophages, resulting in impaired clearance of red blood cells in the spleen. Because red pulp macrophages express a number of genes important in the capturing and recycling of iron, Spi-C-null mice exhibit ectopic deposition of iron in their spleens. Taken together, these 2 examples demonstrate that, in part, specificity in macrophage function is regulated by tissue-specific signaling and transcriptional pathways.

Macrophage Activation and Functional Heterogeneity

The ability of macrophages to enact distinct activation programs and the inherent plasticity of these activation states provides another mechanism by which functional specificity is achieved in tissue and recruited macrophages. Although there are a variety of ways to classify macrophages into distinct subtypes, the simple scheme first proposed by Saimon Gordon is quite useful in understanding the functional importance of these cells in health and disease. In this schema, macrophages are divided into states of classical activation (also known as M1), alternative activation (also known as M2), or deactivation. Because these 3 macrophage activation states correspond to their functional phenotypes during infection with intracellular or extracellular pathogens, it provides a useful framework to understand how the expressed transcriptome or proteome of these cells correspond to their physiological functions. Although segregating macrophages into these 3 states is experimentally very useful, it also has its limitations, as macrophage phenotypes in vivo are likely to exhibit plasticity along the whole continuum of classical activation, alternative activation, and deactivation.

Classically activated macrophages develop in response to Th1 cytokine interferon (IFNγ) and microbial triggers, such as bacterial lipopolysaccharide (LPS), which activate toll-like receptor (TLR)4 signaling. As illustrated in Figure 2a, macrophages maturing along this pathway express a microbicidal program that confers immunity against intracellular pathogens. Whereas the respiratory and inflammatory burst of classically activated macrophages is essential for rapid clearance of invading pathogens, aberrant and chronic expression of this macrophage activation program contributes to the pathogenesis of metabolic diseases, including atherosclerosis and obesity-induced insulin resistance. Congruent with this
idea, genetic ablation of genes involved in the maturation or expression of the classically activated phenotype, including IFNγ, IFNγ receptor, inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNFα), and interleukin (IL)-6, results in dramatic protection from atherosclerosis and/or diet-induced insulin resistance.38,39 The identification of short chain saturated fatty acids as endogenous activators of TLR4 signaling provides a molecular explanation of how a protective host response becomes maladaptive in obesity,40 a state characterized by chronic inflammation and dysregulation of fatty acid homeostasis.

Maturation of alternatively activated macrophages in tissues is controlled by Th2-cytokines IL-4 and IL-13. Because the initial studies showed that IL-4 inhibited respiratory and inflammatory burst in human monocytes and macrophages, it was suggested that IL-4 might simply antagonize the classical pathways regulated by IFNγ in phagocytes.41,42 However, the identification of mannose receptor (MRC1) in 1992 as a highly induced marker in IL-4-activated macrophages,43 along with the observation that IL-4 induces the expression of major histocompatibility complex (MHC) class II molecule,44 led to the concept of “alternative activation” as a distinct macrophage program associated with unique set of phagocytic receptors, with discrete secretory and functional activity.45 Over the last decade, work by various investigators has identified the unique transcriptional signature of alternatively activated macrophages (Figure 2a),46–51 confirming Sionon Gordon’s initial hypothesis that alternative activation is a functionally distinct state.

Among the genes induced in alternatively activated macrophages, Arg1 (arginase 1) is the best studied and serves as a hallmark of alternative macrophage activation.29 Induction of arginase 1 diverts arginine metabolism away from production of nitric oxide to the synthesis of ornithine and polyamine, which are necessary for the reparative functions of these cells.52 Interestingly, this has the net effect of depleting cellular arginine and reducing production of reactive nitrogen species, conditions that are exploited by certain pathogens for intracellular growth.53,54 In addition to Arg1, various phagocytic receptors and lectins (Mrc1, Clec7a, and Clec10a), chemokines (Ccl2 and Ccl7), cytokines (IL1rn), and secreted products (Chi3l3/Chi3l4 and Retnla) are specifically induced during alternative macrophage activation (Figure 2a).29,55 The availability and specificity of these markers allows one to follow the development and trafficking of alternatively activated macrophages in tissues.56

