Editorial

PPARγ Ligands as Modulators of Inflammatory and Catabolic Responses in Arthritis. An Overview

Peroxisome proliferator activated receptors (PPAR) are ligand activated transcription factors belonging to the nuclear hormone receptor superfamily, which includes the classical steroid, thyroid, and retinoid hormone receptors as well as many orphan receptors[1]. So far, 3 PPAR isotypes have been identified and are commonly designated PPARα, PPARß (also referred to as PPARδ, NUC1, or FFAR) and PPARγ. The actions of PPAR were originally thought to be limited to the control of lipid metabolism and homeostasis. Recent studies, however, showed that PPAR activation can regulate inflammatory responses and cellular proliferation and differentiation as well as apoptosis[2-4]. There is also evidence to support a role of PPARγ in various physiopathological conditions including cancer, atherosclerosis and diabetes. The role of PPAR in these diseases has been the subject of excellent reviews[5-7], but none has specifically focused on the potential role of PPARγ in rheumatic diseases. We review the evidence suggesting that PPARγ ligands may modulate inflammatory and catabolic responses and address their potential role in arthritic diseases.

PPAR MOLECULAR ACTION MECHANISM AND TISSUE DISTRIBUTION

Binding of PPAR ligands leads to PPAR activation and heterodimerization with retinoic X receptor (RXR), the receptor of 9-cis-retinoic acid (9c-RA). The PPAR/RXR heterodimers bind to specific peroxisome proliferator response elements, or PPRE, located upstream of responsive genes[8] (Figure 1). The PPRE consist of a direct repeat of 2 copies of a hexameric nucleotide sequence AGGTCA-like separated by one single nucleotide. Genes containing PPRE motifs include acyl-CoA oxidase (ACO)[8], liver fatty acid-binding protein (L-FABP)[9], and peroxisomal bifunctional enzyme[10]. Transcriptional activation or repression of the target gene is more complex than simple binding of the PPAR/RXR complex to the PPRE. Indeed, as with other nuclear receptors, regulation of gene transcription is modulated by factors referred to as coactivators and corepressors. Coactivators and corepressors act as bridging proteins between nuclear receptors and the transcriptional machinery and enhance or decrease transcriptional activation, respectively. In addition to RXR, a number of PPAR interactive proteins have been shown to associate with PPAR. These cofactors include p300/CBP[11], PGC-2[12], Ara 70[13], the steroid receptor coactivator-1[14], the heat-shock protein 70[15], LXR[16], a 165 kDa PPARγ-binding protein designated PBP[17], as well as c-jun p65[18], and nuclear factor of activated T cells (NF-AT)[19].

The expression of PPARα, ß, and γ varies widely from tissue to tissue, suggesting that the PPAR subtypes play different biological roles. PPARα is expressed in tissues with high rates of fatty acid catabolism, such as liver, heart, muscle, and kidney[9,20]. PPARß displays a ubiquitous expression pattern with varying levels in different organs[20,21]. While high expression of PPARγ is found in white and brown adipose tissue, it is also detected at moderate levels in the large intestine and mammary glands[22]. PPAR are also expressed in monocytes/macrophages[23-25], T lymphocytes[26], and vascular wall cells[24,27-30]. In addition, PPAR are present in the joint connective tissue cells including chondrocytes, synoviocytes, and osteoclasts[31-35].

PPAR LIGANDS

PPAR can be activated by a number of compounds (Table 1) that may be classified in natural ligands such as eicosanoids and fatty acids and their derivatives and in synthetic ligands such as the fibrate class of hypolipidemic drugs, the thiazolidinedione class of antidiabetic drugs, and some nonsteroidal antiinflammatory drugs (NSAID).

PPARα is activated by a diverse array of saturated, monounsaturated, and polyunsaturated fatty acids. A search for natural PPARα ligands[36] identified palmitic acid, docosahexaenoic acid (DHA), eicopentaenoic acid (EPA), oleic acid, linoleic acid, and arachidonic acid as endogenous activators of PPARα. The highest affinity, naturally occurring PPARα ligand to be identified to date is the lipoxygenase metabolite 8-S-hydroxyeicosatetraenoic acid (8S-HETE)[37-40]. The role of the chemotactic agent leukotriene B_4 (LTB_4) as a PPARα ligand is controversial[37,39,40]. Among the synthetic ligands, the hypolipidemic agent WY-14643 is the highest affinity ligand described. This compound does not bind to or activate PPARγ or ß and therefore serves as a useful pharmacological tool to selectively modulate PPARα activity. In addition, some NSAID have been shown to bind and activate PPARα[41]. The arachidonic acid analog 5,8,11,14-eicosatetraynoic acid (ETYA) and some agonists and antagonists of the leukotriene membrane receptors also bind and activate PPARα[42].

