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REVIEW

Peroxisome proliferator-activated receptor-γ agonists as potential anti-inflammatory agents in asthma and chronic obstructive pulmonary disease

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Summary

Inhaled corticosteroids are the most effective therapy for chronic persistent asthma and have a role in the treatment of chronic obstructive pulmonary disease (COPD). However, corticosteroids have reduced efficacy in some patients with asthma and fail to halt the progressive deterioration in lung function characteristic of COPD. Additional or alternative drug treatments to corticosteroids are required to improve control of inflammation in patients with therapy resistant airway disease. Peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists have displayed potent anti-inflammatory properties in experimental models of asthma and other airway diseases and as a result have the potential to become an additional treatment for asthma and COPD. We review the evidence from these experimental models and their applicability to asthma and COPD and the requirements for future clinical and experimental research.

Introduction

Inhaled corticosteroids are the safest and most effective treatment available for asthma [1, 2], but do not produce a beneficial response in all patients with mild, moderate or severe disease [3–7]. Active cigarette smoking is an important cause of corticosteroid insensitivity in asthma [8–10]. In chronic obstructive pulmonary disease (COPD), inhaled corticosteroids reduce the frequency and length of exacerbations and may also reduce mortality in severe disease [11]. However COPD can be described as corticosteroid resistant [12] because treatment with corticosteroids cannot prevent disease progression, i.e. decline in lung function or reverse the underlying, predominately neutrophilic pulmonary inflammation [12, 13]. There is therefore a need for additional and/or alternative anti-inflammatory therapies for patients with asthma or COPD that fail to gain the expected benefits from treatment with inhaled corticosteroids.

Thiazolidinediones are a class of drugs currently licensed for the treatment of non-insulin dependent diabetes mellitus that act via the nuclear transcription factor, peroxisome proliferator-activated receptor-γ (PPAR-γ). Thiazolidinediones have come to the fore as a potential new anti-inflammatory treatment on the basis of research carried out in experimental models of inflammatory diseases. This review will concentrate on the research carried out using PPAR-γ agonists in experimental models relevant to the treatment of asthma and other inflammatory airway diseases.

Peroxisome proliferator-activated receptors

Peroxisomes are intracellular organelles, which perform diverse metabolic functions including β-oxidation of fatty acids and have a role in cholesterol metabolism. PPARs were first described following the observation that certain compounds (e.g. fibrates, phthalate esters, non-steroidal anti-inflammatory drugs) could increase the number and activity of liver peroxisomes after chronic high dose administration to rodents [14, 15]. PPARs have since been classified as members of the nuclear receptor family of transcription factors, which also contains vitamin D, corticosteroid, thyroid and retinoic acid receptors.

Peroxisome proliferator-activated receptor structure

PPARs possess four structural domains (Fig. 1). The A/B region is a ligand-independent transcriptional activation domain (also known as activation-function 1/AF-1). The C domain encodes the DNA binding domain that contains

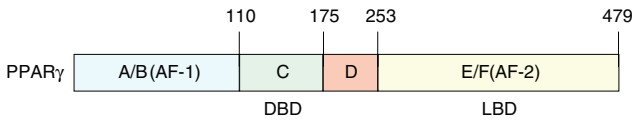

Fig. 1. Peroxisome proliferator-activated receptor (PPAR). AB, activation domain; AF1, activation function 1; C, DNA binding domain; D, hinge region; DBD, ligand binding domain.

two zinc finger motifs. The E domain is responsible for ligand binding, dimerization, nuclear translocation and association with activators and repressors of transcription through its transactivation domain (activation function 2/AF-2). The D domain codes for a hinge which is thought to allow movement of the ligand-binding domain relative to the DNA binding domain.

