PPARs at the crossroads of lipid signaling and inflammation

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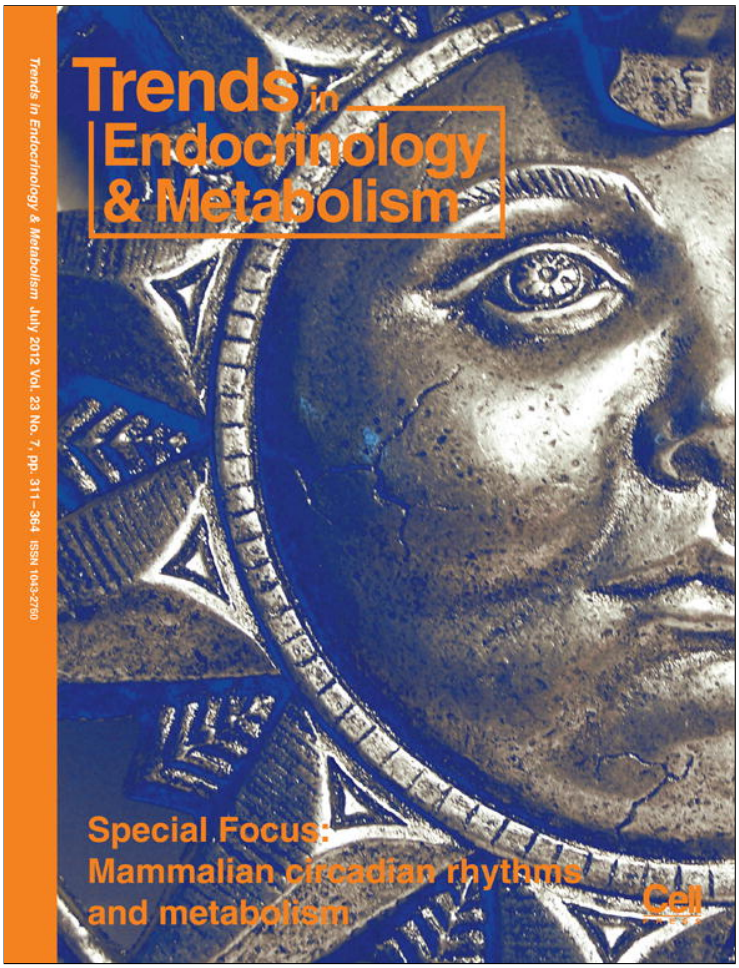

Review

PPARs at the crossroads of lipid signaling and inflammation

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Nuclear receptors (NRs) are ligand-dependent transcription factors whose activation affects genes controlling vital processes. Among them, the peroxisome proliferator-activated receptors (PPARs) have emerged as links between lipids, metabolic diseases, and innate immunity. PPARs are activated by fatty acids and their derivatives, many of which also signal through membrane receptors, thereby creating a lipid signaling network between the cell surface and the nucleus. Tissues that play a role in whole-body metabolic homeostasis, such as adipose tissue, liver, skeletal muscle, intestines, and blood vessel walls, are prone to inflammation when metabolism is disturbed, a complication that promotes type 2 diabetes and cardiovascular disease. This review discusses the protective roles of PPARs in inflammatory conditions and the therapeutic anti-inflammatory potential of PPAR ligands.

Fatty acids and inflammation

There may not be a single biological process in which lipids do not have regulatory roles. This is particularly true for inflammation, which is a normal protective mechanism in infection and injury [1]. Normally, inflammatory responses are controlled to avoid excessive damage to the host, and they resolve in a timely manner. If not properly controlled, inflammatory responses promote acute or chronic diseases characterized by excessive production of arachidonic acid-derived eicosanoids, inflammatory cytokines, and adhesion molecules [1]. There are several conditions where inflammation is a driving force for disease or exacerbation of a pathologic condition, such as obesity, nonalcoholic steatohepatitis (NASH), atherosclerosis, acute cardiovascular events, and Crohn's disease.

Polyunsaturated fatty acids and their derivatives

Eicosanoids are signaling molecules that control inflammatory and immune processes and might have effects on cardiovascular disease, blood pressure, and arthritis. They are produced from the oxidation of either omega-6 (n-6) or omega-3 (n-3) 20-carbon essential fatty acids (FAs), FAs that humans cannot synthesize and must obtain from the diet. Eicosanoids belong to the families of prostaglandins, prostacyclins, thromboxanes or leukotrienes [2–4]. The n-6 arachidonic acid-derived eicosanoids and cytochrome P450 products are generally proinflammatory (Figure 1a),

Glossary

5-aminosalicylic acid (5-ASA): anti-inflammatory drug used to treat ulcerative colitis and Crohn's disease.
13(S)-hydroxyoctadecadienoic acid (13-S-HODE): a fatty acid resulting from the non-enzymatic oxidation of linoleic acid.
13(S)-hydroperoxide of linoleic acid (13-S-HPODE): a lipoxygenase-activating hydroperoxide non-enzymatically derived from linoleic acid through the action of reactive oxygen species.
13-oxooctadecadienoic acid (13-Oxo-ODE): a product of 13-HODE by a NAD^+-dependent dehydrogenase.
15(S)-hydroxyeicosatetraenoic acid (15-S-HETE): a major arachidonic acid metabolite from the 15-lipoxygenase pathway.
15(S)-hydroperoxyeicosatetraenoic acid (15-S-HPETE): a polyunsaturated FA, produced by the action of 15-lipoxygenase on arachidonic acid, that mediates the activation of activator protein-1.
Arachidonic acid (AA): an n-6 polyunsaturated fatty acid present in membrane phospholipids that is involved in cellular signaling as a lipid second messenger.
Activated adipose-tissue macrophages (AAMs): also known as M2, AAMs promote angiogenesis and tissue repair, and produce anti-inflammatory cytokines.
Activator protein-1 (AP-1): a heterodimeric transcription factor that controls differentiation, proliferation, and apoptosis.
Classically activated macrophages (CAMs): also known as M1, CAMs are immune effector cells that are proinflammatory and fight infection.
Fatty acid transport protein (FATP): Also termed cluster of differentiation 36 (CD36), is a long-chain fatty acid transporter that is present at the plasma membrane.
T helper 2 cells (Th2): these cells assist other white blood cells in immunologic processes, including B-cell maturation and the activation of cytotoxic T cells and macrophages.
Hepatic stellate cells (HSCs): cells found in the perisinusoidal space and involved in liver fibrosis.
Inflammatory bowel disease (IBD): a group of inflammatory conditions of the colon and small intestine, including ulcerative colitis and Crohn's disease.
Intestinal epithelial cells (IECs): IECs cover the small and large intestines. They take part in digestion and immunity, and function as a barrier and a first-line pathogen recognition system.
Inhibitor of nuclear factor of κ light polypeptide gene enhancer in B cells (IκB): an inhibitor of NF-κB.
Interleukins (ILs): cytokines involved in inflammation and the regulation of the immune system.
Linoleic acid (LA): a polyunsaturated n-6 fatty acid used in the biosynthesis of AA and some prostaglandins.
Lipopolysaccharides (LPS): immune response-triggering molecules found in the outer membrane of Gram-negative bacteria.
Omega-3 polyunsaturated fatty acids (n-3 PUFAs): anti-inflammatory FAs containing a double bond at the third carbon atom from the end of the carbon chain.
Oxidative phosphorylation: a mitochondrial process that produces ATP through the oxidation of nutrients
Regulatory T cells (Tregs): also known as suppressor T cells, Tregs are specialized to actively suppress immune activation.
Resolvins: compounds synthesized by the human body from EPA and DHA, via the COX-2 pathway. Resolvins are nonclassic eicosanoids that reduce cellular inflammation by inhibiting the production and transportation of inflammatory cells and chemicals to the sites of inflammation.
Sumoylation: a post-translational modification that modulates functions such as nuclear–cytosolic transport, transcriptional regulation, and protein stability.

