COMMENTARY

Suppression of macrophage inflammatory responses by PPARs

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The proinflammatory response of macrophages to stimuli, such as lipopolysaccharide (LPS) or IFNγ, can be blocked by ligands for peroxisome proliferator-activated receptors (PPARs). However, the mechanisms, and even the validity, of this antiinflammatory role for PPARs has recently come under question. The paper by Welch *et al.* (1) in this issue of PNAS addresses some of these concerns, and suggests that the proposed antiinflammatory effects may result from activation of at least two members of this family of molecules.

PPARs represent a subgroup of the nuclear receptor superfamily that contributes to lipid and carbohydrate balance, and hence, homeostatic regulation of energy supplies. They appear to act as lipid sensors and to regulate transcription of lipid metabolizing enzymes, many within the peroxisome (hence their imposing name). The three subtypes of PPAR (α, β or δ, and γ) have different tissue distribution, different (though overlapping) ligand specificity, and mediate their effects by regulating different patterns of gene expression. PPARα is found mainly in heart, liver, and kidney, i.e., tissues with high rates of lipid oxidation, regulates fatty acid catabolism, and has been suggested to act as a "lipostat" to match lipid delivery to oxidative capacity. PPARδ is ubiquitously expressed, but is the least well understood of the group, in terms of either ligands or target genes. By contrast, PPARγ is found (and can be up-regulated) in many cell types, but particularly in adipocytes and macrophages. By appropriate induction of gene expression as well as cooperitivity with other nuclear receptors, PPARγ has been suggested to play a key role in adipose tissue development and cholesterol import and export (2). It can be activated by thiazolidinedione (TZD) drugs (e.g., rosiglitazone), which are used to improve insulin sensitivity in diabetic patients. In addition, over the last 5 years, an antiinflammatory effect has been ascribed to PPARγ activation in macrophages resulting from suppression of their production of proinflammatory mediators. Originally, this was shown by experiments in which, for example, induction of inducible NOS (iNOS), cyclooxygenase (COX_2), proteases, and inflammatory mediators by LPS or IFNγ was reduced by ligands of PPARγ, including rosiglitazone and 15 deoxy $\Delta^{12,14}$ prostaglandin J_2 (hereafter termed 15$\delta$$PGJ_2$) (3, 4). The potential antiinflammatory effects of PPARγ activation have since received significant attention, not in the least because of the availability, and in some cases antiinflammatory properties, of pharmacologic activating ligands. However, the effects and mechanisms have also become controversial. In particular, there appeared to be a discordance between ligand-binding affinities for the PPARγ and antiinflammatory effect as well as a significant lack of understanding as to how the PPARγ may be acting, or even whether the PPARγ is required for the antiinflammatory effects of the putative activating ligands (5–7).

The proposed antiinflammatory effects of PPARs may result from activation of at least two family members.

Previous studies by Chawla *et al.* (6) had examined responses to PPARγ ligands in macrophages derived from PPAR$\gamma^{-/-}$ embryonic stem (ES) cells and found that LPS stimulation of proinflammatory genes (iNOS and COX_2 for example) were still suppressed. In their own examination of this discrepancy, Welch *et al.* (1) used a cre–lox system to delete PPARγ from macrophages *in vivo* and also examined the cells for gene expression by microarray techniques. In thioglycollate-elicited macrophages, even a relatively high concentration of the PPARγ activator rosiglitazone induced increased expression of only a very few genes. Most of these were involved in lipid homeostasis and included the type B scavenger receptor, CD36.

The relatively low number of genes seen to be activated in this system were remarked on by the authors and even included candidates (such as LXRα and ABCA1) seen to be poorly responsive here although shown to be up-regulated in other studies. It seems reasonable that the mononuclear phagocyte class in general might exhibit a selective response to PPAR activators in contrast to a higher and more varied in adipocytes. In addition, the thioglycollate-elicited macrophage derives from incoming monocytes and has already been somewhat preprogrammed by the inflammatory response that initiates the elicitation raising important questions about macrophage heterogeneity in regard to PPAR-induced regulation. Importantly, however, several of these same activated genes showed increased mRNA expression to a ligand that is more specific for PPARδ (GW0742) and indeed, rosiglitazone was still able to up-regulate their mRNA in the PPAR$\gamma^{-/-}$ macrophages, presumably by acting on PPARδ.

As expected, in cells stimulated with LPS or IFNγ, PPARγ ligands led to suppression of so-called inflammatory gene expression. At low concentrations of rosiglitazone, the response in the PPAR$\gamma^{-/-}$ cells was lost, supporting a role for this receptor in antiinflammatory effects. By contrast, at higher concentrations the suppressive effect was maintained, even in the absence of PPARγ. Moreover, under these conditions, the response was similar to that induced by the selective PPARδ agonist. The authors conclude that both PPARs can induce similar antiinflammatory effects and respond to some of the same ligands (including rosiglitazone and 15$\delta$$PGJ_2$) albeit in different dose ranges. They further imply the concurrent involvement of PPARγ and PPARδ as one contributory explanation of the discrepant observations in the literature. At this point, confirmation of the dual roles of these two PPARs in macrophages will require additional experiments, including concurrent deletion of both. Nevertheless, the likely role for PPARs in macrophage-induced inflammatory responses demands further investigation and also raises important questions with regard to their participation in other cell types including myeloid and even lymphoid dendritic cells.