Screening for ligands of PPARγ revealed that PPARγ binds primarily to polyunsaturated fatty acids, such as linoleic acid and linolenic acid. Fatty acids found in fish oil and marine mammals, such as DHA and EPA, bind and activate PPARγ[40,43]. The linoleic acid metabolites derived from the 15-lipoxygenase pathway, i.e., 9-hydroxy-octadecadienoic acid (9-HODE) and 13-HODE, also function as PPARγ agonists[44]. The PGD_2 dehydration product 15-deoxy-delta-12-14-PGJ_2

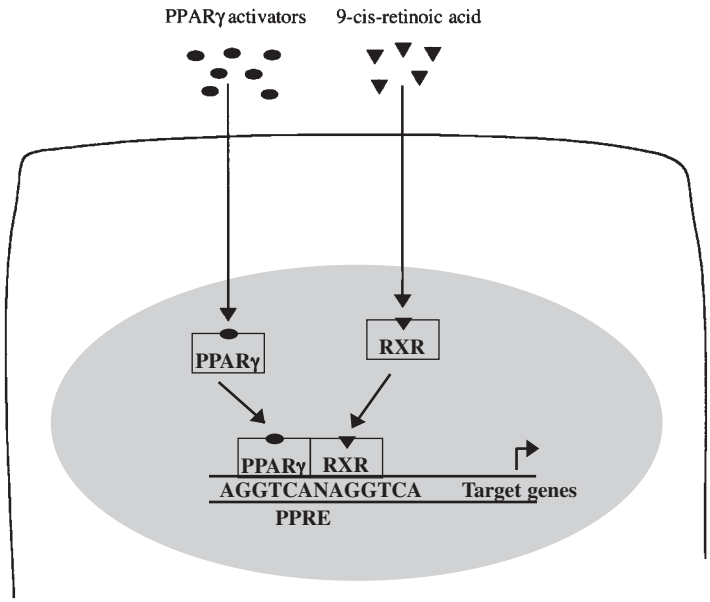

Figure 1. The PPAR action mechanism. After ligand binding and activation, PPAR heterodimerizes with the retinoic X receptor (RXR), the receptor for 9-cis retinoic acid (9c-RA). This heterodimeric complex binds to the PPAR responsive element (PPRE) and drives the transcription of target genes.

(15d-PGJ_2) was the first endogenous PPARγ ligand identified[45,46]. This prostaglandin is currently widely utilized as a naturally occurring PPARγ activator. Further, lipoxygenase metabolites 12- and 15-hydroxyeicosatetraenoic acid (HETE) are potent activators of PPARγ and are produced by interleukin 4 treated macrophages[47]. In addition, the insulin-sensitizing antidiabetic thiazolidinediones (TZD) bind and activate PPARγ. One such compound, rosiglitazone (BRL 49653) binds with a high affinity to PPARγ[45,48]. All TZD tested to date, i.e., BRL 49653, pioglitazone, and troglitazone, bind and activate the PPARγ isotype with Kd that parallel their antidiabetic activity *in vivo*[45,48-50]. In addition to TZD, other synthetic compounds have been identified as PPARγ activators. It has been shown that several NSAID, such as indomethacin, ibuprofen, fenoprofen, and flufenamic acid, bind and activate PPARγ and promote adipocyte differentiation[41,51,52].

In contrast to the other subtypes, no selective drugs to PPARß/δ have been identified so far. Many natural and synthetic compounds have been shown to bind and activate PPARß/δ, in particular polyunsaturated fatty acids[37,40] and the semisynthetic PGI analog carbaprostacyclin[37]. Lastly, the eicosanoid PGA1 preferentially activates this subtype, suggesting that endogenous PPARß/δ ligands are derived from the cyclooxygenase (COX) pathway[37,38]. More information relating to the structure of PPAR, their putative ligands, and their many biological activities can be found in recent reviews[42,53,54].

PPAR AND MONOCYTES/MACROPHAGES

The pathogenic role of monocyte/macrophage derived cytokines in chronic inflammatory diseases is now generally accepted. This is supported by the clinical improvement observed after specific neutralization of interleukin 1ß (IL-1ß) or tumor necrosis factor-α (TNF-α) in patients with arthritic diseases[55-58]. In addition to proinflammatory cytokines, activated monocyte/macrophages produce a large array of mediators including metalloproteinases, reactive oxygen radicals, and arachidonic acid derivatives that may ultimately lead to serious impairment of joint function[59].

Recently, several articles reported that both PPARα and γ are expressed in monocytes/macrophages and suggested that these transcription factors may be involved in the regulation of proinflammatory responses. Jiang, *et al*[60] reported that treatment with PPARγ activators, including 15d-PGJ_2, troglitazone, or certain members of the NSAID class, inhibited the release of proinflammatory cytokines IL-1ß, TNF-α, and IL-6 in phorbol myristol acetate treated human monocytes. The results with the NSAID are of particular interest as it has been observed that some NSAID have incremental therapeutic benefits in the treatment of osteoarthritis (OA) and rheumatoid arthritis (RA) at concentrations 100 to 1000-fold higher than that required for *in vivo* inhibition of COX and prostaglandin production. Thus, it seems likely that in addition to inhibition of COX, NSAID may induce their beneficial effects through a PPARγ mediated suppression of proinflammatory cytokine production. However, it is important to point out that some NSAID are *bona fide* activators of both PPARα and PPARγ, whereas others do not activate PPAR and still display antiinflammatory effects[41]. This calls for caution when interpreting

Table 1. PPAR ligands.

