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Parsing the Role of PPARs in Macrophage Processes

A thesis submitted in partial satisfaction of the
requirements for the degree Master of Science
in
Biology

by
Daniel Toobian

Committee in charge:

Professor Pradipta Ghosh, Chair
Professor Alisa Huffaker, Co-Chair
Professor Keefe Reuther

2021

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University of California San Diego

2021

I dedicate this thesis to my late mother, as she is the main motivation for pursuing my career in
biomedical sciences.

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ABSTRACT OF THE THESIS

Parsing the Role of PPARs in Macrophage Processes

by

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Master of Science in Biology

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Cells are richly equipped with nuclear receptors, which act as ligand-regulated transcription factors. Peroxisome proliferator activated receptors (PPARs), members of the nuclear receptor family, have been extensively studied for their roles in development, differentiation, and homeostatic processes. In the recent past, there has been substantial interest in understanding and defining the functions of PPARs and their agonists in regulating innate and adaptive immune responses as well as their pharmacologic potential in combating acute and

chronic inflammatory disease. In this review, we focus on emerging evidence of the potential roles of the PPAR subtypes in macrophage biology. We also discuss the roles of dual and pan PPAR agonists as modulators of immune cell function, microbial infection, and inflammatory diseases.

INTRODUCTION

Peroxisome proliferator activated receptors (PPARs) are ligand-dependent transcription factors that are structurally conserved members of the nuclear receptor superfamily [1]. PPARs influence a variety of cell signals including cellular differentiation and development [2; 3; 4], lipid metabolism [5], the insulin signaling network [6], homeostasis [7] and tumorigenesis [2; 3; 8]. In 1960, scientists showed an increased number of peroxisomes in the livers of rats treated with hypolipidemic drugs. A decade later, this increase was attributed to certain members of the nuclear receptor family. In 1990, Issemann and Green cloned these receptors for the first time and demonstrated that hepatocarcinogens promote the proliferation of peroxisomes in rodents through these receptors, and thus named them **P**eroxisome **P**roliferator **A**ctivated **R**eceptors (PPARs) [9]. Three PPAR isoforms have been identified thus far: PPAR α , PPAR β/δ and PPAR γ . They each have distinct patterns of function and tissue distribution, and are expressed in various cell types including immune cells [6; 10; 11], epithelial cells [12] and endothelial cells [13; 14]. All PPARs utilize a common domain organization (Figure 1A) with a slightly variable amino-terminal that contributes to transcriptional activation function, and a central highly conserved DNA binding domain that contains a zinc motif [15]. A ligand-binding domain at the carboxy-terminal end confers their ligand-binding property, regulates ligand-dependent transcriptional activation and repression functions, and contributes to receptor homo- or heterodimerization (Figure 1B-1C) [16; 17].

PPAR γ is the most extensively characterized and researched member of the PPAR subfamily and consists of two isoforms, PPAR γ 1 and PPAR γ 2, that are expressed mainly in adipose tissue. They mediate adipocyte differentiation by regulating the expression of genes that are involved in lipid metabolism and insulin signaling [18]. PPAR α is the second most studied

PPAR, expressed mainly in liver and immune cells which regulate lipid catabolism, especially during fasting conditions [19]. The third is PPAR δ , (aka PPAR β) which is highly active in skeletal muscle where it is also involved in regulating fatty acid catabolism [20; 21]. PPAR δ activation also increases insulin sensitivity, improves lipid homeostasis, and prevents weight gain. Though PPARs are extensively studied, their role in molecular and cellular signaling in immune cells has limited understanding.

PPARs also regulate the functions of the innate immune system such as macrophage function and differentiation [5; 17; 22; 23]. Thus, there has been substantial interest in understanding and defining the functions of PPARs and their agonists in regulating gene expression in macrophage biology and how that relates to acute and chronic inflammatory diseases [22]. Few studies attempted to discuss the role of PPARs in macrophage function, and the discussion is generally limited to PPAR γ [6; 10; 22; 24]. Since all three isoforms of PPARs regulate each other's expression through feedback loops, it is worthwhile to understand their role together. In this review, we begin with a brief introduction of PPAR signaling and mechanism, and then highlight recent developments that provide insight into how isoforms of PPAR and their agonists can regulate several steps involved in the initiation, proliferation, and resolution of inflammatory responses in macrophages, especially in the context of microbial infection and inflammatory diseases.

TRANSCRIPTIONAL MECHANISM OF PPARS

PPARs regulate several metabolic and inflammatory signaling pathways during infection through both positive and negative regulation of gene transcription [22; 25]. The positive regulation comes from direct binding of PPARs to peroxisome proliferator hormone response elements (PPREs) present in the vicinity of target genes. PPARs predominantly bind as heterodimers with retinoid X receptors (RXRs), either in presence or absence of ligands (Figure 1C), to stimulate transcription activity [23; 25]. For example, PPAR α increases expression of carnitine palmitoyl transferase (CPT)-I, an enzyme located in the mitochondrial outer membrane controlling fatty acid β -oxidation, [23; 25]. Additionally, PPARs negatively regulate target genes by constitutively binding, along with nuclear co-repressors, to the PPREs of target genes which often function as transcriptional repressors in absence of ligands (Figure 1C). For example, NCoR and SMRT decrease transcriptional activity of PPAR γ thus preventing iNOS induction by LPS. Also, PPARs bind directly to transcriptional factors involved in inflammation including NF- κ B and AP1, inhibiting their transcriptional activity. This phenomenon is termed as 'trans-repression' (Figure 1C, 2A) [23; 25].

Trans-activation is mainly regulated by recruiting co-activators (Figure 1C), which enhance activation of PPAR-regulated genes. For example, PPAR coactivator-1 α (PGC-1 α) is a co-activator of both PPAR α and PPAR γ . Repression, trans-repression, and trans-activation mechanisms of transcriptional control of PPARs has been extensively studied and reviewed elsewhere. In the following section, we discuss recent progress in understanding how PPARs and their agonists regulate the metabolic and inflammatory signaling of macrophages in response to infection and inflammatory diseases.

INFLAMMATION AND INFECTION

Inflammation is a host response that targets invading infectious agents and tissue injury through recruitment of immune cells and repair machinery. Macrophages detect pathogen associated molecular patterns (PAMPs) present on microbes using pattern recognition receptors (PRR) [26; 27; 28]. For example, toll-like receptor 4 (TLR4) is a PRR that recognizes lipopolysaccharides (LPS) present on gram-negative bacteria cell walls [28]. In addition to recognizing diverse microbial components, many toll-like receptors also detect endogenous danger signals associated with tissue injuries and inflammatory diseases. Upon microbial invasion or purified ligand stimulation, TLRs promote rapid activation of major signal dependent transcription factors: nuclear factor- κ B (NF- κ B), activator protein 1 (AP1), and interferon regulatory factors (IRFs) [10; 28] (Figure 2A). These transcription factors work together to rapidly induce genes that trigger the initial inflammatory response, promote antimicrobial activity, and activate development of acquired immunity. It is important for macrophages to sustain sufficient inflammation to kill invading microbes. This sustained inflammation is maintained by several cytokines upregulated during initial stimulation which promote a forward transcriptional loop due to the autocrine and paracrine effects of cytokines [26; 29; 30]. However, sustained inflammation can lead to collateral tissue damage [30]. Therefore, negative feedback loops are essential to limit the extent of inflammation and promote resolution.