By ligand-induced heterodimerization with the retinoid X receptor (RXR) the

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PPARs are thought to target cognate DNA response elements (PPREs). However, despite this apparent commonality of mechanism and some overlap of response, the overall regulatory output of the different PPARs appears significantly disparate both within a given cell as well as between different cell types. A detailed understanding of how this specificity of response is regulated is so far lacking. In the case of macrophages, additional questions arise in relation to putative endogenous receptors for PPARγ, and now PPARδ as well, in the generation of specific macrophage responses or even so called macrophage "phenotypes." Much effort is being placed on the role of natural PPAR ligands in the development of macrophage and foam cells in the atherosclerotic plaque (see ref. 2) with particular emphasis on alterations of lipid homeostasis. With the developing interest in non- or antiinflammatory states in macrophages, one might reasonably question the role of this group of receptors in their generation.

For decades, studies of macrophages focused on their role as key cells of the innate immune system and their fundamental activities in killing and removing foreign organisms and cell debris, with a little antigen presentation thrown in. As such, the response to macrophage stimulation was usually seen as proinflammatory and proimmunogenic. More recently, alternative forms of macrophage activation have been proposed with both noninflammatory or even antiinflammatory consequences. Thus, in the 1960s, a state of macrophage activation was defined in relation to response to infectious agents, particularly Mycobacteria, and shown to involve IFNγ and later, other cytokines, in conjunction with stimulation by LPS or other Toll-like receptor (TLR) ligands. This so-called classically activated macrophage has up-regulated iNOS and COX_2, and produces a host of potentially pro-inflammatory molecules including cytokines, chemokines, eicosanoids, growth factors, oxygen and nitrogen radicals, and proteases. TLR activation by itself also induces a proinflammatory cell recently termed an "innately activated macrophage" (8).

Additional attention is now being placed on macrophages that are inactivated with regard to inflammatory mediator production. General suppression is achieved after stimulation with IL-10, but exposure to IL-4 or related cytokines initiates a so called "alternatively activated macrophage" (8), sometimes called an M2 macrophage (9). This cell shows diminished capacity to produce inflammatory mediators, the oxidative burst, iNOS, etc., in response to LPS. Importantly some molecules are selectively up-regulated in these cells, particularly the mannose receptor (which was suppressed in IFNγ-activated cells) and some additional potential surface markers (FIZZ1 and Ym1; ref. 10). The *in vivo* representative of the alternatively activated macrophage appears in parasite (helminth) infections, in keeping with the induction in these circumstances of Th-2 cytokines such as IL-4. Exposure of macrophages (and other cell types) to apoptotic cells or the phosphatidylserine that they expose on their surface also leads to a suppressed, and in this case also suppressive (i.e., affecting neighboring cells) macrophage with regard to proinflammatory mediator production (11).

At this point in time the possible role of PPARs in these noninflammatory "phenotypes" is not at all clear. IL-4 is known to up-regulate PPARγ and, as a consequence, enhance the antiinflammatory effects of PPARγ ligands. A recent paper describes the mRNA expression profile of alternatively activated macrophages from helminth-infected mice (12), and it would be relevant to compare this with the microarray data from Welch *et al.* with rosiglitazone stimulation, not to mention a possible defect in induction of the alternately activated macrophage phenotype in $PPAR\gamma^{-/-}$ cells. However, the problem with the latter experiment may well be the putative compensatory effect of PPARδ. A role for one or both PPARs in development of such macrophage phenotypes also raises the perennially difficult question of the presumptive natural ligands for these receptors. It is generally stated that lipids in oxidized low-density lipoprotein serve as agonists for PPARγ, such as 9-hydroxyoctadecanoic acid (9-HODE) or 13-HODE, oxidized phosphatidylcholine derivatives as well as products of arachidonate metabolism through 12,15 lipoxygenase (early experiments implicating such an action also for leukotriene B_4 seem to be less likely). Molecules such as these may well be produced during macrophage stimulation and, in fact, up-regulation of 15 lipoxygenase is a response to a number of stimuli, including IL-4. The spectrum of likely candidates for PPARδ activation in macrophages is less clear but might well prove important.

Another unknown in investigating PPAR alteration of the pattern of macrophage proinflammatory gene activation is how long the effect may last or how easily it is reversed. Exposure of mononuclear phagocytes to activators of PPARs has been implicated in the development of foam cells in the atherosclerotic plaque, intuitively a relatively persistent state but one whose reversibility would be much desired. The persistence of inflammatory gene product inhibition after activation of PPARγ and/or PPARδ by external or internal ligands is less well characterized and, in any event, would require knowledge of supplies of ligand, receptor, internal regulation, transcriptional control, etc. It is noteworthy that optimal suppression was seen with 18–24 h of pretreatment with rosiglitazone in the Welch study. In addition, although their data do not formally address the possibility that absence of PPARγ might release an endogenous and ongoing suppression of proinflammatory gene expression, the unstimulated $PPAR\gamma^{-/-}$ macrophages did not seem to show a marked difference from controls in inflammatory gene expression at baseline, or even for that matter after LPS stimulation. Because the cells were derived from an ongoing inflammatory lesion (the thioglycollate stimulated peritoneum) this might imply a lack of endogenous regulation in this system via PPARγ. However, once again a redundant effect of PPARδ might be involved; the *in vitro* culture of elicited macrophages before they were studied might alter the ongoing regulation, or this type of inflammatory response might not be ideal to investigate such a possibility.

As the uncertainties surrounding a regulatory role for PPARs in macrophages are sorted out, the participation of these molecules in the pluripotential capacity of mononuclear phagocytes makes them not only subjects for investigation but also of potentially important pharmacologic manipulation (13) in the targeted and controlled manipulation of inflammatory responses.

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