In contrast, macrophage deactivation is an active process that switches off classically and alternatively activated macrophages, resulting in decreased antigen presentation and increased immunosuppression. IL-10, glucocorticoids, and transforming growth factor-β are potent stimulators of macrophage deactivation, as evidenced by downregulation of MHC class II molecules, inhibition of antigen presentation and suppression of inflammation.29 Similarly, phagocytosis of apoptotic cells renders macrophages refractory to the classical stimulus LPS by enhancing the release of deactivating cytokines IL-10 and transforming growth factor-β.57–59 In addition, as discussed below, ingestion of apoptotic cells activates nuclear receptor signaling in macrophages, resulting in suppression of inflammation. Although significant strides have been made in understanding the molecular mechanisms by which glucocorticoids and IL-10 promote macrophage deactivation,60–62 the paucity of cell surface makers that can prospectively identify these cells in tissues has greatly hampered our understanding of their physiological functions in vivo.

**PPARs, Macrophage Cholesterol Metabolism, and Vascular Function**

The expression of PPARγ in murine and human macrophages, which was first reported in 1998 by 3 groups,63–65 led to a decade of intensive investigations into its regulatory role in macrophage activation and metabolism. The initial studies from the Glass and Seed laboratories indicated that pharmacological activation of PPARγ attenuated expression of macrophage inflammatory programs.63,65 Because PPARγ is robustly expressed in macrophages taking residence in atherosclerotic plaques,64,66 it was postulated that pharmacological activation of PPARγ would decrease plaque inflammation and burden. In contrast, studies from the Evans laboratory had implicated PPARγ in the promotion of atherosclerosis by demonstrating that constituents of oxidized low-density lipoprotein (LDL), 9- and 13-hydroxyoctadecenoic acids, transcriptionally activated PPARγ.64,67 Because activation of PPARγ by oxidized LDL enhanced expression of CD36, a scavenger receptor mediating uptake of oxidized LDL, the implications were that PPARγ activation would establish a positive-feedback loop that favored the development of atherosclerotic lesions.68

This apparent paradox was resolved by completion of pharmacological and genetic studies in murine models of atherosclerosis. A number of groups reported that treatment of Ldlr−/− or ApoE−/− mice with synthetic PPARγ agonists, such as TZDs (troglitazone or rosiglitazone) or GW7845 (a tyrosine-based PPARγ agonist), inhibited development of atherosclerotic plaques.69–71 This was accompanied by decreased expression of inflammatory markers in the affected vessel wall. Congruent with these studies, transplantation of PPARγ-deficient bone marrow into Ldlr−/− mice increased atherosclerotic plaque burden.72 Interestingly, transcriptional activation of PPARγ was found to couple oxidized LDL uptake to cholesterol efflux via induction of a transcriptional cascade involving the nuclear receptor liver X receptor (LXRα) and the cholesterol efflux pump Abca1.72–74 Thus, based on these preclinical studies, enthusiasm was high for atheroprotective effects of TZDs in diabetic patients. Whereas smaller clinical trials provided support for this notion, metaanalyses of various studies failed to demonstrate atheroprotective effects of rosiglitazone.75,76 However, in contrast to therapy with the antidiabetic agent glimepiride, treatment with the TZD pioglitazone did significantly lower the rate of progression of coronary atherosclerosis.77,78

Whereas murine macrophages do not express appreciable amounts of PPARα, PPARδ is highly expressed by both mouse and human macrophages. The ability of PPARδ to sense native fatty acids led to its identification as a sensor of the triglyceride-rich lipoprotein very-low-density lipoprotein in macrophages and implicated this receptor in atheroma lipid metabolism.79 Unexpectedly, adoptive transfer of PPARδ-
null bone marrow into \( Ldlr^{-/-} \) mice revealed dramatic reduction in lesion area at the level of the aortic arch, which was independent of its known role in cholesterol metabolism. Loss of PPAR\( \delta \) was shown to decrease expression of various inflammatory markers, such as monocyte chemotactic protein (MCP)-1, IL-1\( \beta \), and matrix metalloproteinase (MMP)-9, genes that are targeted by the repressor protein B-cell lymphoma (BCL)6. In agreement with this, genetic deletion of PPAR\( \delta \) or its activation by synthetic ligand disrupted the interaction between PPAR\( \delta \) and BCL6, hence releasing this repressor to inhibit macrophage inflammatory response (see Figure 5c below). Together, these early studies, which were spawned by an interest in nuclear receptor function in vascular disease, firmly established a role for PPAR\( \gamma \) and -\( \delta \) in the regulation of macrophage lipid metabolism and inflammation.

