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Licensing PPARγ to work in macrophages

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Abstract

The mechanisms that direct cell-type specific PPAR gene programs are poorly understood. In this issue of Immunity, Szanto et al identify STAT6 as a transcriptional switch that licenses PPARγ-dependent gene expression in macrophages and dendritic cells.

Macrophages are central components of the innate immune system that are critical for host defense. Found in almost all tissues, they exhibit wide heterogeneity and acquire a variety of functional phenotypes depending on the external milieu. For example, dendritic cells and macrophages present foreign antigens and coordinate inflammatory responses triggered by microbial pathogens through the production of pro-inflammatory factors. In other contexts, they clear apoptotic cells and facilitate tissue remodeling and resolution of inflammation through production of anti-inflammatory mediators. Classical activation of macrophages (M1 phenotype) is induced by T helper 1 (Th1) cell inflammatory cytokines such as TNFα and IFNγ and by pathogen activation of Toll-like receptors (TLRs). M1 activation leads to a coordinated inflammatory response that primes cells to deal with pathogens. Alternative activation of macrophages (M2 phenotype) can be triggered by Th2 cell-activated T-cells, mast cells, basophils, eosinophils, or macrophages through release of the cytokines IL-4 or IL-13. Alternative activation has been implicated in parasitic infections, allergy, tissue repair, and inflammation. Although it is useful to lump macrophages into the M1 and M2 categories for the purposes of broad discussion, it is likely that a continuum of phenotypes between these rigid categories is adopted by endogenous macrophages depending on the cellular context.

In this issue of *Immunity*, Szanto et al. elucidate a mechanism whereby alternative macrophage activation leads to enhanced PPARγ-dependent gene expression. PPARγ is a ligand-activated transcription factor that was originally characterized as a “master regulator” of adipogenesis. PPARs form obligate heterodimers with retinoid X receptors (RXRs) that bind to cis-regulatory elements (PPREs) found in proximal promoters, introns, or distal regions of their target genes. In adipose cells, PPARγ regulates the expression of genes involved in differentiation, lipid uptake, and triglyceride storage. PPARγ is also the target of a popular class of antidiabetic drugs, thiazolidinediones that act as direct ligands of the receptor.

In addition to adipose tissue, PPARγ is highly expressed in macrophages and is induced during monocyte differentiation and dendritic cell maturation. It has been recognized for several years that the gene expression programs induced by PPARγ ligands in adipocytes and macrophages are only partially overlapping, raising the question of how cell-type

specificity is accomplished. Lazar and colleagues have recently reported that binding sites for the transcription factor PU.1 are present together with PPREs in many macrophage-expressed PPARγ target genes. This characteristic distinguishes them from adipocyte-selective target genes, which commonly have C/EBPα binding sites adjacent to the PPREs (Lefterova et al., 2010).

The molecular basis for differential engagement of PPARγ responses between different types of macrophages and dendritic cells has also been an important question in the field. Glass and colleagues reported a number of years ago that the Th2 cell cytokine IL-4 was a strong inducer of PPARγ expression in macrophages (Huang et al., 1999). Subsequent studies reported that an active PPARγ pathway is a prominent feature of alternatively activated (M2) macrophages and that M2-type responses were compromised in the absence of PPARγ expression (Odegaard et al., 2007). PPARγ expression is important for the full expression of certain genes characteristic of M2 macrophages, especially the gene encoding arginase I, a direct PPAR target (Odegaard et al., 2007; Gallardo-Soler et al., 2008)). However, the degree to which PPARγ activity is required for the establishment of broader IL-4 responses and the various biological functions of alternatively activated macrophages has continued to be an active area of investigation (Marathe et al., 2009). In particular, the transcriptional underpinnings of IL-4-PPARγ crosstalk in alternatively activated macrophages have remained poorly understood.

Szanto et al. began by investigating how the PPARγ pathway was altered in various types of macrophages and dendritic cells. They found that activation of macrophages with IL-4 drove the expression of PPARγ itself and enhanced target gene expression in response to the PPARγ ligand rosiglitazone. In contrast, classical activation of the cells with IFNγ, TNFα or LPS inhibited the response to rosiglitazone, despite the fact that increased PPARγ expression was also observed with LPS treatment. Crosstalk between IL-4 and PPARγ signaling was further supported by global gene expression analysis. Remarkably, the authors found that rosiglitzone induced 635 genes in the presence of IL-4, but only 120 genes in the absence of IL-4. Moreover, both the magnitude of induction and the number of genes regulated by PPARγ were affected by IL-4. Thus, robust activation of PPARγ signaling in macrophages and dendritic cells was highly dependent on IL-4 stimulation, and this could not simply be explained by differences in PPARγ expression. Importantly, the requirement for IL-4 in PPARγ responses was also observed in mouse and human macrophages as well as in dendritic cells. These findings suggested the existence of one or more transcription factors that “gate” or “license” the PPARγ response in myeloid cells.

The authors went on to address the reciprocal question of the degree to which PPARγ was required for the gene expression response to IL-4. In distinction to the strong requirement of PPARγ target genes for IL-4 costimulation, the IL-4 transcriptome was modestly affected in the absence of the gene encoding PPARγ in macrophages. For example, induction of the alternative activation markers YM1 or FIZZ1 by IL-4 proceed normally in wild type or PPARγ-deficient peritoneal or bone marrow-derived macrophages.