Class	Ligands	PPAR α	PPAR β	PPAR γ
Natural				
Fatty acids	Arachidonic acid	+	+	+
	Linoleic	++	+	–
	Eicosapentaenoic	++	+	++
	Docosahexaenoic	++	+/–	++
	9-HODE	ND	ND	++
	13-HODE	ND	ND	++
Eicosanoids	PGA_1	+	++	+
	PGJ_2	+	+	++
	15d-PGJ_2	+	+/–	+++
	8 (S)-HETE	+++	–	–
	12-HETE	++	ND	ND
	15-HETE	ND	ND	+
	LTB_4	+/–	ND	ND
Synthetic				
Hypolipidemic drugs				
	Wy 14643	+++	+	+
	Clofibrate	++	+/-	+
	Ciprofibrate	++	–	+
	Gemfibrozil	++	–	+
Antidiabetic thiazolidinediones				
	BRL-49653	–	–	+++
	Pioglitazone	–	–	++
	Ciglitazone	–	–	++
	Englitazone	–	–	+
NSAID				
	Indomethacin	+	–	+++
	Ibuprofen	+	–	+
	Fenoprofen	++	–	+

Natural and synthetic activators of PPAR. Natural PPAR agonists comprise fatty acids, oxidized fatty acids, and eicosanoids. Synthetic PPAR agonists comprise the hypolipidemic drugs (fibrates), the antidiabetic thiazolidinediones (TZD) and certain nonsteroidal antiinflammatory drugs (NSAID). + indicates activator of this isoform in transactivation assay; –, not an activator of this isoform in transactivation assay; +/– conflicting data reported in the literature. ND: not determined[37–41, 43–45, 47,48].

results obtained using these molecules in respect to the role of PPARγ in their actions.

In activated macrophages, treatment with natural or synthetic PPARγ activators provokes a resting phenotype, resulting in a global inhibition of proinflammatory mediators and downregulation of the inducible nitric oxide (NO) synthase, scavenger receptor A, and the matrix metalloproteinase-9 (MMP-9) or gelatinase B genes[61]. Further analysis using isolated multimerized sites revealed that this inhibition takes place at the transcriptional level by interfering with the transcription factors AP-1, STATs, and NF-κB (Figure 2). Marx, *et al*[24] showed that PPARγ activators inhibit MMP-9 gelatinotic activity and gene expression in monocyte derived macrophages. This is of particular interest given that MMP-9 was found in synovial tissues and fluids of patients with OA and RA[62] and was reported to closely participate in the destruction of cartilage[63]. Shu, *et al*[64] examined the effect of PPARγ activators on MMP-9 and IL-8 expression in human monocytic THP-1 cells, and found that PPARγ activators reduced the spontaneous and lipopolysaccharide (LPS) induced MMP-9 production. In contrast, the production of IL-8 was unaffected, suggesting that, rather than exerting a negative regulation on all genes driven by AP-1, NF-κB, and STATs, PPARγ activators may regulate only a subset of genes. PPARγ activators were also shown to inhibit iNOS activity and protein expression in stimulated RAW264.7 murine macrophages[65]. This inhibition was suggested to be mediated by modulation of the stress protein heme oxygenase 1. Finally, Chinetti, *et al*[25] showed that macrophage treatment with rosiglitazone or 15d-PGJ_2 resulted in apoptosis induction, likely by interfering with the NF-κB mediated antiapoptotic pathways.

The ability of PPARγ ligands to inhibit the expression of proinflammatory genes suggests a potential protective role of PPARγ in arthritis. Nevertheless, comparison of the potency of different PPARγ ligands in inhibiting inflammatory gene expression in macrophages shows that relatively high concentrations of TZD, which are considered highly selective for