MACROPHAGE POLARIZATION AND PPARS

Macrophages are phagocytic innate immune cells, whose functions include scavenging microbes and apoptotic and necrotic cells, as well as playing a role in lipid homeostasis and tissue remodeling. Macrophages possess functional heterogeneity, in that they uptake different functions depending on signaling factors and metabolic changes [26; 27; 28; 30]. For example, upon sensing invading pathogens and Th-1 cytokines such as IFN γ , macrophages assume immune reactive form (pro-inflammatory/classical activation state, M1) to phagocytose pathogens. In contrast, after encountering Th-2 cytokines such as IL4 and IL13, macrophages assume immune tolerant form (anti-inflammatory/alternative activation state, M2) to help with tissue repair and angiogenesis in injured tissues [27; 30]. Impaired functions of both immune reactive and immune tolerant states of macrophages could lead to host tissue damage and development of chronic disease [10; 30; 31; 32; 33].

PPAR γ activation suppresses the immunoreactive state of macrophage as reported by suppression of immune reactive cytokine markers such as NOS2, TNF α , IL6, IL1 β and MCP1 in murine macrophages [34], whereas its activation promotes immunotolerant state markers such as CD36, IL13, Arg1, Ym1, Fizz1, CD206, IL4, and IL10 in murine macrophages [22; 35; 36; 37; 38]. PPAR γ deficient mouse macrophages also showed an increase in Th1 cytokines such as TNF- α , IL1- β , IL-6, IL-12 and a reduction of Th2 cytokine IL10 when induced with LPS [39]. PPAR γ also inhibits the expression of HIF1 α , which plays key role in inducing the immune reactive phenotype, and promotes Arginase 1 expression, which is a hallmark marker of the immune tolerant macrophage, in mice [40]. The evidence makes it clear that PPAR γ is in charge of, or at least promotes, the immune tolerant state of macrophages. This is further evidenced by how PPAR γ responds to infection *in vivo*.

As stated, the function of immune tolerant macrophages includes post-infection repair, which includes the cleanup of debris [41]. PPAR γ agonists have been shown to increase Fc γ receptor-mediated opsonized phagocytosis in murine alveolar macrophages [42; 43] demonstrating a possible pathway in which PPAR γ controls the cleanup process. In terms of repair, one study showed that PPAR γ deficient mice had an increase in pulmonary collagen deposition following influenza infection [44], demonstrating PPAR γ 's role in proper tissue repair post-infection. Again, PPAR γ shows itself to be a good promoter of the immune tolerant macrophage phenotype.

Aside from immune-reactive and immune-suppressive polarization, PPAR γ has also demonstrated effects on tissue-resident macrophage polarization. One of the most common tissue-resident macrophages are Kupffer cells [45]. Kupffer cells are generally localized to hepatic sinusoids [46]. PPAR γ activation has been shown to decrease the number of pro-inflammatory NO⁺ Kupffer cells, particularly through downregulation of TNF α and upregulation of IL10 [47]. PPAR γ has also been associated with the differentiation of alveolar macrophages. Monocytes are known to differentiate into alveolar macrophages after exposure to granulocyte-macrophage colony-stimulating factor (GM-CSF) [48]. GM-CSF activity leads to the expression of PPAR γ , which has been shown to be essential for monocyte differentiation into alveolar macrophages in mouse models [49]. PPAR γ has also been shown to inhibit the production of macrophage colony-stimulating factor (M-CSF), which regulates monocyte differentiation into alveolar macrophages [50].

PPAR γ can also affect macrophage polarization in a ligand-independent manner through trans-activation. For example, after alternative activation through exposure to IL4, macrophages displayed a remodeled and more accessible chromatin profile, an upregulation of PPAR γ , and no

changes in RXR levels. Upon subsequent stimulation with IL4, PPAR γ bound to DNA independently of ligands through the recruitment of P300 and RAD21, leading to further anti-inflammatory activity [51].

As for PPAR α , one study demonstrated that activating human cells with PPAR α agonist WY-14 643 led to an upregulation of Th1 cytokines such as IL-1 β -induced inflammatory cytokines [52]. Furthermore, extracts from PPAR α deficient mice demonstrated higher levels of IL13 and GATA-3 [53], which is a vital transcription factor for Th2 differentiation [54]. This demonstrates how PPAR α promotes the immune reactive state through inhibiting the immune tolerant state of macrophages. However, PPAR α has also been shown to be involved in tissue repair. Activation of PPAR α using WY 14,643 led to a reduction of acute injury and vascular leakage in perforated mouse lungs [55]. Additionally, PPAR α activation contributes to rapid repair of intestinal epithelium during SIV infection in macaque models [56]. While this does seem contradictory, there is nothing in these studies that suggest that these repairs are related to macrophage polarization, so the idea that PPAR α promotes the immune reactive macrophage phenotype is not ruled out.

Out of all the three members of the PPAR family, PPAR δ has the least amount of research conducted on it. There is evidence relating PPAR δ to the promotion of Th2 cytokines, suggesting its essential relationship with alternative activation of macrophages, however. IL-13 and IL-4 are examples of Th2 cytokines that become active through STAT6 activation [57]. Additionally, adipocytes secrete Th2 cytokines involved in alternative activation, as macrophages incubated with adipocyte conditioned medium (CM) displayed an inhibition in pro-inflammatory Th1 cytokines such as MCP-1 and TNF α while displaying an upregulation of immune tolerant marker genes such as Mgl1 and Mgl2 [58]. When PPAR δ -deficient mice were

incubated with adipocyte CM, there was an inhibition of STAT6 activity, inhibiting alternative activation of macrophages. There was also an inhibition of transcription of immune tolerant markers Mgl1, Mgl2, and Mrc2 and an upregulation of Th1 cytokines such as MCP-1, TNF α , and IL-6 [59]. This study suggests that PPAR δ expression in macrophages is essential for adipocyte-induced activation of immune tolerant state of macrophages. This hypothesis is further corroborated by other studies. GW501516, a PPAR δ agonist, inhibits transcription of Th1 cytokines such as IL-6, IL1 β , TNF α , and NF- κ B as well as neutrophil and macrophage infiltration in mice [60]. PPAR δ activation has also been shown to suppress IFN γ in mice [60; 61]. Another study directly demonstrated that transferring PPAR δ -deficient bone marrow into wild type mice led to an inhibition of alternative activation of macrophages [62]. We conclude that PPAR δ , similarly to PPAR γ , promotes the immune tolerant phenotype and inhibits the immune reactive phenotype of macrophages, while PPAR α promotes the immune reactive phenotype while inhibiting the immune tolerant phenotype. The members of the PPAR family indirectly regulate each other on their effects on macrophage differentiation through competing cytokines.

MACROPHAGE FUNCTION AND PPARS

Upon infection, macrophages surge at the place of infection and assume a pro-inflammatory, immune reactive state. Immune reactive macrophages are programmed for phagocytosis and killing of the invading pathogen by producing large amount of reactive oxygen species (ROS). Since this infectious environment is low in oxygen, immune reactive macrophages program themselves to survive in low oxygen (hypoxic) conditions [63]. Within immune reactive macrophages, both aerobic glycolysis and pentose phosphate pathways are induced upon activation (Figure 3). Glycolysis promotes glucose uptake to produce pyruvate (Figure 3). However, under hypoxic conditions, NADH cannot be oxidized to NAD⁺, a required electron acceptor for the further oxidation of pyruvate. Therefore, in hypoxic conditions, pyruvate is first reduced to lactate, accepting electrons from NADH, and thereby regenerating the NAD⁺ needed for glycolysis to continue [64]. In the immune reactive macrophage's mitochondria, the electron transport chain is dampened, promoting production of mitochondrial reactive oxygen species (mtROS) due to incomplete electron transfers (Figure 3). Additionally, induction of pentose phosphate pathways in immune reactive macrophages generates more NADPH, which is needed for the NADPH oxidase to generate cytosolic ROS and nitric oxide [65].