Peroxisome proliferator-activated receptor family

The PPARs differ in gene and chromosome origin, display varied effects and have different tissue distributions, however the three known isoforms (PPAR-α, PPAR-δ, PPAR-γ) display strong structural and sequence homology. PPAR-α is expressed in heart, liver, kidney, adipose tissue and skeletal muscle, PPAR-δ is widely expressed in tissues such as spleen, bowel, heart, brain, muscle, lung, adrenal glands and adipose tissue [14–16] and PPAR-γ is found at highest concentrations in adipose tissue. PPAR-γ is also expressed in the lung epithelium, submucosa and airway smooth muscle and expression appears to be up-regulated in response to inflammation [17].

PPAR-γ has three isoforms; PPAR-γ_1, PPAR-γ_2 and PPAR-γ_3 due to differing transcription start sites and variable splicing [18, 19]. Study of PPAR-γ's function was initially hindered by the lack of PPAR-γ gene deleted (PPAR-$\gamma^{-}/^{-}$) animals as the PPAR-$\gamma^{-}/^{-}$ genotype results in death *in utero*. However work carried out using PPAR-γ heterozygous (PPAR-$\gamma^{-}/^{+}$) animals and PPAR-$\gamma^{-}/^{-}$ cell lines has facilitated understanding of its role in control of adipogenesis, glucose metabolism and inflammation.

Peroxisome proliferator-activated receptor-γ agonists

The metabolic products of polyunsaturated fatty acids such as α-linoleic, γ-linolenic, arachidonic and eicosapentaenoic acids and eicosanoids such as 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ_2), 9-hydroxyoctadecanoic acid (9-HODE), and 13-hydroxyoctadecanoic acid (13-HODE) have been proposed as the endogenous ligands for the PPAR-γ receptor. Commonly prescribed drugs that can stimulate PPAR-γ include fibrates [16], retinoids [20], thiazolidinediones and non-steroidal anti-inflammatory drugs [15] ((NSAIDs) although only at supratherapeutic doses). The ligand-binding pocket of PPAR-γ is quite large (>1300 Å) and is accommodating to a large number of unrelated molecules, which goes some way to explaining how PPAR-γ can be activated by a variety of both endogenous and synthetic ligands.

Thiazolidinediones are potent PPAR-γ agonists and were designed to exploit the beneficial effect of PPAR-γ stimulation in the treatment of diabetes mellitus. However concerns have also been expressed over the PPAR-γ specificity of some experiments utilizing thiazolidinediones. Thiazolidinediones have been used in concentrations greater than that required for maximal stimulation of PPAR-γ [21]. The K_D for the thiazolidinediones is in the nanomolar range, however many studies demonstrate immunological effects only at doses of 10–20 μM or above [21, 22]. The ligand binding affinities of the thiazolidinediones for PPAR-γ also appear inversely related to their anti-inflammatory efficacy [23]. Synthetic agonists also differ in their ability to activate intracellular processes [24] and different concentrations of the same drug can produce different effects in the same model [25]. Work is currently being carried out using partial agonists of PPAR-γ with the hope of gaining more understanding of the effects of PPAR-γ on inflammation. We would suggest that experiments utilizing thiazolidinediones and claiming to demonstrate PPAR-γ mediated effects without additional evidence to show that PPAR-γ is necessary for the demonstrated action should be interpreted with caution.

Mode of action of peroxisome proliferator-activated receptor-γ agonists

PPAR-γ agonists are utilized in experiments to investigate the effect of PPAR-γ stimulation. However they appear to act via more than just PPAR-γ stimulation (Figs 2a–g). How then do PPAR-γ agonists produce their effects?

Transactivation (Fig. 2a)

All the members of the nuclear receptor family form dimers with another nuclear receptor to interact with DNA. PPAR-γ commonly forms heterodimers with retinoic X receptor (RXR). PPARs bind to portions of DNA known as peroxisome proliferator response elements (PPREs) [16]. The binding of an agonist to a PPAR induces a conformational change allowing dissociation of co-repressor molecules and association with co-activators. This change facilitates the formation of heterodimers and interaction with PPREs. This process, known as transactivation, could result in reduced inflammation through increased anti-inflammatory gene transcription.