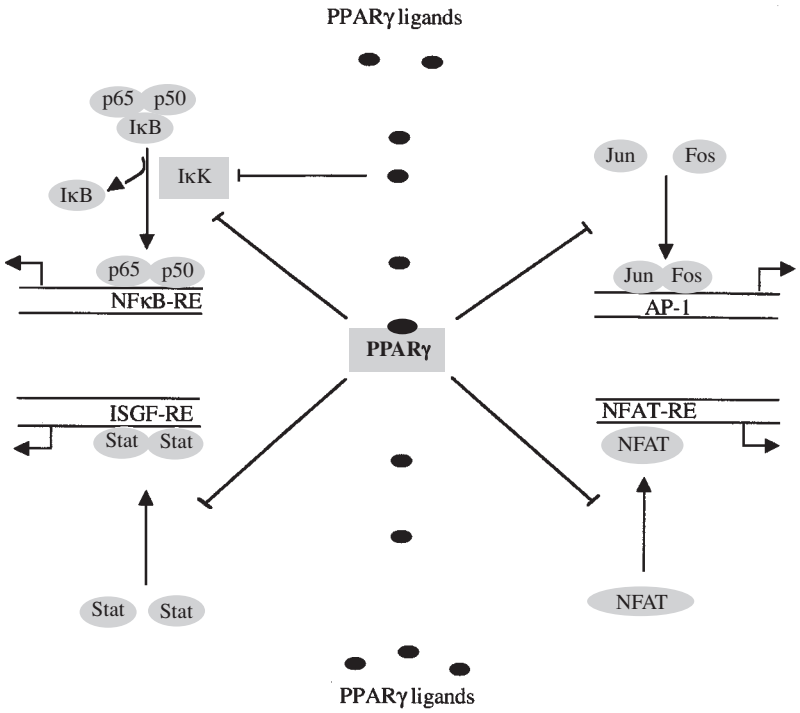

Figure 2. Mechanism of transcriptional repression by PPARγ activators. PPARγ activators downregulate catabolic and proinflammatory gene transcription by antagonizing NF-κB, AP-1, NF-AT, and STATs signalling pathways. Stimulatory effects are indicated by an arrow, whereas inhibitory effects are indicated by a stop sign (⊥). TRE: TPA responsive element; ISGF-RE: interferon stimulated gene factor response element; NF-κB-RE: nuclear factor κB-responsible element; NFAT-RE: nuclear factor of activated T cells responsive element.

PPARγ, were needed to promote effects similar to those of the less specific PPARγ ligand 15d-PGJ_2, suggesting that PPARγ independent mechanisms might be involved in these processes.

PPARγ AND ARTICULAR TISSUE

Proteolytic degradation of articular cartilage is a hallmark of many arthritic diseases such as RA and OA, and MMP are believed to play a central role in this process[66,67]. Among MMP, collagenase-1 (MMP-1), stromelysin-1 (MMP-3), and collagenase-3 (MMP-13) are considered key enzymes in the pathological destruction of cartilage and bone. Accordingly, numerous studies describe elevated level of these enzymes in OA and RA joints. Both chondrocytes and synovial cells express MMP-1, -3, and -13 and proinflammatory cytokines such as IL-1ß and TNF-α induce or enhance their production. These cytokines are also potent inducers of NO, which is suggested to actively participate in joint destruction.

Recent studies on PPAR expression patterns have shown that PPARα and γ are expressed in human articular cartilage and cultured chondrocytes. To determine whether PPARγ regulated the responses of normal human articular chondrocytes to inflammatory cytokines, the effect of PPAR activators on IL-1ß induced production of NO, a key mediator in articular inflammation and cartilage damage, was analyzed[32]. PPARγ, but not PPARα activators, dose dependently inhibits IL-1ß induced NOS expression and synthesis. PPARγ activators also inhibited the induced NOS (iNOS) expression in chondrocytes from patients with OA. This is unique, in that the iNOS expression in OA chondrocytes has been shown to be refractory to inhibition by classical iNOS expression inhibitors such as transforming growth factor-ß (TGF-ß) or dexamethasone[68]. PPARγ ligands also inhibited IL-1ß induced MMP-13 expression and production in chondrocytes. In addition to IL-1ß, other cytokines produced in arthritic joint tissues, such as IL-17 and TNF-α, may contribute to joint destruction through production of NO and MMP-13. As expected, PPARγ activators inhibited the production of NO and MMP-13 in response to TNF-α and IL-17, suggesting that PPARγ activators may target common pathways leading to NO and MMP-13 production. We also showed that the reduction of iNOS and MMP-13 expression by PPARγ agonists occurs at the transcriptional level, at least in part, through inhibition of AP-1 and NF-κB activity[32].

Although the exact mechanisms remain unclear, activated PPARγ could downregulate AP-1, NF-κB, and STATs activity by titration of essential transcription cofactors, such as CBP/p300 and SRC-1[29,60]. PPAR may also antagonize AP-1 and NF-κB activity through protein-protein interaction. Such a mechanism was shown to be utilized by retinoic acid receptor and glucocorticoid receptor with AP-1[69] or NF-κB[70].

Bordji, *et al*[31] showed that in addition to inhibition of NO production, 15d-PGJ_2 and troglitazone counteracted the IL-1ß induced decrease in proteoglycan synthesis in rat chondrocytes. Additional evidence for a protective effect of PPARγ activators in cartilage comes from the study of Curtis, *et al*[71], where they assessed the effects of another class of PPARγ activators on bovine chondrocytes. These authors showed that the n-3 fatty acids EPA and DHA specifically reduced the expression of IL-1ß, TNF-α, COX-2, and the proteoglycan degrading enzymes (aggrecanases). However, it is still uncertain if these fatty acids induce their protective effects through binding and activation of PPARγ in chondrocytes.