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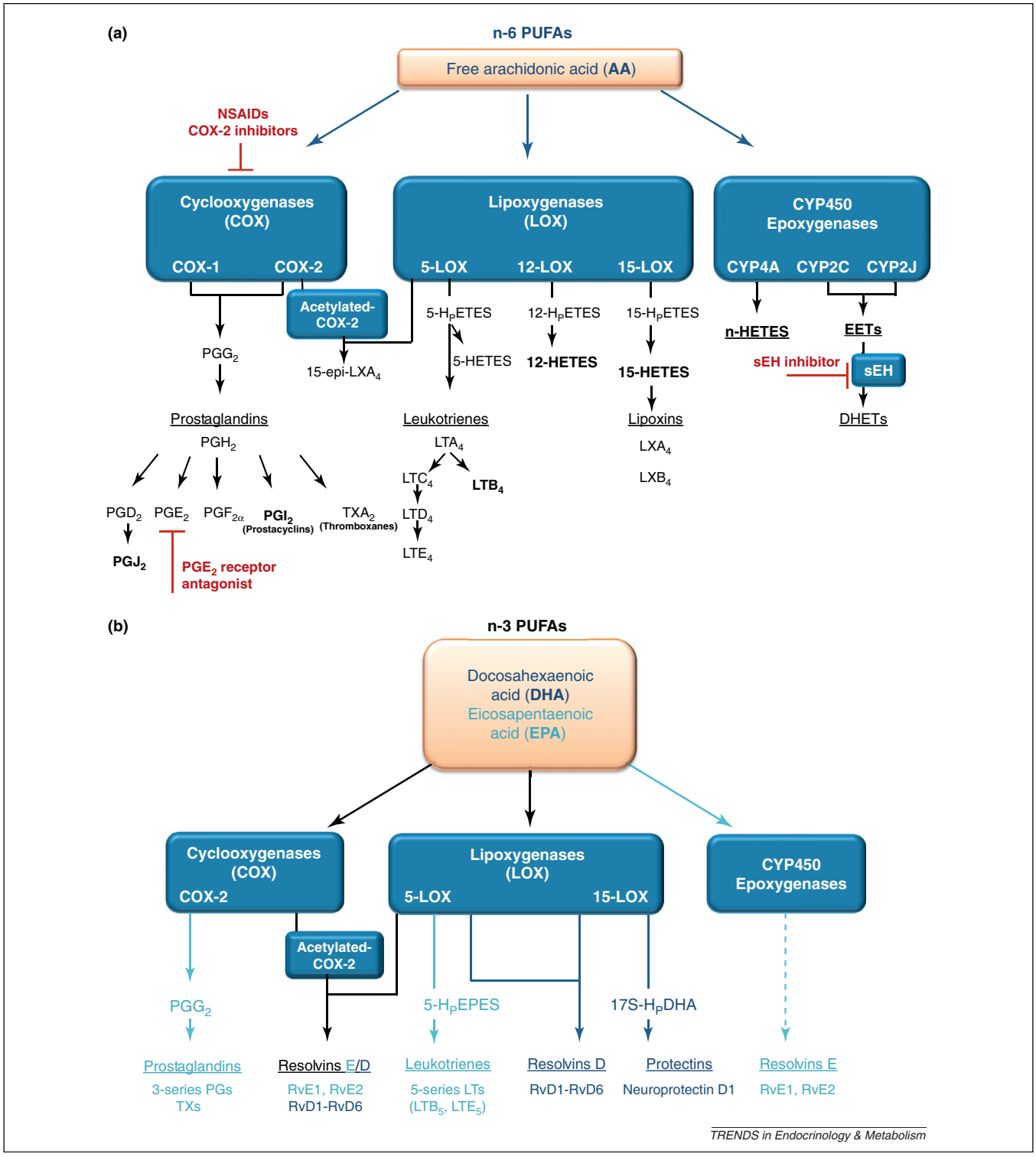

Figure 1. n-6 Arachidonic acid, n-3 docosahexaenoic acid (DHA)-derived and eicosapentaenoic acid (EPA)-derived compounds. **(a)** Cyclooxygenase, lipoxygenase, and epoxygenase products derived from arachidonic acid are shown. They are generally proinflammatory, with the exception of prostaglandin E2 and the inflammation-resolving lipoxins. Inhibitors are indicated in red. **(b)** Cyclooxygenase, lipoxygenase, and epoxygenase products derived from DHA (dark blue) and EPA (light blue) are shown; they are generally anti-inflammatory. Peroxisome proliferator-activated receptor (PPAR) ligands and classes of molecules containing entities that have been identified as PPAR ligands are indicated in bold. The following molecules are PPAR ligands: unsaturated fatty acids (FAs), saturated FAs, LTB4, 8-HETE, 8,9-EET, 11,12-EET, oleoylethanolamide (OEA), palmitoylethanolamide (PEA), and 16:0/18:1 GPC are PPARα ligands; unsaturated FAs, saturated FAs (weak), carbaprostacyclin, and very low density lipoprotein components are PPARβ/δ ligands; unsaturated FAs, oxidized and nitrated FAs, 15-HETE, 9-HODE, 13-HODE, 13-Oxo-ODE, 15-deoxy-$\Delta^{12,14}$-PGJ_2, phospholipid cyclic phosphatidic acid (CPA), and oxidized low-density lipoprotein components are PPARγ ligands. Abbreviations: NSAIDs, nonsteroidal anti-inflammatory drugs; RvD, resolvin D.

Table 1. Selected examples of interplay between cell-surface GPCRs and nuclear PPARs

Ligand and mode of interaction	Receptor/s	Model	Mechanism studied and outcome	Ref.
LTB_4 Binding to BLT2 and PPARα	PPARα	Cell lines, human peripheral blood PMNs, mice	LTB4 is a physiologically relevant PPARα agonist. PPARα decreases secretion of LTB4 and stimulates its breakdown. LTB4 plays a central role in the regulation of inflammation through its ability to exert both proinflammatory effects via BLT2 and anti-inflammatory effects via PPARα.	[23]
	BLT2 and PPARα	Primary pleural mesothelial cells recovered from transudative pleural effusions	Pleural mesothelial cells express both BLT2 and PPARα and mount an integrated response to LTB4 with a prevalence of BLT2 activities in the presence of an inflammatory milieu within the pleura.	[85]
LTE_4 Binding to a MK571-sensitive GPCR; no binding to PPARγ	MK571-sensitive GPCR ($CysLT_3R$?) PPARγ (indirect activation)	LAD2 cell line isolated from the bone marrow of a patient with MC leukemia. Primary human mast cells (hMCs)	LTE_4 activates hMCs by a pathway involving cooperation between an MK571-sensitive GPCR thereby linking extracellular LTE_4, PPARγ-dependent ERK activation, inducible expression of COX-2, and generation of PGD2. The indirect mechanism of PPARγ activation remains to be elucidated. This LTE_4-selective receptor-mediated pathway may explain the unique physiologic responses of human airways to LTE_4.	[86]
GW1929 (synthetic) Binding to PPARγ; no binding to GPCR	PPARγ	Spontaneously hypertensive rats (SHR rats)	PPARγ modulates the expression/ activity of GPCRs. PPARγ-mediated improvement in hypertension may involve transcriptional regulation of GPCR kinase-2 (GRK-2) activity.	[87]
Acetate and propionate Binding to GPCR43; no binding to PPARγ	GPCR43 and PPARγ	C57BL/6J mice Primary adipocytes and stromal-vascular cells from adipose tissues 3T3-L1 cells and differentiation into adipocytes	Acetate and propionate stimulate the expression of GPCR43 and PPARγ in differentiated adipocytes, with stimulation of fat accumulation. GPCR43 and its ligands function as regulators of adipogenesis in adipocyte development and differentiation.	[88]
Lysophosphatidic acid (LPA) Binds to and activates PPARγ and LPAx receptors	PPARγand LPA GPCRs	RAW264.7 monocytic cells	LPA activates PPARγ and LPA_x receptors. LPA can couple activated tumor cells or platelets to PPARγ stimulation and gene regulation in neighboring as well as distal target cells.	[89]
Farnesyl phosphate and diphosphate Binding to both LPA GPCRs and PPARγ	PPARs and LPA GPCRs	RH7777 cells stably transfected with either LPA1, LPA2, or LPA3 and PC3 prostate cancer cells endogenously expressing all three EDG-family LPA GPCRs	Farnesyl phosphates are endogenous ligands of lysophosphatidic acid receptors (LPA_x) and PPARs. Inhibition of LPA GPCRs and activation of PPARs. Both farnesyl phosphate and farnesyl diphosphate potently and specifically antagonize LPA-elicited intracellular Ca^{2+}-mobilization mediated through the LPA3 receptor and activate PPARγ-mediated gene transcription.	[90]