PPARs modulate both glycolysis and NADPH oxidase induced ROS (Figure 3). PPAR γ diminishes glycolysis in mice by promoting expression of 6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3 (PFKFB3), which in gluconeogenesis pathway, converts fructose-2,6-bisphosphate to fructose-6-phosphate and increases accumulation of fructose-6-phosphate (Figure 3) [66]. This indicates that PPAR γ prevents macrophages from polarizing towards the glycolysis dependent immune reactive state, further confirming its anti-inflammatory effect on

macrophages. Interestingly, PPAR α , but not PPAR γ , is essential for NADPH oxidase induced ROS generation in both humans and mice [67]. This is further evidence that PPAR α contributes to immune reactive macrophage polarization [68].

Immune tolerant macrophages, on the other hand, obtain their energy from fatty acid oxidation and oxidative phosphorylation for tissue repair and tissue remodeling [69]. In addition, macrophages can induce the constituent electron transport chain, which is required for oxidative phosphorylation and drives pyruvates into the Krebs cycle (Figure 3). Both PPAR α and PPAR γ promote the gene expression of several molecules/enzymes involved in oxidation of fatty acid. For example, PPAR α and PPAR γ , in humans, promote expression of mitochondrial fatty acid transporter carnitine palmitoyltransferase 2 (CPT2) and the hydroxyacyl-coenzyme A (CoA) dehydrogenase trifunctional multienzyme complex subunit beta (HADHB) [70], which catalyzes the final step of β -oxidation (Figure 3).

Considering PPAR α promotes fatty acid oxidation, it may seem as though PPAR α is anti-inflammatory. However, the promotion of fatty acid oxidation through PPAR α also leads to its inhibition through proinflammatory phospholipid by-products. PPAR α has been shown to promote leukotriene B4 (LTB4) synthesis through β -oxidation in rats. Interestingly, LTB4 and other fatty acid derived molecules are ligands for PPAR α , thus this feedback loop promotes their own catabolism and leads to resolution of inflammation [71] (Figure 4). Even though PPAR α contributes to fatty acid oxidation, it only does so to regulate and inhibit itself to allow for less inflammation. Taken together, it emerges again that PPAR α is essential for promoting the immune reactive state of macrophage whereas PPAR γ is essential for promoting the immune tolerant macrophage. PPAR α demonstrates its ability to regulate its own pro-inflammatory abilities through self-inhibition when inflammation resolution is necessary.

BACTERIA AND PPARs

When it comes to bacterial infection, PPAR γ activation appears to correlate with poor outcome. PPAR γ activation in both humans and mice has been shown to decrease the number of neutrophils and macrophages as well as compromise bacterial clearance, which could worsen complications such as influenza-associated pneumonia [72]. Several studies have shown that known PPAR γ agonists contribute to caspase-associated apoptosis of monocytes, T cells, and B cell progenitors [73; 74; 75]. PPAR γ has also been shown to attenuate neutrophil migration and activation [76]. This may explain how PPAR γ can cause immunosuppression to the extent of increasing risk of infection. This is further corroborated by a study that demonstrated how PPAR γ knockout mice had an increased effector response when infected with *E. coli* [77]. This may also explain why PPAR γ agonists have shown an increased risk in cardiovascular dysfunction in humans [78], as there are many species of bacteria that can increase risk of cardiovascular disease. PPAR γ can certainly be seen as a therapeutic target for infection and immune related diseases, but should be done so with the consideration of its indication of poor prognosis through inhibition of bacterial clearance.

On the other hand, PPAR α activation has been shown to be protective against bacterial infections. Infection with *Francisella tularensis* heavily upregulates fatty acid metabolism, which we already know is regulated by the PPAR α pathway [79]. This may be indirect evidence that infection with *F. tularensis* leads to PPAR α activation. PPAR α activation using gemfibrozil has been shown to decrease the bacterial load of *Mycobacterium tuberculosis* infected mice while also inhibiting *M. abscessus* induced hypersecretion of pro-inflammatory cytokines [80]. One more study using mice demonstrated similar results using *Pseudomonas aeruginosa* [81]. Another study demonstrated that PPAR α -deficient mice have a decreased survival rate during

bacterial sepsis as well as impaired liver metabolism [82]. Again, in contrast to PPAR γ , PPAR α demonstrates a pro-inflammatory phenotype, with it promoting cells' abilities to kill bacteria, especially considering we previously mentioned PPAR α 's ability to induce NADPH oxidase formation of ROS, which is essential for bacterial clearance.

As for PPAR δ , there is unfortunately a lack of direct evidence on its impact on bacterial clearance. Considering we do know its similarities to PPAR γ in its promotion of immune tolerant phenotype macrophages, we can hypothesize that its activation also inhibits bacterial clearance. However, more studies would need to be conducted to confirm this.

VIRUSES AND PPARs

In infection, too much inflammation can devastate the body. For example, influenza infection can lead to a “cytokine storm”, a hyper-induction of immune response that can lead to complications and lung pathogenesis [83]. Considering excessive inflammation is tied to influenza related mortality, PPAR γ has been considered as a therapeutic target to limit such harmful inflammation [84]. In a recent study, it was demonstrated that in mouse alveolar macrophages, PPAR γ mRNA levels were reduced after influenzaA infection and respiratory syncytial virus infection [24]. In contrast, the spike protein of SARS-CoV-2 upregulates PPAR γ in macrophage-like RAW264.7 cells [85]. In another independent study, infection with MERS-CoV upregulated PPAR γ in human macrophages [86]. It appears that PPAR γ is upregulated in certain viral infections while downregulated in other viral infections. Regardless, PPAR γ plays a significant role in the prognosis of viral infections in general.

One study demonstrated that PPAR γ reduced the secretion of influenza-induced proinflammatory cytokines TNF-a, IL-8, and RANTES in humans [87]. PPAR γ activation also leads to decreased mortality in obese mice infected with influenza [88]. Furthermore, HIV infection in mice also leads to the hyper-induction of proinflammatory genes such as TNF α , IL-1 β , IFN γ , CCL2, CCL3, CXCL10, and iNOS, all of which has been shown to be attenuated using PPAR γ agonists rosiglitazone and pioglitazone [89]. While PPAR γ activation leads to poor prognosis in bacterial infection, it apparently leads to good prognosis in viral infection through its inhibition of hyperimmune response.

PPAR α activation contributes to rapid repair of intestinal epithelium during SIV infection in macaque models [56]. Interestingly, certain viral infections, such as Zika virus, have been shown to modulate and dysregulate PPAR α signaling pathways in human cells [90]. The core

protein of hepatitis C virus (HCV) was also found to inhibit PPAR α expression in humans [91]. Another study confirmed that both PPAR α and PPAR γ are downregulated during HCV infection, and then further downregulated during co-infection with HIV in humans [92]. Furthermore, activation of PPAR α has been shown to inhibit STING activation of type I interferons as well as increase herpesvirus replication in infected mouse cells [93]. It appears that PPAR α activation and an increase in viral load and pathogenesis are heavily correlated, again showing an inverse relationship between bacterial infection prognosis and viral infection prognosis. PPAR agonists and antagonists may be key therapeutic strategies depending on the type of infection.