**PPARs and Alternative Macrophage Activation**

As mentioned above, the Th2 cytokines IL-4 and IL-13, which engage distinct signaling receptors, provide the instructive cue for maturation of alternatively activated macrophages. Binding of IL-4 or IL-13 to their cognate receptors, IL-4R\( \alpha \)/IL-2R\( \gamma c \) or IL-13R\( \alpha 1 \)/IL-4R\( \alpha \), respectively, initiates a cytoplasmic signaling cascade that culminates in tyrosine phosphorylation of transcription factor STAT6 (signal transducer and activator of transcription 6). In turn, phosphorylated STAT6 dimerizes and translocates to the nucleus where it induces the expression of its target genes, including markers (Arg1, Chi3l3, Mrei1, Mgl1, and Retnla) and regulators (Pparg, Ppard, and PGC-1\( \beta \)) of alternative activation. Whereas the instructions for alternative macrophage activation are provided by IL-4 and IL-13, the acquisition and long-term maintenance of this phenotype requires the metabolic regulators PPAR\( \gamma \), -\( \delta \), and the coactivator protein PGC-1\( \beta \) (PPAR\( \gamma \) coactivator-\( \beta \)).

The ability of immune cells to perform their effector functions is tightly linked to and controlled by their metabolic state. For instance, almost a century ago, it was noted that anaerobic glycolysis is required for fueling the microbial metabolic program of classically activated macrophages, which is transcriptionally regulated by hypoxia-inducible transcription factor (HIF)-\( \alpha \). These observations raised the possibility that aerobic metabolism might fuel alternative macrophage activation, as it is best suited to fuel long-term activation of macrophages during helminth infection. In a series of studies, it was demonstrated that stimulation of macrophages with IL-4 induces uptake and oxidation of fatty acids and bioenergetics of mitochondria, programs that are transcriptionally controlled by PPAR\( \gamma \) and the coactivator protein PGC-1\( \beta \) in macrophages. Interestingly, STAT6, in an IL-4–dependent manner, controls the entire cascade of metabolic genes and regulators (Figure 3), indicating that the switch over to oxidative metabolism is a bona fide component of alternative macrophage activation. Congruent with this idea, deletion of PPAR\( \gamma \) in macrophages gravely impairs their ability to induce oxidative metabolism, as evidenced by reduced rates of \( \beta \)-oxidation of fatty acids and the blunted mitochondrial biogenic response. Consequently, PPAR\( \gamma \)-null macrophages are unable to clear this metabolic checkpoint which is required for full expression of the alternative phenotype, both in vitro and in vivo.

The shift toward oxidative metabolism is accompanied by an influx of fatty acids, nutrients that serve as the substrates for \( \beta \)-oxidation and as activators of PPARs. In this regard, PPAR\( \delta \) functions as a sensor of fatty acids to facilitate the acquisition of the immune phenotype of alternatively activated macrophages. For instance, in a PPAR\( \delta \)-dependent manner, monounsaturated fatty acids, such as oleic acid, were found to synergize with IL-4 to enhance the expression of alternative activation signature genes Arg1, Clec7a, Chi3l3 and Pdcd11g2 (programmed cell death 1 ligand 2). The direct activation of Arg1 and Mgl1 promoters by PPAR/RXR heterodimers provides a molecular explanation for the observed synergy between metabolic regulators and canonical

**Figure 3.** Cooperation between IL-4/STAT6 signaling and PPARs directs alternative macrophage activation. Stimulation of macrophages with the Th2 cytokines IL-4 and IL-13 leads to activation of JAKs, resulting in tyrosine phosphorylation of STAT6. Phosphorylated STAT6 translocates to the nucleus, where it activates the transcriptional program for alternative activation, including the induction of the nuclear receptors PPAR\( \gamma \) and -\( \delta \), and the coactivator protein PGC-1\( \beta \). Cooperation among STAT6, PPAR\( \gamma \), and PGC-1\( \beta \) reprograms macrophages for oxidative metabolism by upregulating fatty acid oxidation and mitochondrial biogenesis, whereas transcriptional synergy between STAT6, PPAR\( \delta \), and PGC-1\( \beta \) is necessary for full expression of their immune phenotype.
cytokine signaling pathways. Furthermore, PPARδ is also required for the proliferative and antiinflammatory effects of IL-4 on macrophages. Together, these studies suggest a model of how the local cytokine and fatty acid milieu favors alternative macrophage activation, and provide a framework for understanding how cytokine and nutrient signals are integrated into a macrophage transcriptional response (Figure 3).