although prostaglandin E2 (PGE2) inhibits the generation of inflammatory leukotrienes and promotes the production of the anti-inflammatory nonclassic eicosanoid lipoxin A4 [1]. Diets rich in fish-derived n-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increase the incorporation of these FAs into the phospholipids of immune cell membranes, and give rise to eicosanoids such as prostaglandin E3 and the anti-inflammatory derivatives of DHA and EPA, the resolvins (Figure 1b). Thus, FA type determines the pro- or anti-inflammatory properties of the lipid-derived mediators produced, impacts upon receptor signaling, and influences cytokine production [1].

Signaling through cell-surface receptors

Lipid mediators such as those described above are ligands for the PPARs, as well as for cell-surface G protein-coupled receptors (GPCRs) and Toll-like receptors (TLRs) [2,5,6] whose dysfunctions can cause inflammatory and immune disorders [5,7] (Glossary).

GPCRs respond to extracellular stimuli and initiate intracellular signaling in many physiological and pathophysiological processes. FAs can bind to and activate GPCRs: long-chain FAs activate GPR119, medium- and long-chain FAs activate GPR43, GPR120, and GPR40, and short-chain FAs activate GPR41. The inflammatory mediator leukotriene B_4 (LTB_4) acts via the leukotriene B_4 receptor (BLT2), another member of this seven transmembrane domain receptor superfamily. The interplay between GPCRs and PPARs presents various facets, such as activation by the same ligand, which can result in antagonistic effects, activation by different ligands to mount an integrated response, and modulation of each others expression (Table 1).

TLRs recognize structurally conserved molecules derived from pathogens, trigger innate immune responses, and prime antigen-specific adaptive immunity. They are also associated with inflammatory and autoimmune diseases. In macrophages, saturated FAs trigger inflammatory responses via TLR2/TLR4. By contrast, long-chain n-3 FAs inhibit TLR2/TLR4 expression, activity, and downstream signaling, thereby contributing to the anti-inflammatory response [8] by at least two mechanisms: inhibition of TLR2/TLR4 expression, and PPAR activation. The connection between TLR signaling and PPAR expression and activation is documented especially for PPARγ (Table 2). The stimulation of TLR signaling by bacterial molecules, for instance the binding of lipopolysaccharides (LPS) to TLR4, promotes inflammation by triggering ubiquitination and then degradation of corepressor complexes bound at the promoter of inflammatory genes, and by concomitant activation of the NF-κB p65–p50 heterodimer, which enters the cell nucleus and binds to the NF-κB response element in the promoter of the inflammatory genes. If the cells are exposed simultaneously to a PPARγ ligand, a fraction of the receptor molecules are conjugated with SUMO1 in the ligand-binding domain. The SUMOlated and ligand bound PPARγ binds to NCoR-containing repressor complexes and inhibits their degradation by the 19 S proteasome, thus maintaining active gene repression [9].

The examples given in Tables 1 and 2 show that some lipid mediators bind either to cell-surface (GPCR, TLR) or nuclear receptors (PPARs), whereas others concomitantly activate both types of receptors, resulting in highly diverse connections that serve to fine-tune many homeostatic pathways, but are also associated with metabolic disturbances and inflammation [10].

Signaling through nuclear receptors: PPARs as lipid sensors and master regulators

PPARs and their expression patterns

PPARs compose a three-member subfamily including PPARα, PPARβ/δ, and PPARγ. Transcriptional activity is mediated by PPAR:retinoid X receptor (RXR) heterodimers that bind to specific DNA sequence elements termed PPREs in the regulatory region of their target genes. PPARs control expression of genes that function in lipid and carbohydrate metabolism, vascular biology, tissue repair, cell proliferation and differentiation, and sexual dimorphism [11].

PPARα, PPARβ/δ, and PPARγ exhibit isotype-specific but partially overlapping expression patterns. Tissues that perform significant catabolism of FAs, such as brown adipose tissue, liver, heart, kidney, and intestine, express high levels of PPARα [11]. PPARβ/δ has important functions in the skin, gut, placenta, skeletal and heart muscles, adipose tissue, and brain [11]. PPARγ is found in two isoforms, γ1 and γ2, that differ at their N termini. The shorter PPARγ1 has a relatively broad expression pattern including the gut, brain, vascular cells, and immune and inflammatory cells, whereas PPARγ2 is found at high levels mainly in adipose tissues [11].

Endogenous ligands of PPARs

A variety of natural compounds, including n-3 and n-6 FAs, eicosanoids, and a few endocannabinoids and phospholipids, have been identified as PPAR ligands (Figure 1). Unsaturated FAs, saturated FAs (weaker), leukotriene B4, 8(S)-hydroxyeicosatetraenoic acids, 8,9-epoxyeicosatrienoic acids, 11,12-epoxyeicosatrienoic acids, oleoylethanolamide (OEA), palmitoylethanolamide (PEA), and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (16:0/18:1GPC) are PPARα ligands. Unsaturated FAs, saturated FAs (much weaker), prostacyclin, 4-hydroxy-2-nonenal (4-HNE), 4-hydroxydodeca-(2E,6Z)-dienal (4-HDDE) and very low-density lipoprotein components are PPARβ/δ ligands. Unsaturated FAs, oxidized and nitrated FAs, 15-HETE, 9/13-HODEs, 13-oxo-ODE, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, phospholipid cyclic phosphatidic acid (CPA), and oxLDL components are PPARγ ligands [9,10,12–14]. In addition to these natural ligands, a wide range of synthetic ligands have been developed, some of which are used to treat dyslipidemia (fibrates) and diabetes (thiazolidine-2,4-diones or TZDs) [10]. A list a synthetic agonists and antagonists of each PPAR isotype can be found in [10]. Ligand-activated PPARs regulate metabolic activities leading to FA catabolism, lipid storage, and/or other effects, such as those affecting inflammation [10]. Activity of these ligands depends on their presence in cells or tissues enriched in PPARs, their binding specificity toward the different PPARs and the availability of coregulators that can act either as coactivators or corepressors of transcription [15,16]. Given the variety and anatomic distribution of endogenous PPAR ligands, and the combinations in which they occur depending on physiological (e.g., abundance and composition of food, physical activity) and pathophysiological conditions (e.g., hyperlipidemia, hypertension, diabetes, chronic inflammation, cancer, and atherosclerosis), it is difficult to evaluate thoroughly the roles of each PPAR ligand in a given cell at a fixed time-point, and this remains a major challenge in the field. It is tempting to speculate that the diversity of PPAR functions has been acquired in association with the rich variety of ligands.