Transrepression (Fig. 2b)

Alternatively PPAR-γ stimulation may produce its effects via inhibition of inflammatory gene transcription (transrepression) and there are a number of ways that this may occur [14, 26, 27].

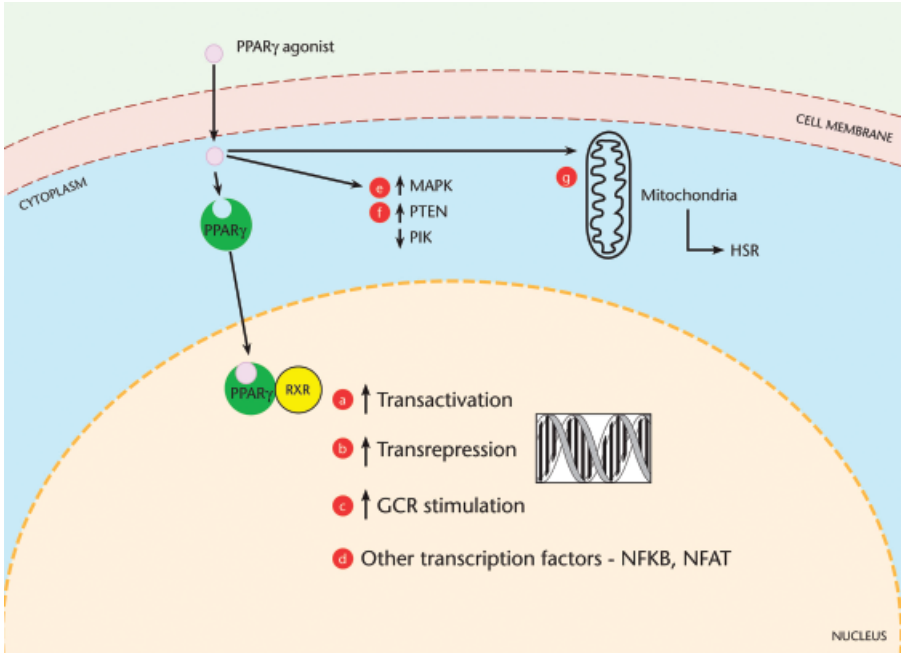

Fig. 2. Potential mode of anti-inflammatory actions of peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists: (a) PPAR-γ agonist binding to the PPAR-γ receptor results in formation of heterodimers with retinoid X receptor (RXR) and interaction with peroxisome proliferator response elements (PPRE's) in DNA leading to anti-inflammatory gene transcription. (b) PPAR-γ receptor can suppress inflammatory gene transcription via sequestration of shared co-activators, binding with inflammatory transcription factors and preventing their association with DNA or preventing their release from inhibitor molecules or following SUMOylation (addition of a *s*mall *u*biquitin related *mo*difier protein) of PPAR-γ and PPAR-γ binding to inhibitory complexes on DNA preventing their removal and inflammatory gene expression. (c) PPAR-γ appears able to exert part of its anti-inflammatory effects via the glucocorticoid receptor. (d) PPAR-γ can bind to other transcription factors to exert some of its observed effects e.g. binding of NF-κB to induce apoptosis. (e) PPAR-γ agonists can act via intracellular kinases. (f) PPAR-γ agonists act via PTEN (phosphate tensin homologue deleted on chromosome 10) and its associated pathway. (g) PPAR-γ agonists stimulate the 'heat shock reaction' via mitochondria resulting in suppression of inflammatory responses.