**Macrophage Activation and Metabolic Disease**

A growing body of evidence suggests that inflammation in metabolic tissues plays a key pathogenic role in the development of obesity-induced insulin resistance. Since the initial description of white adipose tissue being infiltrated by macrophages in obese animals, numerous studies have shown that stimuli that increase recruitment of classically activated macrophages promote adipocyte dysfunction and potentiate insulin resistance. However, adipose tissue of lean animals is also populated with macrophages, the majority of which express the alternative phenotype. These observations led to the postulate that retention of alternatively activated macrophages might be beneficial in metabolic disease. Indeed, using mouse models in which alternative activation was impaired, such as the macrophage-specific PPARγ or δ knockout mice or PPARδ-null bone marrow chimeric mice, we and others have demonstrated that loss of alternatively activated macrophages in tissues increases susceptibility to diet-induced obesity, insulin resistance, and glucose intolerance.

Consistent with their complementary functions in alternative activation, PPARγ and δ have nonredundant roles in the acquisition and maintenance of alternatively activated macrophages in adipose tissue and liver. For instance, although adipose tissue of obese macrophage-specific PPARγ-null mice contained fewer macrophages, there was selective loss of those that were alternatively activated, indicating a specific requirement for PPARγ in sustaining this macrophage subtype in adipose tissue. However, in this same model, alternative activation of Kupffer cells was not significantly impacted. This contrasts with the results obtained with macrophage-specific, global or bone marrow chimeric PPARδ knockout mice. Albeit to varying degrees, in these models, both liver and adipose tissue macrophages exhibited dependence on PPARδ for alternative activation. In this case, although the total number of macrophages in adipose tissue or liver were not significantly different, their ability to express the alternative markers was significantly impaired. Together, these data provide the first evidence for how PPARγ and δ regulate macrophage activation in a depot-specific manner. Thus, in future studies, it will be important to identify the factors and underlying mechanisms that contribute to this observed tropism.

**PPARs, Apoptotic Cell Clearance, and Macrophage Deactivation**

Deactivation is an active process that potently suppresses the immunogenic and inflammatory programs of macrophages, resulting in resolution of inflammation and containment of tissue damage. Although the molecular basis for macrophage deactivation is not fully understood, some factors that promote macrophage deactivation have been identified. Acquired deactivation results after exposure of macrophages to glucocorticoids, IL-10 or TGF-β, whereas engulfment of certain pathogens, such as *Trypanosoma cruzi*, or apoptotic cells results in innate deactivation (Figure 2c). Recent studies demonstrate involvement of 2 nuclear receptors, PPARδ and LXRα, in macrophage deactivation after engulfment of apoptotic cells.

Resident and recruited macrophages are the professional phagocytes that rapidly clear dying cells. The timely removal of apoptotic cells by macrophages, before they undergo secondary necrosis, serves to protect the neighboring cells from noxious contents of dying cells and ensures that tolerance to self antigens is maintained. Because engulfment of apoptotic cells brings in large amounts of cellular lipids, we and others reasoned that this might serve as a signal for the tolerogenic responses of macrophages. Indeed, PPARδ and LXRα were found to function as the sensors of apoptotic cells in macrophages, thereby coordinating prompt clearance of dying cells and suppressing autoactive immune responses (Figure 4).

Using both global and macrophage-specific PPARδ-null mice, Mukundan et al demonstrated that PPARδ orchestrates timely clearance of apoptotic cells to ensure that tolerance to self is maintained. Engulfment of apoptotic cells, but not necrotic or opsonized cells, led to induction of PPARδ, thereby positioning this receptor to sense and coordinate clearance of dying cells. In agreement with this idea, genetic deletion of PPARδ reduced apoptotic cell clearance by 50% to 75%, whereas its activation by synthetic PPARδ agonist, GW0742, enhanced disposal of dying cells in both mice and human macrophages. The decreased expression of various opsonins, molecules secreted by macrophages that bridge apoptotic cells to their cognate phagocytic receptors, provided a molecular explanation for the observed phagocytic defect in PPARδ-null macrophages. In particular, complement 1q (C1q) gene, which is mutated in monogenic forms of the autoimmune disease systemic lupus erythematosus, was demonstrated to be a direct transcriptional target of PPARδ/RXR heterodimers (Figure 4). Congruent with this, incubation of macrophages with purified human C1q or wild type sera largely rescued the phagocytic defect of PPARδ-null macrophages.