PPAR and inflammation

Metabolic syndrome and inflammatory diseases

PPARs have emerged as targets of drugs used to treat various components of metabolic syndrome, a cluster of

Table 2. Selected examples of interplay between TLRs and PPARγ in different cell types

Cell/tissue	Model	Aim	Results	Refs
Intestinal mucosa	HT-29 human IECs	Evaluate attenuation of inflammation by PPARγ in IECs comparing costimulation with LPS and PPARγ ligand versus stimulation with LPS alone.	PPARγ and TLR4 pathways are antagonistic in their actions on NFκB signaling.	[91]
Intestinal epithelial cells	CMT-93 cells TLR-$2^{-/-}$ mice	Study whether PPARγ is involved in probiotic-mediated effects.	*Lactobacillus crispatus*-derived H_2O_2 acts as a signal-transducing molecule activating PPARγ in IECs. This protective effect is less pronounced in TLR-$2^{-/-}$ mice.	[92]
	Caco-2 cells TLR4-deficient mice Patients with UC	Determine the role of bacteria and their signaling effects on PPARγ regulation during inflammatory bowel disease (IBD).	TLR4 signaling enhances PPARγ expression in an IKK-β-dependent fashion. Murine and human intestinal flora induce PPARγ expression in colonic epithelial cells of control mice. PPAR-γ expression is considerably impaired in patients with UC.	[66]
Nasopharyngeal mucosa Nasopharyngeal epithelial cells	Detroit 562 cells	Evaluate whether the upper respiratory tract commensal *Neisseria lactamica* (*Nlac*) affords anti-inflammatory mucosal protection.	*Nlac* suppresses pathogen-induced inflammation in the nasopharyngeal mucosa, mediated through TLR-1/2 stimulation, by activating PPARγ and inhibiting NF-κB activity.	[93]
Immune cells	Dendritic cells	Evaluate the effects of the PPARγ agonists 15d-PGJ2 and troglitazone on the immunogenicity of human monocyte-derived DCs upon stimulation with TLR ligands.	PPARγ activation inhibits TLR-induced DC maturation via inhibition of the NF-κB and mitogen-activated protein (MAP) kinase pathways.	[94]
	Monocytes *db/db* mice	Examine the anti-inflammatory effects of pioglitazone on TLR2 and TLR4 expression in human monocytes exposed to Pam and LPS.	Pam- and LPS-induced TLR2 and TLR4 expression are inhibited by the PPARγ agonist pioglitazone in human monocytes and db/db mice.	[95]
	Macrophages	Evaluate the effect of PPARγ agonists on IFN-β production in peritoneal primary macrophages in response to LPS and poly(I:C).	PPARγ negatively regulates IFN-β production in TLR3- and 4-stimulated macrophages by preventing IRF3 binding to the IFN-β promoter.	[96]
	Thioglycollate-elicited macrophages	Examine the mechanisms by which different members of the nuclear-receptor superfamily repress proinflammatory programs of gene expression using TLR signaling as a model system.	Combinations of agonists for PPARγ, GR and LXRs result in additive or synergistic inhibition of a subset of TLR4-target genes both in cultured macrophages and *in vivo*. GR represses inflammatory response genes by disrupting p65/interferon regulatory factor (IRF) complexes required for TLR4- or TLR9-dependent transcriptional activation. PPARγ and LXRs repress targets by p65/IRF3-independent mechanisms and cooperate with the GR to synergistically transrepress distinct subsets of TLR-responsive genes.	[97]
	Fetal liver-derived macrophages	Investigate molecular mechanisms underlying the formation of lipid droplets during *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) infection, focusing on the role of PPARγ.	BCG-induced PPARγ expression and lipid body formation are diminished in macrophages from TLR2-deficient mice, suggesting a key role for TLR2. Link between the innate immune receptor TLR2 and PPARγ that coordinates lipid metabolism and inflammation in BCG-infected macrophages.	[98]
	Mouse peritoneal macrophages Human $CD14^+$ monocytes	Evaluate whether ligand-activated NRs repress both basal and pathogen-enhanced HIV-1 replication in macrophages by directly repressing HIV-1 transcription and by improving the local proinflammatory response to pathogens.	PPARγ represses both basal and TLR-activated HIV-1 transcription through transrepression mechanisms preventing the association of NF-κB p65 and AP-1 with the HIV-1 LTR, as well as by preventing the clearance of NCoR from the LTR. Ligand activated PPARγ also potently represses the expression of pro-inflammatory cytokines that have been shown to augment HIV-1 replication.	[99]
Vascular smooth muscle cells (VSMC)	VSMC cultures Experimental model of subarachnoid hemorrhage model in rat (SAH)	Investigate whether oxyhemoglobin (OxyHb) can induce the expression of TLR4 in VSMCs, and evaluate the effect of rosiglitazone on OxyHb-induced inflammation in VSMCs. Investigation of the anti-inflammation properties of rosiglitazone in basilar arteries in a rat experimental SAH model.	OxyHb exposure induces TLR4. Rosiglitazone suppresses TLR4 expression and cytokine release via the activation of PPARγ. Rosiglitazone suppressed the SAH-induced inflammatory responses in basilar arteries by inhibiting the TLR4 signaling.	[100,101]

risk factors that includes dyslipidemia, insulin resistance, hypertension, inflammation, and coagulation disorders that promote type 2 diabetes (T2DM) and/or cardiovascular events in affected patients. Metabolic syndrome is often associated with obesity and is characterized by macrophage infiltration and activation in adipose tissue and liver. In fact, inflammation is a major determinant of health complications seen in overweight and obesity, which underscores the link between nutrition, metabolic organs, and the immune system [17–19]. Chronic low-grade inflammation, characterized by increased circulating inflammatory cytokines and acute-phase proteins, reflects a weak activation of the innate immune system, and this affects the metabolic organs, arteries, heart, and brain. All three PPAR isotypes have demonstrated anti-inflammatory effects in these conditions [20,21].

Multiple mechanisms account for the anti-inflammatory effects of PPARs

PPARs affect inflammation through direct and indirect mechanisms. For instance, secreted LTB_4 (Figure 1a) triggers inflammatory responses through cell-surface receptors. LTB_4 is also a physiological ligand of PPARα, whose activation stimulates the expression of genes encoding cytochrome P450 and β-oxidation enzymes responsible for the neutralization and breakdown of LTB_4 [22,23]. Through this mechanism, LTB_4 limits its own activity via PPARα, and this eventually contributes to the resolution of inflammation. In addition, PPARα upregulates the expression of IκB, which blocks the nuclear translocation and activation of the proinflammatory transcription factor NF-κB [24]. Upregulation of soluble interleukin-1 receptor antagonist (IL-1ra), which is stimulated during hepatic inflammation and promotes anti-inflammatory effects, is also controlled by PPARα [25]. In the female liver, sex-specific sumoylation of PPARα results in the repression of the cytochrome P450 gene *Cyp7b1*, which may in turn result in a protective effect of PPARα in estrogen- and inflammation-induced cholestasis and toxicity [26]. The best-known mechanism by which PPARα (as well as the other isotypes) inhibits many inflammatory genes is transrepression. Transrepression is based on the tethering of the PPARs to master regulators of inflammation such as NF-κB, activator protein 1 (AP-1), nuclear factor of activated T cells (NFAT), and signal transducers and activators of transcription [27]. A mechanism of transrepression is stabilization by ligand-activated PPARs of corepressor complexes at the promoter of inflammatory genes, which represses their transcription.

Unexpectedly, activated PPARα inhibits the expression of glucocorticoid response element (GRE)-driven genes by interfering with the recruitment of glucocorticoid receptor alpha (GRα) to GREs [28,29]. For instance, WY-14643 activated PPARα prevents glucocorticoid-induced hyperinsulinemia in mice fed a high-fat diet. Interestingly, this interference potentiates anti-inflammatory effects, offering a rationale for the control of inflammation by GRα and PPARα by inflammatory gene repression (via the additive effect of PPARα and GRα) [28,29]. Similarly, the role of PPARα in estrogen-mediated anti-inflammatory effects in the lungs was revealed by a weakened response to estrogen in PPARα null mice. Although the molecular mechanism remains to be clarified, the possible relevance of the cooperation between PPARα and the estrogen receptor in other models of human inflammatory disease warrants in-depth analysis [28,29].