PPARS AND INFLAMMATORY BOWEL DISEASE

Inflammatory bowel diseases (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) negatively impact the quality of life of millions of people [94]. CD consists of inflammation of the mouth, anus, and intestines, while UC consists of inflammation in the mucosal layer of the colon [95]. Common pro-inflammatory cytokines are associated with IBD such as TNF α , IL-1 β , IL-6, IFN γ , and IL-12 [96] which are predominantly secreted by inflammatory immune cells including neutrophils and macrophages. Unsurprisingly, anti-inflammatory drugs are a common treatment for IBD.

Colon RNA seq data revealed the fact that both PPAR α and PPAR γ are down regulated during IBD disease progression [97; 98]. Considering PPAR γ demonstrates anti-inflammatory abilities and is highly expressed in the intestines [99], many researchers see PPAR γ as a good treatment candidate target. Rosiglitazone, pioglitazone, troglitazone and AS002, known PPAR γ agonists, have demonstrated protection and recovery from pathogenic inflammation in colitis mouse models [100; 101]. However, several PPAR γ agonists have failed in clinical trials.

Regarding PPAR α , there is conflicting evidence on its role in IBD. One study showed how the PPAR α -UGT pathway increased de novo bile acid synthesis, exacerbating mouse model colitis [102]. Another study used a recombinant protein (rSj16) taken from bacteria and demonstrated its effects on inhibiting PPAR α as well as protecting against DSS-induced colitis in mice [103]. When mouse models were treated with fenofibrate, PPAR α activation increased in parallel to colonic inflammation [104]. Although, it should be noted that fenofibrates alter many different metabolic pathways [105]. On the other hand, several studies conclude the opposite. When mice were treated with PPAR α agonist Wy-14643, there was a decrease in susceptibility to colitis [106]. Additionally, verbascoside (VB) acts as a collector of intracellular ROS,

reducing experimental colitis. PPAR α -KO mice showed weaker VB-mediated anti-inflammatory activity compared to wild type, suggesting PPAR α 's protective role against IBD [107]. Also, in PPAR α -KO mice, innate immune cells decreased production of IL-22 and antimicrobial peptides RegIII β and RegIII γ as well as calprotectin. This led to commensal dysbiosis as well as an increased tolerance for gut bacteria that release proinflammatory cytokines [108]. Finally, an additional study demonstrated that dexamethasone induced anti-inflammatory activity is weakened in PPAR α -KO mice [109].

Knowledge of PPAR δ and its role in IBD is severely limited. One study showed that dual activation of PPAR δ and PPAR γ using conjugated linoleic acid (CLA) downregulated both TNF α and NF κ B activation while upregulating TGF- β 1 as well as protecting against DSS and CD4 induced colitis in mice [110]. However, another study demonstrated that PPAR δ upregulates COX-2 in mouse gut epithelial cells, leading to an increase in macrophage-produced proinflammatory cytokines and increased the risk of colonic inflammation [111].

In our previous study we demonstrated that activation of PPAR α or PPAR γ individually is not enough for protection against *Citrobacter*-induced colon infection in mice. However, a dual activation of both PPAR α and PPAR γ using a balanced dual agonist protected mice from *Citrobacter*-induced colon infection [129].

Taking all of this information, it appears that the use of anti-inflammatory PPAR γ agonists prevent excessive inflammation in colon. However, its prolonged use could lead to polarization of gut macrophages towards an immunotolerant state which eventually help the survival and replication of pathogenic gut bacteria and inflate the development of IBDs. Additionally, continuous use of PPAR α agonists alone causes excessive activation of NADPH oxidase and mitochondrial dependent ROS production, potentially leading to collateral host

tissue damage and inflammation. Therefore, balanced activation of both PPAR γ and PPAR α is the key to treat the IBD disease and might be help prevent IBD disease progression, combining the pro-inflammatory effects of PPAR α and the anti-inflammatory effects of PPAR γ .

PPARS AND ATHEROSCLEROSIS

Atherosclerosis is the leading cause of the development of cardiovascular diseases. During hyperlipidemic conditions, lipids sneak into the subendothelial layer of the aortic wall, where oxidation of lipid alters it to form oxidized LDL (oxLDL). Macrophages scavenge on oxLDL to process it. However, excess oxLDL promotes oxLDL accumulation in macrophages, leading to foam cell formation and atherosclerosis development.

PPAR α activation was shown to promote low density lipid (LDL) oxidation in humans and mice [67], as ROS can be responsible for the oxidation of LDL and PPAR α increases ROS levels through NADPH oxidase. PPAR α activation has also been shown to inhibit LPS activation of iNOS [67], the inducible enzyme that produces nitric oxide (NO). Normally, NO is responsible for overall cardiovascular health [112], vasodilation [113], as well as inhibition of LDL oxidation [114]. However, in the presence of an excess of superoxides, NO can react with the superoxides to create peroxynitrite, a reactive peroxide that can lead to ROS and RNS (reactive nitrogen species)[115]. Interestingly, oxLDL has been shown to activate PPAR α [67]. So far, PPAR α has been described as pro-inflammatory, yet it also appears to demonstrate self-regulatory abilities. PPAR α increases ROS/superoxide levels, as previously stated, and uses oxLDL as a signal to know that such an increase has taken place. In an effort to protect the cell from self-destruction from the creation of too much ROS, PPAR α disables an alternate pathway that superoxides can interact with to create even more potentially harmful reactive species, namely the iNOS activation pathway (Figure 4).

Polyphenols, such as punicalagin, gallic acid, and ellagic acid, derived from fruits such as pomegranates, have been shown to activate PPAR γ activity [116]. Activation of PPAR γ using these polyphenols increases transcription and protein activity of Paraoxonase 1 (PON1) in HuH7

hepatocyte cells [116]. There are also several studies that demonstrate a correlation between an activation of PPAR γ , using agonists such as rosiglitazone and several different statins, and an increase in PON1 activity [117; 118; 119; 120; 121]. Once PON1 forms, it associates with high-density lipids (HDL) [122], where it performs several protective functions, such as preventing lipid hydroperoxide formation on HDL as well as protecting the activity of lecithin-cholesterol acyltransferase (LCAT) [123], an enzyme important for HDL antioxidant function and preventing oxidative stress [124]. Furthermore, HDL-associated PON1 has been shown to prevent oxidation of low-density lipids (LDL) in humans [125; 126]. When LDL is oxidized (oxLDL), lipoperoxides and thiobarbituric acid reactive substances are formed, which can cause oxidative damage. PON1 was shown to inhibit accumulation of these harmful agents in mice [127]. By preventing the oxidation of LDL, PON1 and PPAR γ demonstrate anti-atherosclerotic properties [128]. Furthermore, monocyte chemoattractant protein-1 (MCP-1), which is produced from oxLDL, binds to oxLDL in order to attract macrophages to uptake them. PON1 inhibits this production as seen in human endothelial cells [128]. Interestingly, oxLDL also inhibits activity of PON1 in humans [129], possibly in an effort to equilibrate oxidation.

Naturally derived polyphenols have also been shown to increase transcription of paraoxonase 2 (PON2) through activation of PPAR γ . This was further confirmed as known PPAR γ agonist rosiglitazone was shown to stimulate PON2 expression in mouse macrophages [130]. PON2 potentially plays a protective role in the prevention of superoxide and reactive oxygenated species (ROS). Normally within complex 3 of the electric transport chain (ETC), coenzyme Q10 (Q10) donates an electron from QH₂ to cytochrome C. Q10's transition phase, ubisemiquinone, is rather unstable and can sometimes donate the electron to oxygen instead of cytochrome C, when treated with ETC inhibitors, forming superoxides, leading to ROS and

oxidative stress [131]. Interestingly, PON2 is not only localized within the inner mitochondrial membrane where it is associated with complex 3, but it also binds with high affinity to Q10 [132]. Furthermore, PON2 deficient mice were shown to have increased mitochondrial oxidative stress, decreased complex 1 and 3 activities, decreased oxygen consumption, and decreased ATP production [132], demonstrating that lacking PON2 interrupts the ETC. All this information suggests that PON2 associates with Q10, protecting it from destabilization and preventing it from donating electrons to oxygen to form superoxides and ROS. This would mean PON2, and therefore PPAR γ , plays an antioxidant role in preventing oxidative stress through the Q cycle pathway.