Synovial fibroblast cells from patients with RA and OA were shown to express PPARγ. Interestingly, PPARγ activators were shown to induce synovial fibroblast apoptosis *in vitro* in RA but not in OA[34]. To assess the role of PPARγ in synovial fibroblast function, we analyzed the effect of PPARγ activators on the expression of MMP-1. Similarly to chondrocytes, PPARγ agonists prevent IL-1ß induced production of MMP-1. This inhibition occurs at the transcriptional level, at least in part, through reduction of the AP-1 DNA binding activity. As expected, relatively high concentrations of the specific PPARγ activator BRL49653 were required to achieve a significant inhibition of MMP-1 expression. This opens the possibility that alternative or additional pathways are involved in inhibition of catabolic factor production by chondrocytes and synovial fibroblasts.

Although it is clear that 15d-PGJ_2, a downstream metabolite of the PGD_2 pathway, is present *in vivo*[72], it is still difficult to precisely determine its *in vivo* concentration, particularly in articular joint tissues. In this context, it is crucial to note that PGD_2 is among the most abundant prostaglandins in synovial fluid and is produced by macrophages, synovial fibroblasts, and synovial mast cells[73-76]. Therefore, it is likely that PGD_2 derivatives are present in sufficient amounts to activate PPARγ and act as a negative feedback loop for joint inflammation and cartilage catabolism.

Although the pathophysiologic mechanisms of bone loss in arthritic diseases have not been fully elucidated, studies suggest that bone-resorbing osteoclasts play an important role in bone destruction[77]. Osteoclasts also express PPARγ, and PPARγ activators inhibit differentiation of these cells through inhibition of the NF-κB pathway[35]. This suggests that PPARγ activation in osteoclasts may prevent bone loss in arthritic disease. In agreement with this, Fujiwara, *et al*[78] reported that troglitazone prevented bone destruction in the adjuvant arthritis model. Using the same animal model, Kawahito, *et al*[34] reported that PPARγ ligands 15d-PGJ_2 and troglitazone reduced the severity of arthritis with suppression of pannus formation and mononuclear cell infiltration, although this is considered due in part to the induction of synovial fibroblast apoptosis. However, in this study high doses of troglitazone were utilized and yet 15d-PGJ_2 was more protective. Again, this reiterates the well known caveat that the protective effect is most effectively brought about by the less specific 15d-PGJ_2, indicating that PPARγ independent mechanisms might also be operative *in vivo*. To conclude, it is noteworthy that PPARγ activation could contribute to the protective effects of PPARγ ligands; however, given that PPARγ independent mechanisms appear also to be involved, a significant challenge will be to gain a more specific understanding of how these ligands induce their PPARγ independent effects.

PPARγ AND SYNOVIUM INFILTRATING CELLS

In addition to the destruction of articular structures, some arthritic diseases such as RA are characterized by synovial proliferation and accumulation of inflammatory cells. The infiltrating cells are mainly macrophages, T lymphocytes, and neutrophils as well as other cell types, such as dendritic cells[59,79,80].

A direct role of T lymphocytes in joint destruction has been difficult to confirm; accumulating evidence supports that T lymphocytes contribute to the pathogenesis of RA[79,81]. Recent studies revealed that RA is characterized by a predominant Th1 profile with production of a large amount of Th1 cytokines: interferon-γ (IFN-γ), IL-2, and IL-17[82,83]. Recent data from 2 independent groups have revealed that Th lymphocytes express PPARγ and that PPARγ, but not PPARα ligands, inhibit IL-2 production and phytohemagglutinin, anti-CD3, or Ag induced proliferation[19,26]. Such an effect could attenuate the inflammatory process in the joint. Yang, *et al*[19] demonstrated that the inhibition of IL-2 production is PPARγ dependent. In addition, activated PPARγ physically associates with the nuclear factor of activated T cells (NF-AT), a transcription factor that plays an essential role in IL-2 gene expression and prevents its binding and transcriptional activity. Interestingly, this is the first indication of a functional interaction between PPARγ and a T cell-specific transcriptional factor. Ultimately, Harris, *et al*[84] reported that PPARγ activators promote apoptosis in naive T cells.

Although the mechanism leading to the onset of the inflammatory reaction in RA joints is poorly understood, it was suggested that polymorphonuclear neutrophils may play a crucial role in this process. Neutrophils are recruited into the joint space by local production of cytokines and can then contribute to joint destruction by the production of reactive oxygen metabolites, granule enzymes, and cytokines that further amplify the inflammatory response by their effects on macrophages and lymphocytes[80]. Neutrophils were shown to express PPARγ[85], suggesting a potential role of this transcription factor in neutrophil function. Indeed, Vaidya, *et al*[86] recently showed that natural PPARγ activators, including 15d-PGJ_2, inhibit the induction of H_2O_2 by TNF-α or LPS. Given that AD-5075, a potent and specific synthetic PPARγ activator, did not affect H_2O_2 production, these authors suggested that the inhibitory effect of 15d-PGJ_2 on H_2O_2 production in neutrophils was PPARγ independent. This is consistent with the finding that PPARγ mRNA in neutrophils is truncated and does not fully code the protein[85].