Different mechanisms mediating the anti-inflammatory actions of PPARβ/δ have been proposed: inhibition of NF-κB activation, induction of anti-inflammatory mediators such as TGF-β, and the release from PPARβ/δ of the anti-inflammatory corepressor B-cell lymphoma 6 (BCL-6) protein, rendering this cofactor available for gene repression [21]. PPARβ/δ is the most likely candidate to mediate the effect of chylomicron-derived FAs on the expression of angiopoietin-related protein 4 (Angptl4), a protein that is upregulated in macrophages by chylomicron-derived FAs and that protects against the severe proinflammatory effects of saturated fat by inhibiting FA uptake into mesenteric lymph node macrophages [30].

PPARγ has several inhibitory effects on inflammation, including reduction of NF-κB transcriptional activities, reduction in the production of proinflammatory molecules in T lymphocytes, promotion of the expression of anti-inflammatory mediators in the innate immune system, and inhibition of genes encoding proinflammatory molecules in macrophages [31]. Of the three PPARs, PPARγ was the first reported to undergo agonist-dependent sumoylation, which promotes binding to nuclear receptor corepressor 1 protein (NCoR) and stabilizes association with promoter-bound NF-κB, thereby leading to the transrepression of inflammatory genes [32]. PPARγ targeted disruption in myeloid cells worsens metabolic disease [17–19]. Its deletion in hematopoietic and endothelial cells (ECs) in female mice causes them to produce milk containing inflammatory lipids (most probably generated by the increased expression of 12-lipoxygenase and epoxide hydrolase), and these cause inflammation, alopecia, and growth retardation in the nursing pups. Therefore, maternal PPARγ protects the pups by inhibiting the production of inflammatory lipids in the milk [33].

Below, we review recent findings regarding the roles of PPARs in major organs, such as the adipose tissue, liver, skeletal and heart muscles, vessel wall, and intestines.

Tissue functions of PPARs and inflammatory diseases

Adipose tissue

Obesity is a major health problem characterized by low-grade inflammation. Adipocytes and macrophages both produce inflammatory cytokines such as TNF-α and IL-6, and express TLRs. Free FA levels are elevated in obesity, and their effects may be mediated by TLRs, which are thought to connect metabolism to innate immunity [34]. Crosstalk between TLRs and PPARγ is documented by several observations (Table 2) [35], and TLR2 and TLR4 gene expression is upregulated in the adipose tissue of PPARα-null mice [35].

Signaling between macrophages and adipocytes is well documented. In obese animals, macrophages shift to the classically activated macrophages (CAMs), also termed M1 macrophages, produce proinflammatory cytokines and act as effectors of cell killing as adiposity increases. These macrophages produce TNF-α, IL-6, and

the inducible isoform of nitric oxide synthase [18,36,37] (Figure 2). By contrast, adipose tissue from lean animals contains M2 macrophages, also termed alternatively activated adipose tissue macrophages (AAMs), which dampen inflammation and influence insulin-sensitive adipocytes to secrete insulin-sensitizing adipokines such as adiponectin (Figure 2). Eosinophil-derived IL-4 and IL-13 are required to sustain adipose AAMS [38], and maintenance of the lean phenotype promotes oxidative metabolism, which is favored by PPARγ and PPARβ/δ. In addition, tissue maintenance activities are enhanced because M2 macrophages scavenge cell debris, promote angiogenesis and tissue remodeling and repair (Figure 2). In adipocytes, PPARβ/δ inhibits NF-κB activity and reduces the production of the proinflammatory cytokines involved in insulin resistance [39] (Figure 2).

Liver

Chronic overfeeding often results in hepatic lipid accumulation. Liver injury can occur when the triglyceride storage capacity of hepatocytes is overloaded, resulting in the activation of apoptotic and inflammatory pathways, and development of insulin resistance. Kupffer cells (liver macrophages) and hepatic stellate cells accumulate at the site of injury, and tissue repair and fibrosis is initiated. In some cases nonalcoholic fatty liver disease (NAFLD) and its extreme form, NASH, may develop. In NAFLD, Kupffer cells coexist with and participate in intense crosstalk with the dominant population of hepatocytes to regulate lipid metabolism [19]. Different roles have been attributed to Kupffer cells in promoting NAFLD, including production of IL-1β that results in lipid accumulation through the suppression of PPARα-dependent FA oxidation, increased triglyceride synthesis, or TNF-α-stimulated triglyceride storage which aggravates steatosis [40] (Figure 2). In turn, the inhibition of Kupffer cells prevents diet-induced steatosis and insulin resistance, and exerts anti-obesity effects [41]. Contrasting observations suggest that loss of Kupffer cells in diet-induced obesity is associated with reduced expression of the anti-inflammatory cytokine IL-10, increased steatosis, and decreased insulin signaling [15,16]. Further investigation is required to solve this controversy.

PPARβ/δ has a role in attenuating chemically-induced liver toxicity [42]. Furthermore, as in adipose tissue, IL-4 triggers the anti-inflammatory M2 phenotype in Kupffer cells via the PPARβ/δ-dependent expression of alternative