PPAR γ can also inhibit the production of ROS and oxidative stress in other, more direct ways as well. When mouse macrophages were incubated with PPAR γ agonist prostaglandin D2 metabolite 15-deoxy- Δ 12,14prostaglandin J2 (15d-PGJ2), the activities of pro-inflammation transcription factors AP-1, STAT and NF- κ B were antagonized [133]. These three proteins act as transcription factors for nitric oxide synthase (iNOS), therefore PPAR γ inhibits the transcription of iNOS and the accumulation of nitric oxide [134]. Induction of iNOS has been shown to increase ROS levels in mouse RAW264. 7 macrophages as well [135]. PPAR γ again demonstrates a suppressive role against oxidative stress and ROS (Figure 5), which is why it has been such a common target for anti-atherosclerotic therapy. However, considering the previously stated increase in risk of infection, a dual PPAR α/γ agonist would be a safer approach, especially considering both PPAR γ and PPAR α inhibit iNOS related ROS production.

PPAR ANTAGONISTS AND THEIR THERAPEUTIC POTENTIAL

PPAR γ antagonists (Table1) such as GW9662 and T0070907 have been shown to inhibit the uptake of apoptotic cells [136]. As stated before, immune-suppressive macrophage function includes post-infection repair and cleanup and debris [41]. This is further evidence that PPAR γ promotes the immune-suppressive phenotype of macrophages. PPAR γ antagonist use have also been a strategy for therapy, although less common agonist usage. For example, PPAR γ antagonists have shown an ability to increase insulin-sensitivity and as well as inhibit adipocyte differentiation, lipid metabolism, and lipid accumulation in mouse models [137; 138; 139; 140]. PPAR γ antagonist Gleevec has also been shown to increase the browning of white adipose tissue in both humans and mice [141]. Because of this, PPAR γ antagonists have been seen as a potential therapy for type 2 diabetes and obesity. PPAR γ antagonists have also been seen as a potential therapy for cancer, as they have demonstrated antiproliferative effects on cancer cells [142]. This is also because fatty acid metabolism is associated with tumorigenesis [143]. They have especially been seen as therapy for cancers in areas with high amounts of adipose tissue such as breast cancer [144; 145].

As for PPAR α antagonists, there is less research on their therapeutic potential. They have mostly been seen as a potential therapy for different types of cancer. One study demonstrated that PPAR α antagonist NXT629 induces apoptosis in chronic lymphocytic leukemia cells using mouse models [146]. Another study using mouse models demonstrated that PPAR α antagonist GW6471 attenuates tumor growth in renal cell carcinoma [147].

PPAR δ antagonists have even less research backing them, but they are generally seen the same way as PPAR α antagonists in their use in cancer therapy [148]. One study even demonstrates anti-psoriasis therapy in mice [149]. Overall, PPAR antagonists are an interesting

strategy for therapy, however, there does not seem to be significant clinical research on them (Table 2). While there is evidence of their therapeutic benefits, single PPAR antagonists pose the same potential risks of single PPAR agonists. Activating or inhibiting only one member of the PPAR subfamily creates the risk of an overactive or underactive immunity. There is not much research on dual or pan PPAR antagonists, but the use of single PPAR antagonists should be done with these potential risks in mind.

THE FUTURE OF PPAR IN THERAPEUTICS

In terms of clinical study and treatment, more are leaning towards dual and pan agonists for the PPAR family. While several have been discontinued, several new agonists are in preclinical and clinical trials (Table 2). We have demonstrated the dueling relationship between PPAR γ and PPAR α in terms of macrophage differentiation, bacterial and viral clearance, IBD, and atherosclerosis. A PPAR γ/α dual agonist seems to be more promising in terms of therapeutics and activation of both receptors would counter the each other's side effects while still providing better pharmacological effects [150]. Another notable example is how PPAR γ agonists have been used as a therapeutic drug for increasing insulin resistance in diabetic patients as well as lipid metabolism in patients with atherosclerosis. However, clinical trials were halted when patients developed increased risks for congenital heart disease [78]. While studies of PPAR γ/α agonists in relation to diabetes have been done before [151], there has been a greater focus in more recent years on how these dual agonists can treat diabetes with greater efficacy while also limiting the risk of heart failure [78], utilizing both pro and anti-inflammatory effects to our advantage. Another approach for the same problem is using PPAR α/δ dual agonists, such as GFT505, which have been shown to treat type 2 diabetes while altogether avoiding the cardiovascular risk of PPAR γ agonists [152]. The same PPAR α/δ dual agonist has also been shown to demonstrate hepatoprotective properties [153]. Pan PPAR agonists are being studied for many different conditions such suppressing inflammation and increasing lipid oxidation [154], protecting against metabolic disorders and fibrosis [155], and even angiogenesis in ischemic mice [156].

Regarding direct crosstalk between PPARs, there is unfortunately little evidence. Only one study provided evidence for direct crosstalk, demonstrating that PPAR γ inhibits PPAR δ

while PPAR α inhibits PPAR δ as PPAR δ activates PPAR α [157]. There is more evidence regarding indirect crosstalk, such as how all three PPARs inhibit NF- κ B signaling and function as previously mentioned. There is also how PPAR δ increases COX-2 transcription while both PPAR γ and PPAR α inhibit it. However, looking at indirect relationships gives little insight into direct crosstalk, as there are many interfering pathways. More studies must be done on how specific PPAR activation/inhibition affects other PPAR transcription and activity in order to gain greater insight on the outcomes of PPAR agonists as well as dual and pan agonists.

All Chapters, in full, have been submitted for publication of the material as it may appear in *Frontiers in Immunology*, 2021. Toobian, Daniel; Ghosh, Pradipta; Katkar, Gajanan, *Frontiers Media*, 2021. The thesis author was the primary investigator and author of this paper.