To determine how IL-4 signaling was potentiating PPARγ activity on a molecular basis, Szanto et al. employed pharmacological inhibitors to interrogate various signaling pathways known to act downstream of the IL-4 receptor. The finding that WHI-P131, an inhibitor of the Janus kinase (Jak) 3) pathway, antagonized the induction of the lipid binding protein aP2 by rosiglitazone led the authors to hone in on STAT6, a transcription factor known to mediate IL-4 signaling in macrophages. Using macrophages that were genetically deficient in STAT6 expression the authors were able to show that PPARγ signaling in IL-4-treated macrophages was highly dependent on STAT6. For example, PPARγ target genes such as *Fabp4* (encoding aP2) and *Angptl4* (encoding PGAR) showed a muted response to

rosiglitazone in *Stat6*$^{-/-}$ compared to wild type macrophages. Global transcriptional profiling confirmed that a majority of PPARγ-responsive genes required STAT6 for full activation in macrophages.

These findings led the authors to hypothesize that STAT6 might be regulating PPARγ target genes by binding to their regulatory sequences directly. This idea was validated initially by coexpressing STAT6 and PPARγ in transient transfection assays along with an *Fabp4* -luciferase reporter. Promoter activity was additively responsive to STAT6 and PPARγ, consistent with a direct effect on the *Fabp4*promoter. The authors then analyzed the *Fabp4* gene to identify the response elements involved. Previous studies in adipocytes showed that PPARγ binds to a PPRE in the distal region of the *Fabp4* enhancer, approximately 5.4 kb from the transcriptional start site (Tontonoz et al., 1994). Interestingly, Szanto et al. identified an additional, previously unknown response element, which they termed MacPPRE to distinguish it from the adipocyte PPRE. Moreover, this regulatory region contained a highly conserved STAT6 binding site adjacent to the MacPPRE (Figure 1). Mutation of the MacPPRE or STAT6 element eliminated the ability of IL-4 to facilitate activation of the *Fabp4* promoter.

An important remaining question was whether PPARγ or STAT6 could be localized to the region of the MacPPRE in the endogenous *Fabp4* gene in macrophages. To address this possibility, the authors employed chromatin immunoprecipitation (ChiP) assays using antibodies for STAT6 and PPARγ. Indeed, PPARγ was shown to occupy both the adipocyte PPRE and the identified MacPPRE in macrophages. Unexpectedly, however, STAT6 was enriched in the region of the adipocyte PPRE as well as the region of the MacPPRE (which contains the STAT6 element). Although this finding may simply reflect the limited resolution of the ChiP assay, a more provocative interpretation is that STAT6 may interact with PPARγ without having to bind to DNA, perhaps serving as a coactivator in macrophages. In support of this idea, the authors showed that STAT6 could be pulled down with purified PPARγ protein in biochemical interaction assays. Finally, consistent with the common requirement of many macrophage PPARγ target genes for IL-4 signaling, ChIP assays revealed diminished PPARγ occupancy on the *Angptl4, Cd36, and Fabp4*, and *Scd1* promoters in STAT6-deficient macrophages.

In summary, Szanto have outlined a role for the IL-4 dependent transcription factor STAT6 as a “licensing factor” for PPARγ activity in macrophages and dendritic cells. These studies provide additional mechanistic support for the emerging concept that cell type-specific gene regulation is dependent on a combinatorial code of transcriptional regulators. In addition, the work brings insight into how this code is implemented on specific gene promoters. These findings also extend and clarify prior work by positioning PPARγ downstream rather than upstream of IL-4 in the alternative macrophage activation cascade (Odegaard et al., 2007a). Furthermore, the identities of the genes coregulated regulated by IL-4 and PPARγ are suggestive of a discrete role for PPARγ signaling in a transcriptional program for handling lipids after phagocytosis of apoptotic cells or parasites. In agreement with this possibility, recent studies have reported a role for the related nuclear receptor PPARδ in phagocytic responses (Mukundan et al., 2009).

Several questions are raised by the findings of Szanto et al. that will undoubtedly be the focus of additional research in the coming years. For example, what is the role of PPAR-dependent gene expression in the different functions of alternatively activated macrophages and dendritic cells in various biological contexts? What is the relative importance of lipid metabolic and inflammatory gene expression in these settings? What is the natural ligand for PPARγ in macrophages and how does this fit with the biology of IL-4? Lastly, given the centrality of metabolism and inflammation in human disorders such as atherosclerosis and

diabetes, it will be important to determine the relevance of PPARγ-STAT6 interaction for disease pathogenesis, immunological responses, and therapeutic intervention.

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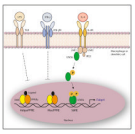

Figure 1.
IL-4 facilitates PPARγ-dependent gene expression through STAT6 signaling. In macrophages PPARγ binds to the adipocyte PPAR response element (AdipoPPRE) as well as the macrophage PPAR response element (MacPPRE) in the *Fabp4* gene (encoding aP2). Maximal PPARγ activity and expression of aP2 is dependent on STAT6 binding to a STAT6 response element (S6RE) adjacent to the MacPPRE.