Angiogenesis, the formation of new blood vessels, is a major pathological feature of RA and appears to be required for pannus development. This neovascularization could maintain the chronic inflammatory state by facilitating the entry of inflammatory leukocytes into the synovial tissue. In addition, the increased endothelial surface area further potentiates the local inflammatory reaction through production of cytokines and proteases[87]. Vascular endothelial cells were also shown to express PPARα and γ[28,88]. Monocyte chemoattractant protein (MCP)-1, a monocyte, B, and T lymphocyte chemoattractant[89], is believed to play an important role in the regulation of inflammation in RA. Injection of recombinant MCP-1 into the knee joints of rabbits led to the accumulation of mononuclear cells within the joint, resulting in histopathological changes resembling RA[90]. Direct support for the involvement of MCP-1 in RA is provided by an animal model study, where an antagonist of MCP-1 has been shown to inhibit arthritis in the MRL-lpr mouse model[91]. Interestingly, PPARγ activators were shown to inhibit the expression of MCP-1 at the transcriptional level[92] in endothelial cells. Similarly, the induction of the endothelial cell derived chemokines, IFN inducible protein of 10 kDa (IP-10), monokine induced by IFN-γ (Mig), and IFN inducible T cell α-chemoattractant (I-TAC) by IFN-γ was also inhibited by PPARγ agonists[30]. Jackson, *et al*[93] demonstrated that PPARγ activators inhibited monocyte binding to human aortic endothelial cells and inhibited expression of vascular cell adhesion molecule-1 (VCAM-1). However, it should be noted that this effect was obtained only with certain PPARγ agonists such as troglitazone and ciglitazone, but not rosiglitazone. This suggests that PPARγ activators might attenuate joint inflammation and destruction through inhibition of leukocyte recruitment within the joint. PPARγ activators also inhibit the endothelial cell release of endothelin-1, a potent vasoconstrictor peptide and vascular smooth cell muscle cell mitogen. PPARγ agonists inhibit endothelin expression at the transcriptional level by negatively interfering with AP-1 DNA binding activities[94]. Further, PPARγ agonists reduce the expression of the vascular endothelial cell growth factor receptors Flt-1 and Flk/KDR[28]. Finally, PPARγ agonists induce endothelial cell apoptosis[88]. Altogether, these data suggest that PPARγ targeting in the joint vasculature may constitute a novel antiangiogenic therapy in inflammatory arthritis.

Epidemiological and clinical studies showed that dietary n-3 fatty acid supplementation resulted in a substantial improvement in the clinical status of patients with RA[95,96]. Some patients were also able to discontinue NSAID while receiving n-3 fatty acids[97,98]. Biochemical studies showed that consumption of n-3 fatty acids resulted in a decrease in the LTB_4 release by neutrophils[95], a reduction in the production of IL-1ß and TNF-α by monocytes[99,100], and partial cyclooxygenase inhibition with a decrease in the synthesis of prostaglandins[101]. A decrease in IL-2 production by T lymphocytes[101] and inhibition of T cell proliferation[101] were also reported. However, the target signalling molecules that mediate suppression of these inflammatory mediators remain unknown. Recently, Kremer[102] showed that a daily intake of the eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids is necessary to achieve a significant improvement. Interestingly, these fatty acids are ligands and activators for PPAR[36,40]. Therefore, it is tempting to hypothesize that the beneficial effects of n-3 fatty acids might result through binding and activation of PPARγ.

PPARγ AND COX-2

Prostaglandins are implicated in the control of cartilage and bone metabolism and are believed to mediate some of the actions of IL-1ß and TNF-α in these tissues. PGE_2 is detectable at high levels in the synovial fluid of patients with RA and OA[103]. Cyclooxygenase is the rate-limiting enzyme in prostaglandin synthesis. At least 2 forms of COX have been identified. The expression of the inducible COX isoform, COX-2, but not the constitutive form, COX-1, was found to be elevated in a disease related pattern in synovial tissue and cartilage from patients with RA or OA[104,105]. Chondrocytes and synovial fibroblasts, along with macrophages, are the source of increased prostaglandin synthesis in arthritis. Consequently, modulation of COX-2 activity has been a major target for therapeutic intervention.

In addition to inhibition of MMP and NO production, we have found that the PPARγ activator 15d-PGJ_2 inhibits IL-1ß induced PGE_2 production and COX-2 expression by human chondrocytes and synovial fibroblasts (unpublished data). This is consistent with the finding that 15d-PGJ_2 prevents LPS induced COX-2 expression in the macrophage-like differentiated U937 cells by interfering with NF-κB activation[73]. However, in bovine arterial endothelial cells (BAEC), which do not express PPARγ, 15d-PGJ_2 does not affect LPS induced COX-2 expression. Interestingly, transfection of PPARγ expression vector acquires this suppressive regulation of COX-2 gene by 15d-PGJ_2, suggesting that the inhibitory effect of 15d-PGJ_2 is mediated through PPARγ[73]. Macrophage and macrophage cell lines produce PGD_2 in a COX-2 dependent manner[73,74], and PGD_2 is spontaneously converted to PGJ_2 derivatives by nonenzymatic dehydration[106]. These observations raise the possibility that PGD_2 metabolites such as 15d-PGJ_2 constitute a negative autocrine loop and inhibit COX-2 expression, at least in macrophages, during the inflammatory process. Indeed, emerging evidence indicates that PGD_2 and 15d-PGJ_2 might display antiinflammatory activity *in vivo*. Gilroy, *et al*[107] analyzed the expression of COX-2 and the production of PGE_2, PGD_2, and 15d-PGJ_2 during inflammation in carrageenin induced pleurisy in rats. They found that COX-2 expression is induced during the first 2 hours and was accompanied by an increase in the level of PGE_2. Surprisingly, the resolution of inflammation was associated with a second peak in COX-2 expression, a low level of PGE_2, and an increase in the level of PGD_2 and 15d-PGJ_2. Consequently, this group has proposed that COX-2 is proin-