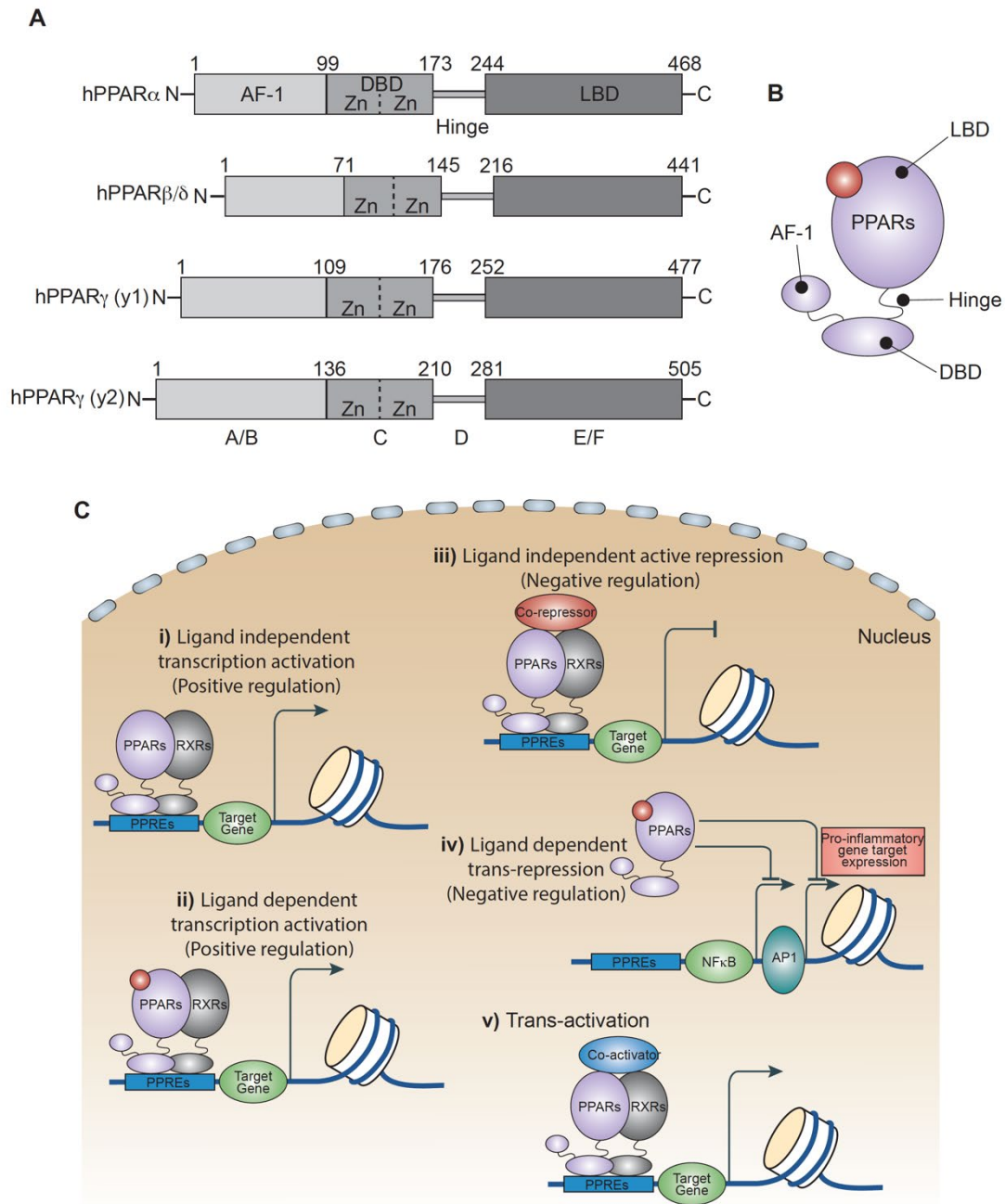


Figure 1

Figure 1. PPAR isoforms and their transcriptional regulatory function. (A) A schematic of the domain architect of PPAR isoforms PPAR α , PPAR β /d and PPAR γ . (B) Cartoon showing ligand binding site in PPARs. (C) Ligand independent and dependent transcriptional regulatory mechanism of PPARs.

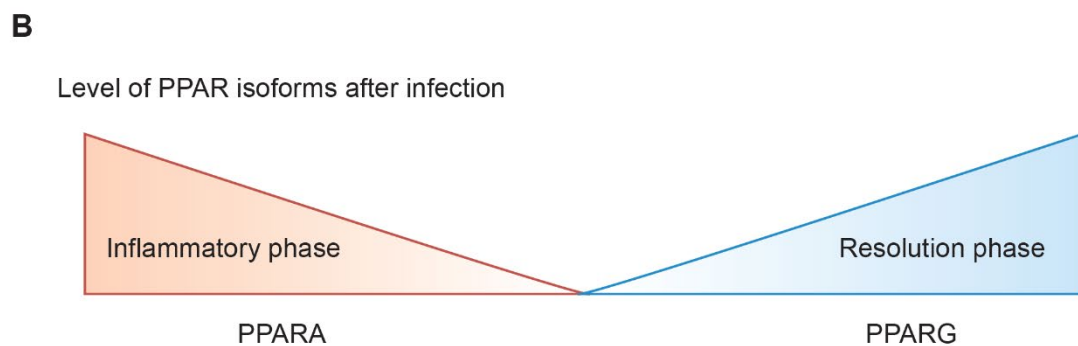
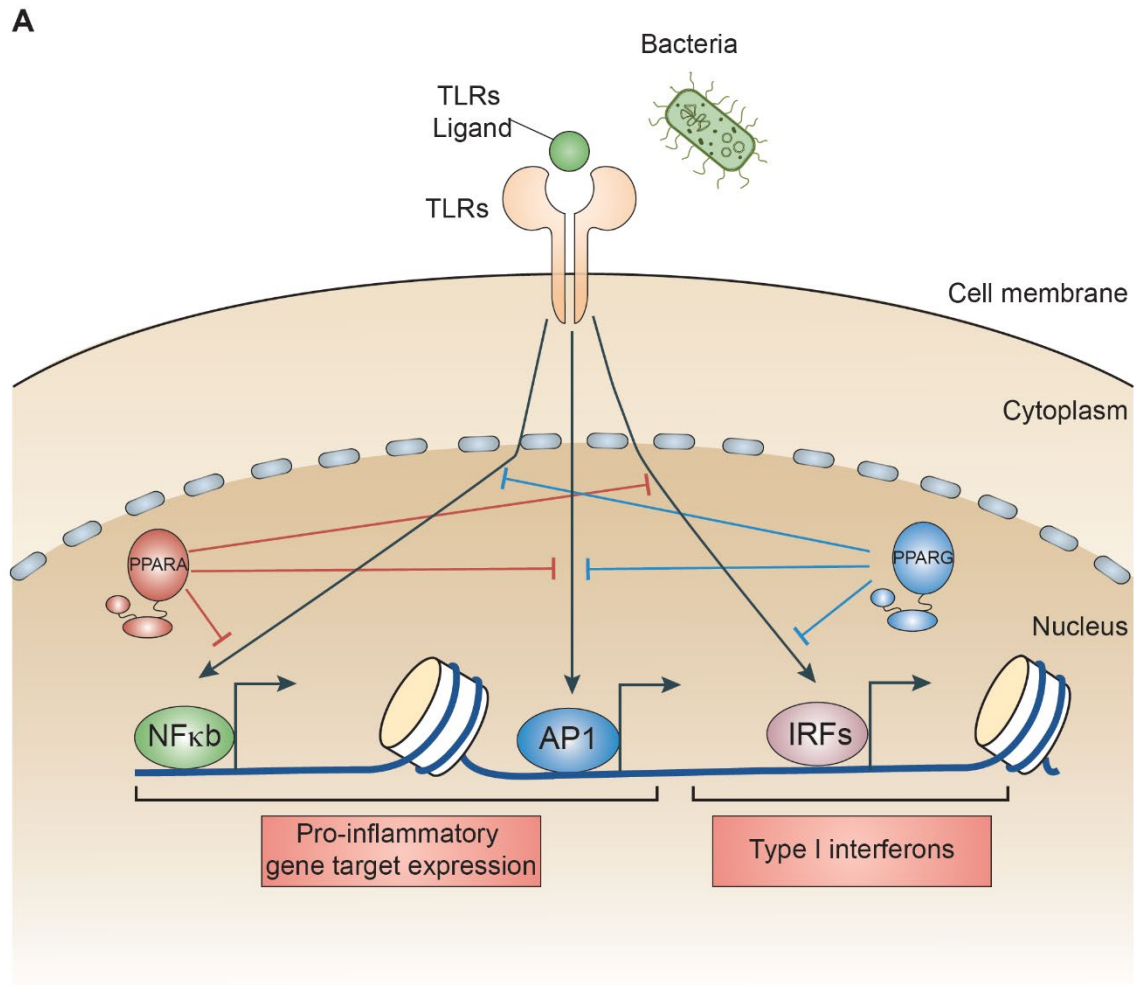


Figure 2. Role of PPARs in bacteria-induced inflammatory signaling. (A) Schematic illustrating LPS/bacteria induced inflammatory signal promoting transcription factors. Whereas PPARs interacts with and modulates transcription factors involved in microbe induced inflammation. (B) Schematic depicting gene expression of PPARs during infection. PPARA level is higher during inflammatory phase whereas PPARG is higher during resolution phase.