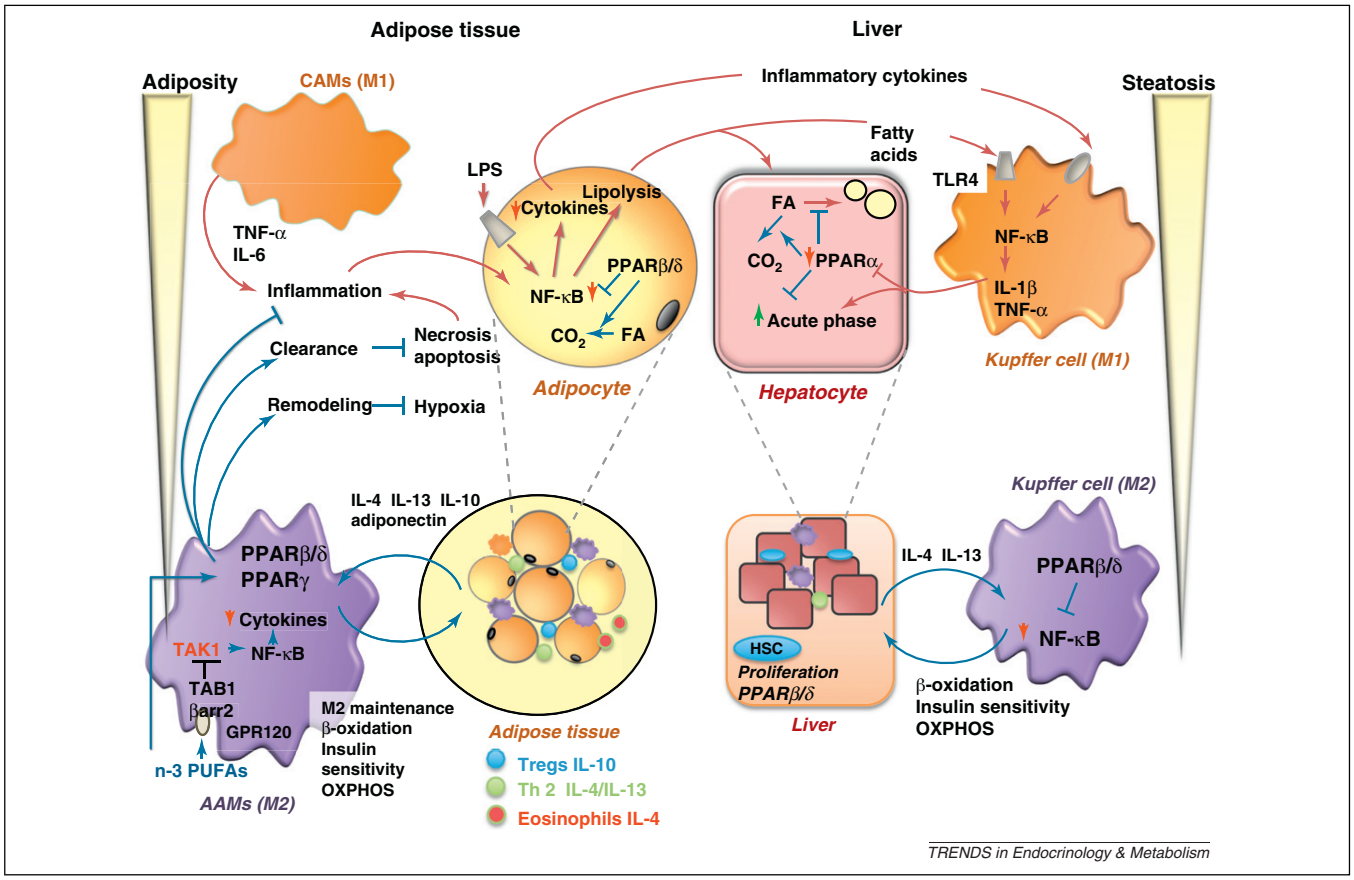

Figure 2. A hypothetical model for the control of inflammatory responses in adipose tissue and liver by alternatively activated macrophages (AAMs). AAMs (M2 macrophages) in adipose tissue from lean individuals influence adipocytes to secrete insulin-sensitizing adiponectin. Maintenance of AAMs depends on PPARβ/δ and PPARγ. The AAM activities protect against insulin resistance, suppress inflammation, promote tissue remodeling to prevent hypoxia, and promote the clearance of apoptotic cells to prevent cellular necrosis. By contrast, classically activated macrophages (CAMs) (M1 macrophages), together with insulin-resistant adipocytes, secrete a variety of inflammatory mediators. PPARβ/δ reduces the inflammatory response by suppressing NF-κB. In Kupffer cells, PPARβ/δ promotes AAM activities which stimulate oxidative phosphorylation, β-oxidation, and insulin sensitivity. However, an inflammatory milieu favors Kupffer cell CAM activities, resulting in the production of inflammatory mediators, which suppress PPARα in hepatocytes. The suppression of PPARα reduces fatty acid oxidation, enhances lipid deposition, and increases acute-phase protein synthesis. Blue lines, anti-inflammatory pathways; red lines, proinflammatory pathways. Abbreviations: FA, fatty acid; Th2, T helper 2 cells; HSC, hepatic stellate cells; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; TLR, Toll-like receptors; Tregs, regulatory T cells; βarr2, β-arrestin 2; NF-κB, nuclear factor κ/light-chain enhancer of activated B cells; TAB1, TAK1 activator binding protein; TAK1, TGF-β activated kinase 1; TNF-α, tumor necrosis factor alpha.

activation signature genes *Arg1, Clec7a, Chi3l3*, and *Tgfb1* (Figure 2) [43]. Consistent with this mechanism, transfer of PPARβ/δ-null bone marrow into wild-type mice reduces the number M2 Kupffer cells and results in hepatic metabolic perturbation and insulin resistance, and treatment of primary hepatocytes with conditioned medium from PPARβ/δ-null macrophages suppresses oxidative metabolism in hepatocytes. Collectively, these data indicate that Kupffer cells are involved in lipid metabolism, and suggest a beneficial role for PPARβ/δ in M2 Kupffer cells, that helps to reduce metabolic syndrome and T2DM [43] (Figure 2). Although PPARβ/δ may have beneficial effects on steatosis and inflammation, its role in fibrosis remains to be clarified. It has been suggested that PPARβ/δ stimulates hepatic stellate cell proliferation during acute and chronic liver inflammation [44].

In preclinical models, PPARγ promotes hepatic steatosis, and loss of PPARγ function in hepatocytes (and to a lesser extend in macrophages) protects from diet-induced fat accumulation in the liver. No beneficial effect on hepatic inflammation was observed after PPARγ deletion in both hepatocytes and macrophages, but there were possible confounding factors in this study related to the high-fat diet [45].

Vascular walls

PPARs exert local and distal anti-inflammatory effects in the arterial cells of the vascular wall and the liver, respectively, preventing atherogenesis. PPARα expression in macrophages modulates local inflammation and cellular cholesterol trafficking [46]; hepatic PPARα represses the production of secreted inflammatory proteins, which modulate systemic inflammation and its associated vascular response [47]. Very interestingly, oscillatory shear stress stimulates the expression of microRNA-21, which inhibits the vascular anti-inflammatory action of PPARα by directly reducing PPARα expression in ECs, thus contributing to the proinflammatory response [48].

PPARβ/δ also attenuates atherogenic inflammation. Its synthetic ligands GW0742 and GW501516 reduce atherosclerosis in low-density lipoprotein receptor (LDLR) null mice, possibly by decreasing monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecule-1, and TNF-α expression [49,50]. Deletion of PPARβ/δ from foam cells increases the availability of Bcl-6, which in turn reduces the atherosclerotic lesion area [49,50]. Similarly, in a proinflammatory model of accelerated atherosclerosis, GW0742 attenuates the process by reducing vascular inflammation via increased Bcl-6 levels and inhibition of proatherogenic and proinflammatory pathways by two key regulators of GPCR signaling, RGS4 and RGS5 [51].

PPARγ is expressed in ECs, macrophages, and smooth muscle cells, all of which are players in atherosclerosis development. In mouse models, the disruption of PPARγ in smooth muscle cells and macrophages increases atherosclerosis [52] and hypercholesterolemia [53], respectively. Inactivation of PPARγ in the ECs causes endothelial dysfunctions under high-cholesterol diet, and this demonstrates that endothelial PPARγ prevents the initiation of atherosclerosis [54]. Together, these findings highlight the anti-inflammatory role of PPARγ in cell types that are crucial to the progression of cardiovascular diseases.

Skeletal muscle and heart

Skeletal muscle lipotoxicity is characterized by inflammation, insulin resistance, and cell death caused by FA overload, especially by saturated FAs such as palmitate [55]. Although the oxidative capacity of skeletal muscle is exceeded when serum FA levels are too high, stimulation of mitochondrial FA oxidation is sufficient to protect from or reverse lipotoxicity [55]. When activated by the monounsaturated FA oleate, PPARα and PPARβ/δ channel palmitate toward triglyceride accumulation and mitochondrial β-oxidation rather than toward the production of deleterious diacylglycerols and ceramides [55,56]. Similarly, treatment of human skeletal muscle cells with the synthetic PPARβ/δ agonist GW501516 increases FA oxidation via PPARβ/δ and AMP kinase [57], and activation of PPARβ/δ by GW501516 prevents palmitate-induced NF-κB activity and insulin resistance in mouse skeletal muscle cells [58]. If palmitate is converted to diacylglycerols, the serine kinase protein kinase C θ (PKCθ), which is abundant in skeletal muscle, is activated leading to impairment of insulin sensitivity and signaling. PKCθ activates IKKβ, which in turn phosphorylates IκB, thereby promoting NF-κB inflammatory pathways (e.g., TLR-2 and COX-2) and various proinflammatory mediators (e.g., IL-6 and TNF-α) [59]. Interestingly, COX-2 upregulation also promotes the production of PGE2, which limits the expression of these two proinflammatory cytokines [58]. Together, these observations suggest that PPARβ/δ downregulates inflammation in muscle cells by reducing diacylglycerol accumulation, thereby attenuating saturated FA activation of NF-κB. In addition, PPARβ/δ stimulates muscle glucose utilization in a pattern similar to that triggered by physical exercise [60].