flammatory in the early phase of inflammation, but may have antiinflammatory activities during the resolution phase by generating antiinflammatory PG. If this cycle is reproduced into other species and humans, this metabolic pathway may constitute a new therapeutic target in inflammatory related disorders including arthritis.

PPARγ AND OTHER INFLAMMATORY DISEASES

The regulatory role of PPARγ ligands in monocytes/macrophage, T lymphocyte and endothelial cell function (Figure 3) suggests that PPARγ may play an additional role in other inflammatory diseases. Indeed, it has been suggested that PPARγ is involved in atherosclerosis, which has much in common with RA.

Atherosclerosis is characterized by macrophage accumulation in the vessel wall and differentiation into lipid loaded foam cells. This process comprises uptake of oxidized low density lipoprotein (oxLDL) by the CD36 scavenger receptor. These cells develop a proinflammatory phenotype characterized by the production of IL-1ß, IL-6, TNF-α, and MMP, which in addition to inflammation promotes smooth muscle cell proliferation. Expression of PPARγ has been reported to colocalize with macrophages in human[23,24] or mouse[108] atherosclerotic lesions. Exposure of human monocytes or monocytic cell lines to oxidized low density lipoprotein induces PPARγ expression, which in turn directly induces the transcription of the CD36 scavenger receptor, thus initiating an autoregulatory loop that promotes intracellular lipid accumulation and foam cell formation[23,108].

Nagy and collaborators[44] observed that 2 components of oxLDL, 9-HODE and 13-HODE, function as PPARγ ligands and activate PPARγ dependent transcription. Taken together, these observations indicate that PPARγ activation might promote atherosclerosis. However, recent evidence suggests that troglitazone slows down atherosclerotic progression in humans[109]. In addition, troglitazone was shown to inhibit vascular smooth muscle cells and intimal hyperplasia[110]. However, further *in vivo* studies are required to determine the exact role of PPARγ in atherosclerosis.

PPARγ activation may also have therapeutic potential in the treatment of inflammatory bowel disease. Indeed, 2 PPARγ ligands, troglitazone and rosiglitazone, were recently shown to attenuate colon inflammation in an established murine model of colitis. In addition, 15d-PGJ_2 or rosiglitazone prevents IL-1 induced expression of IL-8 and the monocyte chemoattractant protein 1, through inhibition of NF-κB activation. Compatible with this, Desreumaux, *et al*[111] recently demonstrated a protective effect of PPARγ and RXR ligands in a different experimental colitis model. The beneficial effect was reflected by a decrease in TNF-α and IL-1ß expression and NF-κB DNA binding activity as well as JNK and p38 activities in the colon.

IS PPARγ THE GENUINE TARGET?

As we have noted, there is one intriguing aspect in these studies. The most pronounced effects were obtained with 15d-PGJ_2, which may not be very selective for PPARγ. When more selective ligands such as TZD were employed, high concentrations were required to achieve similar effects. Thus some of the effects of PPARγ ligands, particularly 15d-PGJ_2, could be mediated by PPARγ independent mechanisms, although PPARγ independent effects of TZD have been also reported[112].