I. Sequestration of shared co-activators. Competition for co-activators would reduce the ability of inflammatory transcription factors to access their target areas of DNA.
II. PPAR-γ could physically interact with other transcription factors. There is evidence for PPAR-γ binding other transcription factors such as nuclear factor-κB (NF-κB), signal transducer and activator of transcription (STAT), nuclear factor of activated T cells (NFAT) or activator protein-1 (AP-1) [16, 26, 28, 29] and preventing their association with DNA sequences.
III. Control of IκB kinase (IKKB kinase). PPAR-γ agonists can stabilize inflammatory transcription factor suppressor molecules via PPAR-γ [30, 31]. For example, inhibition of NF-κB by a PPAR-γ agonist and 15d-PGJ_2 was mediated by (in the case of 15d-PGJ_2) inhibition of IKKB.
IV. Another mechanism by which PPAR-γ mediates transrepression has recently been proposed [27]. SUMOylation of the PPAR-γ ligand-binding domain (SUMOylation = reversible conjugation of a *s*mall *u*biquitin-related *mo*difier protein to another protein, in this case PPAR-γ) was demonstrated to target PPAR-γ to specific complexes on inflammatory gene promoters. These complexes normally serve the function of suppressing inflammatory gene expression until removal following transcription factor binding (such as NF-κB). However SUMOylation of PPAR-γ and subsequent PPAR-γ binding to this inhibitory complex prevented removal of the repressor complexes and hence suppressed inflammatory gene expression.

Glucocorticoid receptor interaction (Fig. 2c)

There may also be interactions between PPAR-γ and other nuclear receptors such as the glucocorticoid receptor (GCR) [32, 33]. Nie et al. [32] demonstrated direct physical interaction between PPAR-γ and the dominant GCR, GCR-α, following the addition of 15d-PGJ_2. The interaction was considered functional because it resulted in the inhibition of eotaxin production by the cells following stimulation *in vitro* with TNF-α. Ialenti et al. [33] also examined PPAR-γ and GCR interaction using a mouse model of footpad inflammation in response to carrageenin injection. GCR blockade with RU486 (a GCR antagonist) removed a substantial portion of the anti-inflammatory effect of the PPAR-γ agonists rosiglitazone and ciglitazone. A PPAR-γ

antagonist used in conjunction with RU486 was necessary to remove all the anti-inflammatory effects. The PPAR-γ agonists rosiglitazone and ciglitazone were also observed to stimulate GCR nuclear translocation in a PPAR-γ deficient cell line leading to the conclusion that PPAR-γ agonists could produce anti-inflammatory effects via GCR activation [33].

Interaction with other transcription factors (Fig. 2d)

There is evidence for PPAR-γ interaction with other transcriptional factors. PPAR-γ has been demonstrated to use nuclear factor-κB (NF-κB) to cause apoptosis in B lymphocytes [34] and PPAR-γ also physically interacts with NFAT preventing its binding to DNA and hence reducing NFAT's transcriptional activity [35].

Intracellular kinases (Fig. 2e)

PPAR-γ agonists exert some of their effects far too rapidly to be working via gene transcription or suppression, and recent evidence has suggested that they can act via mitogen-associated protein kinases (MAPK) [16, 36–40]. MAPK exert control over a large number of intracellular and nuclear processes including histone and regulatory protein phosphorylation and hence gene transcription. PPAR-γ ligands can activate various MAPK members, for example, PPAR-γ ligands can produce phosphorylation of the epidermal growth factor receptor (EGFR) [41]. Some synthetic PPAR-γ agonists (ciglitazone, troglitazone) are more effective MAPK stimulators than others (rosiglitazone, pioglitazone) [36].

Phosphate tensin up-regulation (Fig. 2f)

The tumour suppressor molecule PTEN (phosphate tensin homologue deleted on chromosome ten) is down-regulated in asthma and PPAR-γ stimulation increases its concentration. The administration of PPAR-γ agonists and increased expression of PPAR-γ using adenovirus vectors in an asthma mouse model reduced bronchial inflammation, NF-κB levels and airway hyper-responsiveness as well as increasing PTEN expression [42].