Chronic low-grade inflammation plays a role in cardiac hypertrophy and heart failure [61]. Proinflammatory factors produced under the control of NF-κB, such as TNF-α, MCP-1, and IL-6, which are secreted by cardiac cells under various pathophysiological stimuli, may participate in myocardial inflammation [61]. PPARα, in association with the NAD-dependent deacetylase sirtuin 1 (Sirt1), reduces inflammation and cardiac hypertrophy, and increases FA oxidation [62]. Activated PPARβ/δ dampens LPS-induced TNF-α inflammation signaling in cultured cardiomyocytes, and blocks palmitate-induced inflammatory pathways in mouse heart and human cardiac cells through protein–protein interaction between PPARβ/δ and p65, suggesting inhibition of NF-κB [63,64]. It is tempting to speculate that PPARβ/δ may serve as a therapeutic target to prevent cardiac hypertrophy and heart failure in metabolic disorders [64]. PPARγ attenuates progressive cardiac fibrosis occurring in diabetic cardiomyopathy. In cardiomyocyte- and macrophage-specific PPARγ-null mice infused with angiotensin II to trigger cardiac fibrosis, and then treated with pioglitazone, it is the macrophage and not myocyte PPARγ that attenuates fibrosis [65].

Inflammatory bowel diseases

PPARγ is highly expressed in the colon, where bacterially induced signals increase its expression via TLR4. This

regulation was revealed in mice experimentally colonized with different flora [66]. Interestingly, colonic epithelial cells from ulcerative colitis (UC) patients, but not Crohn's disease (CD) patients express low levels of PPARγ [66]. Both CD and UC depend on interactions between genetic and environmental factors in genetically determined subjects, resulting in an abnormal gut immune response that is directed against luminal constituents. An association between PPARγ polymorphisms and inflammatory bowel disease (IBD) in a Hungarian cohort was reported recently [67], and imbalances between TLRs and PPARγ in response to luminal bacteria may lead to colonic inflammation in some UC patients [68]. Natural and synthetic ligands of PPARγ have provided effective treatment of colitis in experimental models. PPARγ is expressed not only in intestinal epithelial cells (IECs) but also in intestinal macrophages and T cells. Its role in these different cell types has been analyzed by cell-type targeted gene disruption (Table 3). The molecular mechanisms underlying the anti-inflammatory action of PPARγ in IECs are depicted in Figure 3. PPARγ also functions as an antimicrobial factor by maintaining constitutive epithelial expression of a subset of β-defensins, and these have a broad antimicrobial spectrum that includes Gram-positive and Gram-negative bacteria, and fungi [69]. Therefore, maintaining colonic PPARγ activity through therapeutic or nutritional approaches may contribute to the prevention of colonic inflammation by restoring antimicrobial immunity in IBD [69,70] (Figure 3). In fact, activation of PPARγ and PPARβ/δ by conjugated linoleic acid and by dietary punicic acid, both known PPAR ligands, ameliorates intestinal inflammation in mice [69,70].

The role of PPARβ/δ in two mouse models of IBD is controversial and will require additional studies. Deletion of PPARβ/δ exacerbates colitis in one model, although the PPARβ/δ ligand GW0742 had no effect in either wild-type or mutated mice [71,72]. By contrast, treatment with the same PPARβ/δ agonist enhanced colitis in a second model of IL-10-deficient mice [71,72]. Interestingly, PPARβ/δ regulates the differentiation of Paneth cells, one of the principal cell types of the small-intestine epithelium whose dysfunction may predispose to inflammation [73].

Finally, endogenous (suggested by the deletion of PPARα) and exogenous PPARα ligands (Wy-14643) reduce the degree of colitis caused by dinitrobenzene sulfonic acid (DNBS) [74] and dextran sulfate sodium (DSS), probably because of reduced production of the cytokines INFγ, IL-1β, IL-6, and TNF-α [75]. Collectively, these findings suggest that dietary interventions using natural PPAR ligands or novel synthetic molecules should be investigated further in IBD.

Synthetic anti-inflammatory PPAR ligands: therapeutic perspectives

As we have discussed, all three PPAR isotypes are potential targets for drugs to treat chronic inflammatory diseases. Anti-inflammatory effects of synthetic ligands for all three PPARs have been reported and recently reviewed (Table 4) [76]. Fibrates targeting PPARα have been fairly successful at treating dyslipidemia; however, the TZDs that are insulin-sensitizer PPARγ agonists, despite their efficacy, induce deleterious side effects (e.g., peripheral edema, increased risk of congestive heart failure, increased rate of bone fracture, and weight gain), and this has restrained their clinical use and arrested the clinical development of many promising compounds [76].

Today, alternative approaches are being explored, including the development of compounds that simultaneously target more than one receptor type (Table 4). For example, endocannabinoids, which have analgesic and anti-inflammatory effects and bind to cannabinoid receptors, are also natural activators of PPARs [77]. Some have anti-inflammatory effects through PPARα or PPARγ

Table 3. Cell type-specific deletion of PPARγ in colonic epithelial cells and immune cells: effects on dextran sodium sulfate (DSS)-induced colitis

Cell type	Model	Phenotype	Ref
Epithelial cell	Villin Cre$^+$ DSS	• Increased susceptibility to DSS-induced colitis • Colitis more severe in C57BL/6 than in FVB/C57BL/6 genetic background • Suppressed production of IL-10 by CD$^+$ T cells in MLN • Downregulated expression of lysosomal pathway genes (global gene expression analysis) • Reduced expression of ADRP and FABP in absence of induced colitis • Elevated IL-6, IL-1β, and TNF-α levels in colon • Reduced anti-inflammatory effect of dietary punicic acid	[82,102,103]
Macrophage	Lys Cre$^+$ DSS	• Increased susceptibility to colitis (weight loss, diarrhea, rectal bleeding, shortened colon length) • Increased levels of IL-1, IL-22, IL1RL1, CXCL9, CXCL10, CCR1, CCR2 MCP-1, iNOS, SOCS-3, IFN-γ, and MHC class 2 mRNA in IBD colons • Worsened colonic histopathology • Altered the percentages of immune cells in the blood, spleen, MLN, and LP • Increased expression of DC40, Ly6C, and TLR-4 in LP macrophages • Reduced recruitment of macrophages to inflammatory foci in the colon • Abrogated beneficial effects of dietary punicic acid	[103–105]
T cell	CD4 Cre$^+$ DSS	• Acceleration of the onset of disease and body weight loss • Greater epithelial erosion, leukocyte infiltration, and mucosal thickening • Increase in the percentage of CD8$^+$ T cells and reduction of the percentage of CD4$^+$ T cells in blood, spleen, and MLN • Upregulation of leukocyte extravasation marker (adhesion molecules) and inflammatory cytokines in colon (IL-6, IL-1β; SOCS-3) • Downregulation of Krebs cycle and ribosome pathways in colon • Upregulation of apoptosis in colon • Loss of anti-inflammatory efficacy of abscisic acid	[106,107]