The induction and activation of PPARδ by apoptotic cells made it an ideal candidate to serve as a molecular switch for controlling macrophage deactivation. This was indeed the case as apoptotic cells were unable to enhance their own clearance or suppress expression of proinflammatory cytokines in PPARδ-null macrophages. Moreover, the normal switching between the release of cytokines mediating immunity (TNFα and IL-12) to those suppressing autoimmunity (IL-10) was absent in PPARδ deficient macrophages (Figure 4). Consequently, both global and macrophage-specific PPARδ-null female developed spontaneous autoimmunity, as evidenced by higher titers of autoantibodies (including those directed against single stranded DNA, double stranded DNA, cardiolipin and anti nuclear antigen), glomerular immune complex deposition and excessive protein excretion by the kidneys. This lupus-like autoimmune disease could be
further potentiated by exogenous challenge with apoptotic cells or pristane, 2 environmental factors known to exacerbate autoimmunity. Together, these data suggest that, in the context of apoptotic cells, signaling via PPAR\(_{\gamma}\) results in deactivation of macrophages, a program that is necessary for suppression of autoimmune disease in genetic and environmental models of autoimmunity.

**Mechanisms Mediating Inhibition of Inflammation by PPARs**

Macrophages recruited to sites of injury orchestrate both the initiation and resolution phase of inflammation.\(^{29,30}\) For instance, elaboration of chemokines by endothelial cells, lymphocytes, or parenchymal cells results in the recruitment of monocytes to sites of injury, where they differentiate into resident macrophages. Initially, proinflammatory molecules secreted by macrophages serve to protect the host by scavenging, degrading, and clearing cellular and foreign debris. However, if excessive and unabated, this inflammatory response becomes detrimental and contributes to the progression of disease, as is observed in obesity, insulin resistance, and atherosclerosis.\(^{30,395}\) Thus, it is important to understand the molecular pathways that rein in macrophage inflammatory responses. In this context, 3 potential mechanisms have been described in the literature to limit the magnitude and duration of macrophage inflammation (Figure 5).

The first mechanism involves induction of inhibitory feedback loops, which transcriptionally or post-transcriptionally...
interfere with inflammatory signaling pathways. For instance, stimulation of macrophages with IFN-γ, IL-4, or LPS leads to rapid induction of SOCS (suppressor of cytokine signaling) proteins, which can inhibit Janus-associated kinase (JAK)-STAT and LPS signaling in a classic negative feedback loop (Figure 5a). Another negative feedback loop operating at the transcriptional level involves activating transcription factor (ATF)3, a member of the CREB/ATF family of transcription factors. Binding of LPS to TLR4 induces the expression of ATF3, which is recruited to a subset of nuclear factor (NF)-κB target genes, such as Il6, Il12b, Nos2, and Tnf (Figure 5a). Because deletion of ATF3 increases expression of various inflammatory genes in response to LPS, it suggests that ATF3 functions in a negative feedback loop to attenuate inflammation. Although both of these mechanisms concurrently operate in classically activated macrophages, neither has been demonstrated to be a direct transcriptional target of PPARs in macrophages.

The transrepression of NF-κB and AP1 (activator protein 1) target genes by nuclear receptors forms the second mechanism for limiting macrophage inflammation. In resting macrophages, inflammatory genes are actively silenced by recruitment of NCoR (nuclear receptor corepressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptors) corepressor complexes. Thus, for maximal activation of an inflammatory response, 2 steps are necessary: the removal of these corepressor complexes and the recruitment of an inflammatory response, 2 steps are necessary: the removal of these corepressor complexes and the recruitment of inflammatory transcription factors to subsets of genes. For instance, SUMOylation of monomeric PPARγ undergoes SUMOylation, allowing it to selectively interact with components of the NCoR/SMRT complexes. This interaction prevents ubiquitin-mediated degradation of NCoR/SMRT corepressor complexes, resulting in repression of inflammatory genes even in the presence of proinflammatory activating signals (Figure 5b). An important caveat is that PPARγ expression is rapidly downregulated after stimulation with LPS, making it highly likely that sufficient SUMOylated PPARγ exists in these cells to transrepress NF-κB. Thus, it will be important to determine whether alternative activation results in SUMOylation of PPARγ, thereby enabling this receptor to inhibit NF-κB activation.