Intracellular organelles (Fig. 2g)

PPAR-γ agonists have been demonstrated to exert some effects via intracellular organelles independent of PPAR-γ. Ciglitazone and troglitazone increase intracellular calcium by directly stimulating its release from the endoplasmic reticulum [43]. Thiazolidinediones alter mitochondrial respiration but appear to have effects beyond this as illustrated by their ability to induce a heat shock response (HSR) leading to suppression of inflammation [22].

Anti-inflammatory effects of peroxisome proliferator-activated receptor-γ agonists

Following the discovery of PPAR-γ and PPAR-γ's involvement in resolution of inflammation many groups have investigated the relevance of PPAR-γ in specific disease models and patients. As a result PPAR-γ's role in a variety of conditions such as atherosclerosis [44–46], myocarditis [47, 48], inflammatory bowel disease [49, 50], cancer [51–53], dermatology [54], endotoxic shock [55], renal disease [56, 57], acute lung injury [58] and pulmonary fibrosis [59] has come under investigation. Asthma is considered as a prototypic Th2 cytokine-driven disease in which eosinophils play a dominant role. However the contribution of other inflammatory cells to the development and persistence of asthma has gradually been recognized and treatments aimed at many cellular targets and inflammatory pathways appear to be the most effective strategy for producing disease control. We will present the current evidence for the relevance of PPAR-γ agonists as a potential treatment for pulmonary inflammation and their potential role in the treatment of corticosteroid insensitive asthma and COPD.

Effect of peroxisome proliferator-activated receptor-γ agonists on respiratory cell function (Tables 1 and 2)

Eosinophils

PPAR-γ agonists have been used in murine asthma models to investigate their effect on eosinophilic response. Using a mouse model of asthma based on ovalbumin sensitization, treatment with PPAR-γ agonists was found to inhibit the eosinophilic response to allergen challenge [60]. A significant reduction in eosinophilic response was observed in the mice receiving dendritic cells cultured with rosiglitazone, and the specificity of this response was confirmed using a PPAR-γ antagonist. In a series of experiments using the same mouse model, the PPAR-γ agonist ciglitazone demonstrated a similar inhibitory effect on the eosinophilic response following ovalbumin challenge [61]. PPAR-γ and PPAR-α agonists also produced a reduction in airway influx of eosinophils and lymphocytes post challenge [62]. In addition the PPAR-γ agonists rosiglitazone and pioglitazone suppressed bronchoalveolar lavage (BAL), eosinophilia and bronchial hyper-reactivity following ovalbumin challenge [42]. *In vitro* work has demonstrated that PPAR-γ down-regulates eosinophil survival, chemotaxis, antibody-dependent cellular cytotoxicity and degranulation [63].

Dendritic cells

In an ovalbumin mouse model [60] dendritic cell behaviour was observed following culture with and without

Table 1. PPARγ agonist effects on airway cells and animal model

Airway cell	PPAR-γ agonist effect
Eosinophil	⇓ lavage concentration [42, 60–62]
Dendritic cell	⇓ *in vitro* maturation [65],
	⇓ *in vivo* migration [60],
	⇓ T cell activation [60, 65]
Neutrophil	⇓ tissue neutrophilia-pre+post insult [62, 66],
	⟺ no change in BAL [62, 66]
Monocyte/	⇑ CD 36 expression *in vitro* [78, 79],
macrophage	⇓ apoptosis *in-vitro* [76]
	⇓ activation *in-vitro* [39, 72, 77]
T Lymphocyte	⇑ apoptosis [82],
	⇑ survival [25],
	⇓ proliferation [29, 60, 65, 85],
	⇓ cytokine production [28, 29, 80]
B Lymphocyte	⇑ apoptosis [34, 86, 88]
Mast cell	⇓ FcεRI expression [92],
	⇓ histamine release [91]
Bronchial	⇓ mucus production, basement membrane
epithelium	thickness, collagen deposition [94],
	⇓ cytokine and chemokine expression [95, 96]
Airway smooth	⇓ proliferation [97, 98],
muscle	⇓ cytokine and chemokine production [32, 98]
Animal models	⇓ response to insult in asthma model [42, 60–62]