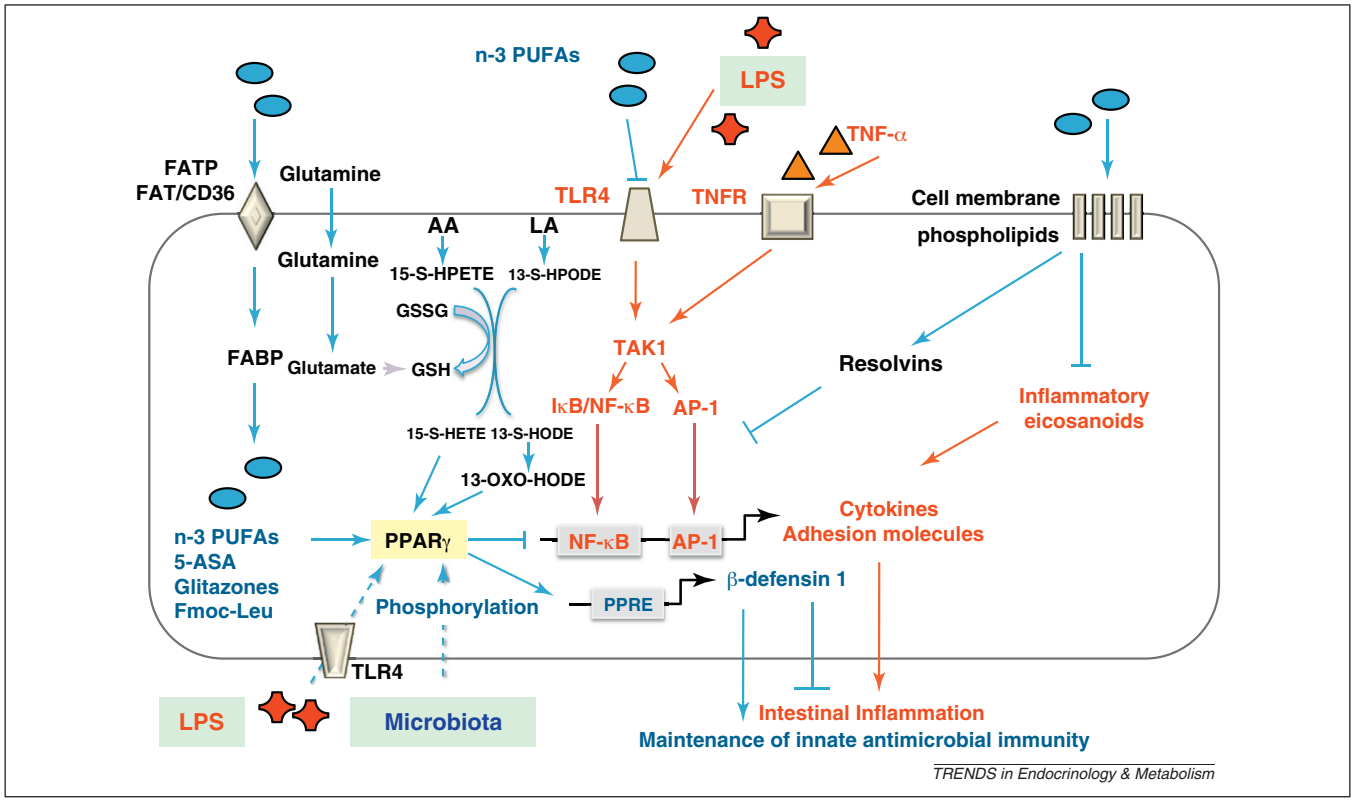

Figure 3. The anti-inflammatory roles of peroxisome proliferator-activated receptor (PPAR)γ in intestinal epithelial cells (IECs). The activities of PPARγ in IECs, macrophages, and T cells help to ameliorate colitis (Table 1). PPARγ in IECs regulates mucosal immune responses and prevents inflammatory bowel disease in experimental animals [82]. In IECs, PPARγ is activated by natural ligands (fatty acids or fatty acid derivatives). Glutamine may contribute to their production [83]. Synthetic ligands (glitazones) can also activate the receptor. PPARγ suppresses the activity of the inflammation-responsive transcription factors NF-κB and AP-1, which are activated by lipopolysaccharides (LPS) and/or TNF-α. The products of their target genes that encode inflammatory cytokines and adhesion molecules promote intestinal inflammation. In conjunction with PPARγ, resolvins also contribute to the anti-inflammatory effects. In colonocytes, PPARγ contributes to β-defensin 1 (DEBF1) expression, and this may contribute to the increased microbial killing by the colonic mucosa [69]. Blue lines, anti-inflammatory pathways; red lines, proinflammatory pathways. Abbreviations: AA, arachidonic acid; FABP, fatty acid binding protein; FATP, fatty acid transport protein; GSH, glutathione; GSSG, glutathione disulphide; LA, linoleic acid; PUFA, polyunsaturated fatty acid; PPRE, peroxisome proliferator response element; TLR4, Toll-like receptor 4.

Table 4. Synthetic anti-inflammatory PPAR ligands

PPAR	Compound	Model	Mechanism studied and outcome	Ref.
PPARα	CP900691	Diabetic cynomolgus monkey	Improvement of plasma lipids, lipoproteins, and glycemic control in diabetic monkeys.	[84]
PPARβ/δ	GW501516	C2C12 and human skeletal muscle cells	Attenuation of fatty acid-induced NF-κB activation and insulin resistance in skeletal muscle cells.	[58]
PPARβ/δ	MBX 8025	Combined dyslipidemic patients	Improvement of multiple metabolic parameters in dyslipidemic overweight patients treated with and without atorvastatin.	[108]
PPARγ	5-ASA	DNBS-induced colitis in mice; organ cultures of human colonic biopsies	Identification of PPARγ as a target of 5-ASA underlying anti-inflammatory effects in the colon.	[78]
PPARγ	GED-0507-34-Levo	DNBS- and DSS-induced colitis in mice	Compound has strong intestinal anti-inflammatory and analgesic properties.	[109]
PPARα/γ	Cevoglitazar	Obese mice; Zucker rats; diabetic cynomolgus monkey	Reduction of food intake and body weight in obese mice and cynomolgus monkeys. Improvement of glycemic and metabolic control. In fatty Zucker rats, cevoglitazar functions through PPARα agonism resulting in increased β-oxidation. In subcutaneous fat, cevoglitazar induces changes similar to those of fenofibrate suggesting export of fatty acids from this depot.	[110] [111]
PPARα/γ	CG301269	*db/db* mice	Improvement of glucose and lipid metabolism without body weight gain by simultaneous activation of both PPARα and PPARγ.	[112]
PPAR γ/cannabinoid receptor	Ajulemic acid (AJA)	Cell cultures; zymosan-A-induced murine peritonitis	Identification of PPARγ as target for AJA, providing a potential mechanism for the anti-inflammatory action of AJA, and possibly other cannabinoids. AJA increases formation of the endogenous proresolving and anti-inflammatory eicosanoid, lipoxin A4.	[113] [114]

(Table 4) [76]. These observations encourage further development of endocannabinoids that are free of psychotropic activity.

5-aminosalicylic acid (5-ASA) is an anti-inflammatory drug used for years in patients with IBD, and its PPARγ-dependent mechanism of action was elucidated recently [78]. An analog of 5-ASA with much higher anti-inflammatory activity, GED-0507-34-Levo, is currently in Phase 2 clinical trials [76].

Antidiabetic PPARγ ligands, such as rosiglitazone, block CDK5-mediated phosphorylation of PPARγ at serine 273, thus increasing the activity of the receptor [79]. Similarly, adipocyte-specific deletion of NCoR reduces the phosphorylation of serine 273 and activates the PPARγ-dependent program in these cells, leading to increased adipogenesis and reduced inflammation [79]. SR1664, a synthetic compound that has a unique mode of binding to PPARγ, presents antidiabetic activity without leading to the serious side effects associated with thiazolidinediones [80]. Future studies will reveal whether a new class of such compounds, which would specifically target Cdk5-mediated phosphorylation of PPARγ, might also promote its anti-inflammatory properties, and if NCoR pharmacologic inhibition can promote insulin sensitization.

Collectively, these studies demonstrate that the development of new classes of PPAR-targeted drugs is feasible and may provide novel therapeutic perspectives for inflammatory diseases.

Concluding remarks

The relatively recent recognition that inflammation is a major contributor to the development of metabolic diseases has prompted many studies into the role of PPARs in immune regulation and the resolution of inflammation. In contrast to 'classic' physiological and metabolic regulatory mechanisms, which largely depend on direct binding of PPARs to the regulatory regions of target genes, the anti-inflammatory effects of PPARs rely primarily on transrepression mechanisms in which protein–protein interactions are crucial and often include B cell, T cell, macrophage and dendritic cell functions [81]. In fact, the transrepression of NF-κB by PPARs, which counteracts the transcriptional stimulation of inflammatory genes, is the most frequent mechanism.

PPARs have emerged as crucial moderators of systemic and cellular metabolic functions in different organs, and now as unavoidable links between lipid signaling and inflammation, and this underscores the necessity of finely tuned crosstalk between the metabolic and innate immune systems. The multifaceted roles of PPARs in these processes rely on the diverse control of gene expression in time and space which also integrates signaling through membrane receptors. Developing molecules that simultaneously target more than one component of the integrated network controlled by PPARs may provide alternative therapeutic perspectives for novel synthetic anti-inflammatory PPAR ligands.

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