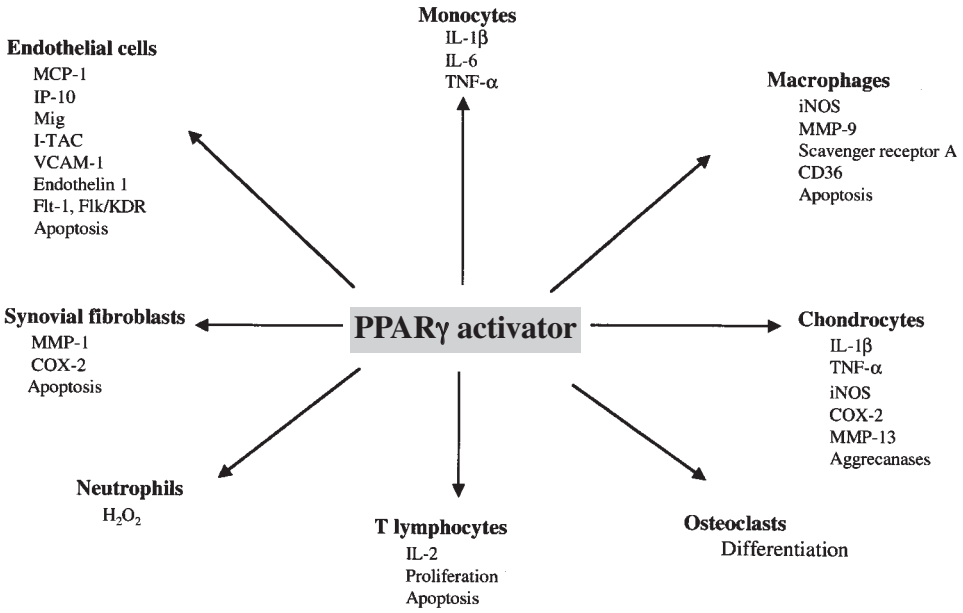

Figure 3. Some PPARγ functions in articular joint cells. PPARγ activators regulate the expression of genes involved in articular inflammation (cytokines and reactive oxygen metabolites), tissue destruction (MMP), and apoptosis.

15d-PGJ$_2$ was shown to prevent iNOS promoter activity and expression in a PPARγ independent manner in rat microglial cells and astrocytes[113]. Moreover, it was shown that 15d-PGJ$_2$ inhibited proinflammatory responses in a PPARγ independent manner by inhibiting cytokine induced degradation of IκBα and IκBß, the endogenous cytosolic inhibitors of NF-κB[114,115], and through direct inhibition and covalent modification of the IκB kinase[116,117]. Recently, 2 independent studies utilized homologous recombination to assess the role of PPARγ and showed that while PPARγ is essential for the regulated expression of CD36, it is not required for the antiinflammatory effects of 15d-PGJ$_2$ and TZD[21,118]. This raises the question whether PPARγ ligand activities are mediated entirely through PPARγ, and emphasizes that caution should be exercised before a function is imputed to PPARγ. Studies, including tissue-specific disruption of the PPARγ gene, are still required to define the exact role of PPARγ and its ligands in the regulation of inflammatory responses.

PPARα AND INFLAMMATION

Emerging evidence suggests that PPARα has the ability to modulate inflammatory responses *in vitro* and *in vivo*. Devchand, *et al*[119] showed that PPARα-null mice display a prolonged response to an inflammatory stimulus, indicating that PPARα has antiinflammatory activity. Treatment of human aortic smooth muscle cells with fibrates that are PPARα activators prevents IL-1ß induced IL-6 and COX-2 expression. In contrast, the PPARγ-specific activator BRL 49653 does not affect IL-6 or COX-2 expression[29]. Recent studies from the same group indicate that PPARα interacts directly with transcription factors NF-κB and AP-1, which may explain the antiinflammatory effects of PPARα ligands[18]. These *in vitro* results correlate with the finding that hyperlipidemic patients treated with fibrate had reduced circulating levels of IL-6, TNF-α, and IFN-α[29,120]. Furthermore, Poynter, *et al*[121] showed that both NF-κB signalling and cytokine (IL-6 and IL-12) release increased with aging and that these changes are suppressed by PPARα. PPARα also mediated fibrate inhibition of TNF-α induced VCAM-1 in human endothelial cells and significantly reduced adhesion of U937 histiocytic cells to cultured human endothelial cells[122]. Collectively, these results indicated that PPARα may exert antiinflammatory functions. However, conflicting data have been published that seemingly describe the opposite effect.

For example, Hill, *et al*[123] reported that PPARα ligands markedly increased TNF-α plasma levels in mice. Utilizing PPARα wild-type (WT) and knockout (KO) mice, they showed that the effects of fibrate on TNF-α expression were mediated by PPARα, indicating that PPARα promotes proinflammatory responses. In addition, PPARα activators were shown to increase TNF-α production[124] and NF-κB activity[125] in other cell types. Moreover, the synthetic PPARα ligand Wy-14643 inhibits nitrite accumulation in activated RAW 264.7 murine macrophages, whereas the natural PPARα ligands LTB$_4$ and 8(S)-HETE stimulate nitrite accumulation[65]. Reasons for the discrepancy between these studies and the seeming ability of PPARα to have pro- and antiinflammatory activities are currently unclear. It is an important future task to establish whether PPARα exerts pro- or antiinflammatory actions and its target cells.

CONCLUSION

Although our knowledge of the physiology of PPARγ has evolved enormously in the last few years, many important questions remain. In particular, it will be important to explore how the expression and activity of PPARγ are regulated and to identify endogenous/physiological ligands and the conditions under which they are produced. Considerable efforts have already been directed toward the identification of synthetic high affinity, high specificity agonists and antagonists for PPARγ. Microarray technology that enables transcript profiling through differential gene expression will certainly facilitate identification of additional genes that are both directly or indirectly regulated by PPARγ. Given the potential involvement of PPARγ independent pathways in mediating the effects of PPARγ agonists, another major challenge will be to identify these signalling pathways. Use of antisense along with tissue-specific disruption of the PPARγ gene should undoubtedly help clarify the exact role of PPARγ. Hence, this information, combined with data from animal model studies, will be of great clinical interest and may be the basis for the development of a future generation of antiarthritic drugs.

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