Lastly, in an atypical manner, PPARδ also functions as an inflammatory switch in activated macrophages. In this case, unliganded PPARδ interacts with the transcriptional repressor BCL6, preventing it from suppressing the expression of its target genes. Intriguingly, addition of PPARδ ligand or its genetic deletion abolishes the interaction between PPARδ and BCL6, thereby freeing BCL6 for repression of inflammatory genes, including Il1b, Ccl2, and Mmp9 (Figure 5c). Because PPARδ deficient macrophages are unable to suppress inflammatory responses during alternative activation or deactivation, it will be important to determine the extent to which interaction with BCL6 or SUMOylation of PPARδ contribute to suppression of macrophage inflammation in these activation programs.

Future Prospects

Over the last decade, a great deal has been learned about the functions of PPARs in macrophages. Whereas the initial studies focused on the transcriptional mechanisms by which PPARγ and -δ regulate macrophage cholesterol metabolism, recent work has elucidated the regulatory roles for these receptors in macrophage activation and repression of inflammation. The pleiotropic functions of PPARs in macrophages, in part, stems from their ability to function as sensors of both native and modified fatty acids. In addition, the signaling pathways that regulate innate and acquired activation of macrophages, such as cytokines or ingestion of apoptotic cells, induce expression of PPARγ or -δ, thereby providing a mechanism for integration of lipid signals into macrophage activation. Congruent with this, many of the effector genes of alternatively activated or deactivated macrophages are co-regulated by the PPARs.

The direct involvement of PPARγ and -δ in the regulation of both alternative macrophage activation and deactivation raises the important question of how these receptors coregulate 2 distinct programs of macrophage gene expression. There are 3 potential mechanisms by which specificity in the PPAR transcriptional response is achieved. First, whereas the tone and direction of macrophage activation is dictated by its local cytokine milieu, signaling via the PPARs solely functions to amplify and sustain a particular transcriptional program. In support of this idea, transcriptional activation of PPARs has been shown to synergize with the Th2 cytokines, IL-4 and IL-13, to induce the transcriptome of alternatively activated macrophages. Second, posttranslational modification of the PPARs can functionally limit the access of these transcription factors to subsets of genes. For instance, SUMOylated monomeric PPARγ represses inflammatory genes, whereas PPARγ/RXR heterodimers transactivate the prototypical PPARγ target genes. Lastly, the induction of specific coactivators directs the recruitment of PPARγ and -δ to activation state-specific target genes. For instance, the induction of PGC-1β by IL-4, and its coactivation of STAT6 and PPARs serve to integrate and amplify the macrophage program of alternative activation. However, despite progress thus far, additional studies will be necessary to fully understand how these and other mechanisms direct recruitment of PPARγ and -δ to activation state-specific target genes.

The underlying mechanisms by which PPARγ and -δ direct macrophage functions in metabolic diseases deserve additional attention. As discussed above, in diet-induced obesity, a PPARγ- and -δ-regulated program of alternative activation prevents metabolic dysfunction in adipose tissue and liver. However, the specific mechanisms by which alternatively activated macrophages maintain metabolic homeostasis is incompletely understood. In addition, the cell types responsible for production of IL-4, the Th2 cytokine required for alternative activation of resident tissue macrophages, need to be identified, and their response to metabolic perturbations needs to be investigated. In the context of atherosclerosis, PPARγ and -δ functions have primarily been studied in early stages of atherosclerosis, which are dominated by infiltration of classically activated macrophages. However, rupture-prone complex atherosclerotic lesions con-
tain a central necrotic core, which is filled with apoptotic cellular debris and surrounded by alternatively activated and deactivated macrophages. Because persistence of apoptotic cellular debris contributes significantly to the pathogenesis of complex necrotic plaques in mice and humans, it would be valuable to perform additional studies to ascertain the functions of PPARγ and -δ in macrophages of late-stage atheromas.

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