Figure 3. PPARs in macrophage function and polarization. The schematics depicts the biochemical steps involved in glycolysis and gluconeogenesis, and how each pathway correlates to different immune states of macrophages. PPARA regulates immune reactive state, glycolysis dominant state (red left side) and PPARG regulates immune tolerant state, gluconeogenesis dominant state (blue right side).

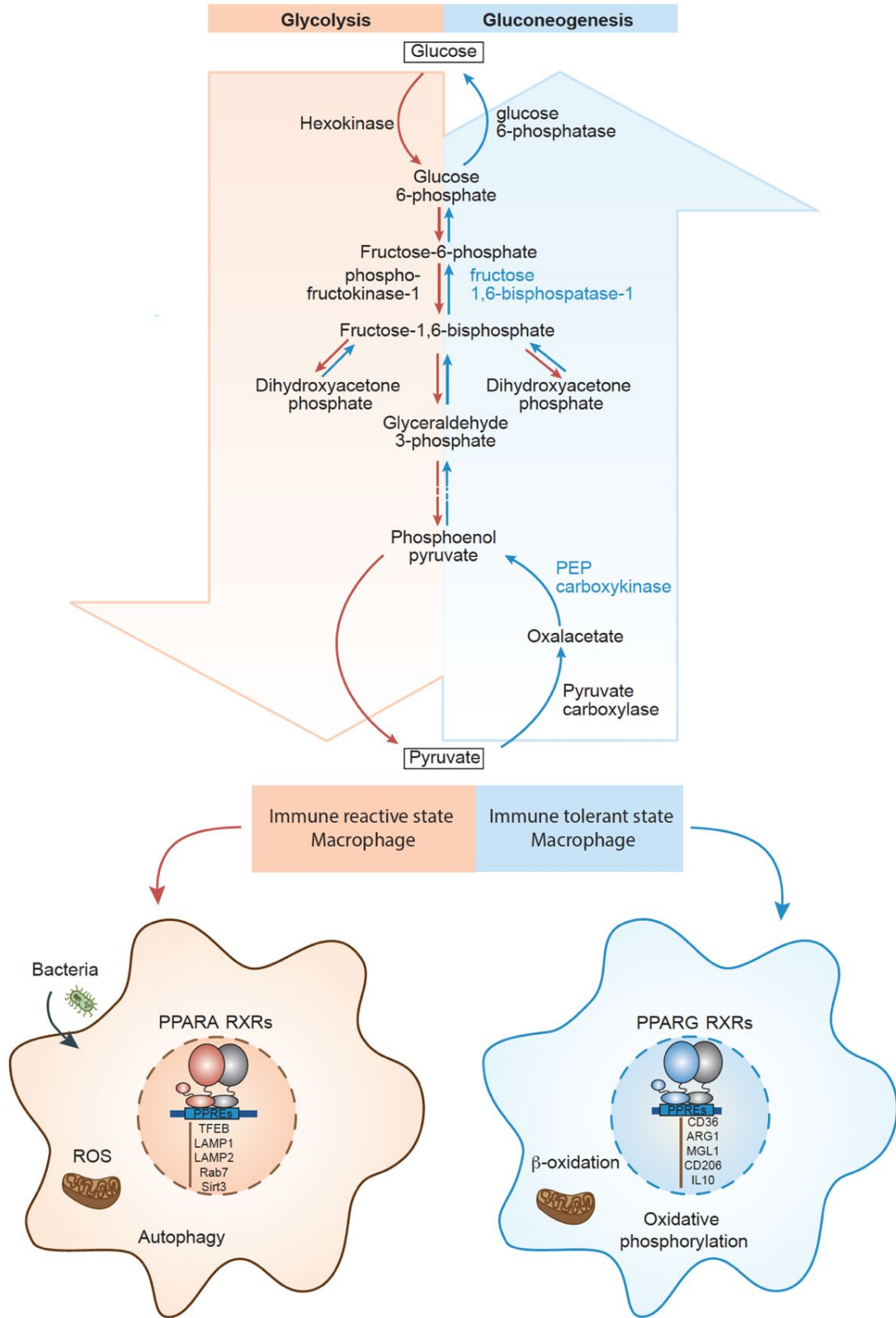


Figure 3

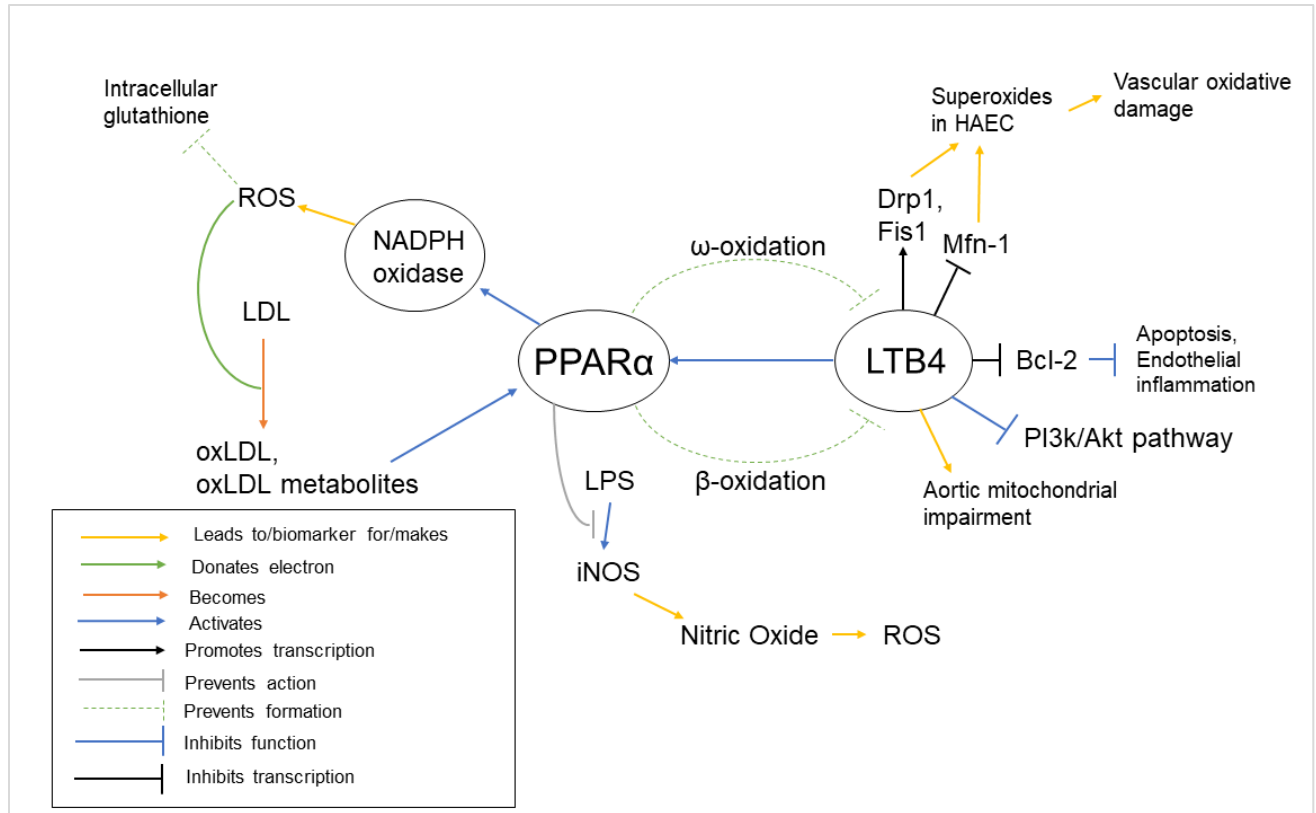


Figure 4. PPAR α Inflammatory Pathways. This schematic visualizes how PPAR α directly and indirectly influences inflammatory pathways using color coded lines and arrows labeled in the box in the bottom left.

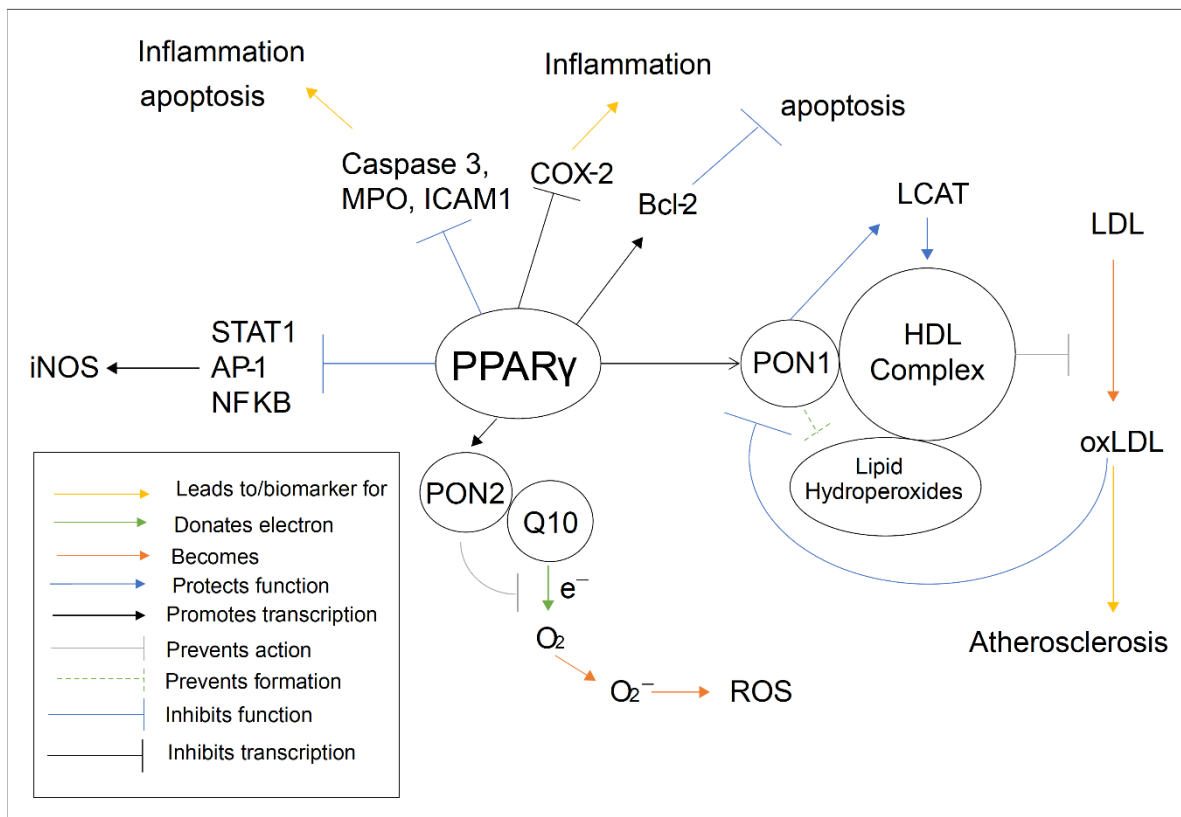


Figure 5. PPAR γ Inflammatory Pathways. This schematic visualizes how PPAR γ directly and indirectly influences inflammatory pathways using color coded lines and arrows labeled in the box in the bottom left.

TABLE 1. PPAR single antagonists and their market status

PPAR Antagonist	Indications	Effect	Status	Reference
PPARα Antagonist				
TPST-1120	Cancer	Inhibits fatty acid metabolism	Phase I clinical trials	[158]
GW6471	Renal cell carcinoma	Inhibits fatty acid metabolism and glycolysis	Preclinical	[147]
NXT629	Chronic lymphocytic leukemia	-	Preclinical	[146]
MK886	Lung adenocarcinoma	-	Preclinical	[159]
PPARγ Antagonist				
GW9662	Cancer, obesity, diabetes	-	Preclinical	[144]
T0070907	Cervical cancer	-	Preclinical	[160]
SR-202	Obesity, diabetes	-	Preclinical	[138]
Betulinic acid	HIV, inflammation, malaria dysplastic nevus syndrome, melanoma	Induces apoptosis, increases ROS and caspase activation	Phase I clinical trials	[161]
Gleevec	Leukemia	Inhibits tyrosine kinase	Approved	[162]
PPARδ Antagonist				
GSK-3787	Psoriasis	-	Preclinical	[149]
SR13904	Cancer	-	Preclinical	[148]
GSK0660	Psoriasis	-	Preclinical	[149]

Table 2. PPAR α / γ dual agonists, their potency and market status.

PPAR α / γ Dual Agonist	EC50 (α)	EC50 (γ)	Status
Muraglitazar	320.0nM 5680nM	110.0nM 243nM	Discontinued [163, 164]
Tesaglitazar	4780nM 1200nM	3420nM 1300nM	Discontinued [164, 165]
Naveglitazar	2816nM	361nM	Discontinued [166]
Ragaglitazar	3200nM	600nM	Discontinued [167]
Farglitazar	250nM 450nM	0.2nM 0.34nM	Discontinued [168, 169]
Imiglitazar	8nM	4nM	Discontinued [170]
Netoglitazone	100nM	3000nM	Discontinued [171]
Reglitazar	1900nM	83nM	Discontinued [172]
MK0767	140nM	83nM	Discontinued [173]
KRP-297	850nM	83nM	Discontinued [174]
TZD18	26nM	14nM	Preclinical [175]
Chiglitazar	1200nM	80nM	Phase II clinical trials [176]
Aleglitazar	50nM 5nM	21nM 9nM	Phase III clinical trials [164, 176]
PLX429	-	-	Preclinical
AVE0847	-	-	Phase II clinical trials
Azaindole- α -alkyloxyphenylpropionic acid	-	-	Preclinical
BVT-142	-	-	Preclinical
O-Arylmandelic acid derivatives	-	-	Preclinical
Amide substituted with α -substituted- β -phenylpropionic acid derivatives	-	-	Preclinical
2-Alkoxydihydro cinnamate derivatives	-	-	Preclinical
LY51029	-	-	Preclinical
α -Aryloxyphenyl acetic acid derivatives	-	-	Preclinical
Tricyclic- α -alkyloxyphenyl propionic acids	-	-	Preclinical
Saroglitazar	0.00065nM	3nM	Phase II clinical trials, Approved in India and Mexico [177]

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