Table 2. PPARγ agonist's effects on cytokines, chemokines and inflammatory mediators

Cytokines	Effect
IL-2	⇓ [35]
IL-4	⇓ [28]
IL-5	⇓ [91]
IL-6	⟺ [106]
IL-8	⟺ [32]
	⇓ [95]
IL-10	⟺ [39]
	⇑ [60]
IL-13	⇓ [42]
IFN-γ	⇓ [81]
Eotaxin	⇓ [32]
MMP1	⇓ [107]
MMP9	⇓ [96]
GM-CSF	⇓ [98]
IFN-γ	⇓ [83]
TNF-α	⇓ [96]
	⟺ [39, 62, 106]

PPAR-γ agonists. Dendritic cells in this model demonstrated increased expression of the chemokine receptor CCR-7, which is involved in dendritic cell migration. Culture with PPAR-γ agonists resulted in reduced CCR-7 expression and reduced migration of dendritic cells into the draining lymph nodes. This was associated with reduced ovalbumin-specific T lymphocyte proliferation. A significant reduction in eosinophilic response was observed in the mice receiving the dendritic cells cultured with rosiglitazone, and the specificity of this response was confirmed using a PPAR-γ antagonist. This agonist effect appeared to be mediated by an increase in T cell derived IL-10 production, and this was partially reversed using an anti-IL-10 receptor antibody. Further work by this group [64] demonstrated that PPAR-γ stimulation reduced TNF-α-induced migration of Langerhans cells from the peripheral circulation into lung draining lymph nodes.

The culture and maturation of blood monocytes into dendritic cells, and their subsequent function as antigen-presenting cells has been studied following addition of a PPAR-γ agonist [65]. Exposure of monocytes and dendritic cells to troglitazone or the PPAR-γ agonist BRL49653 during differentiation did not produce a change in morphology but increased CD86 expression and reduced the expression of CD1a and CD80. This is an unusual dendritic cell phenotype. On further investigation the dendritic cells were non-responsive to known dendritic cell stimulants (lipopolysaccharides (LPS), TNF-α and CD40 ligand (CD40L)) and had reduced expression of CCR-7 and Epstein–Barr virus induced gene-1 ligand chemokine (ELC). These changes were associated with a reduction of the ability of these dendritic cells to stimulate T cell proliferation. Therefore *in vivo* PPAR-γ agonists could alter dendritic cell maturation and function, reduce their ability to enter 'transit' mode and antigen-presenting cell efficiency.

Neutrophils

The interaction between PPAR-γ and neutrophils has been explored in experimental models. Birrell et al. [66] used a murine acute lung injury model based on LPS aerosolization to examine the effect of PPAR-γ agonists on lung neutrophilia and compared its abilities against dexamethasone. Rosiglitazone administered to mice pre-LPS inhibited lung parenchyma neutrophil influx but lung lavage fluid neutrophil levels did not change. However dexamethasone was able to reduce both lavage and parenchymal neutrophilia. This effect was in contrast to the effects of administering rosiglitazone post LPS. In this context rosiglitazone, unlike dexamethasone, was able to reduce tissue neutrophilia compared with control. In keeping with these findings, Trifilieff et al. [62], using the murine ovalbumin asthma model, found no reduction in BAL neutrophilia or TNF-α and keratinocyte-derived chemokine (KC) levels after ovalbumin challenge despite treatment with either PPAR-γ or PPAR-α agonists pre-challenge. Post-challenge effects and tissue neutrophil level was not examined. Birrell and colleagues went on to explore this phenomenon. Rosiglitazone administered pre-LPS reduced tissue granulocyte colony-stimulating factor (G-CSF) and KC (murine IL-8) levels (chemoattractants/neutrophil survival factors), whereas dexamethasone was able to reduce G-CSF, KC and granulocyte–monocyte colony-stimulating factor (GM-CSF) levels in the tissue

and GM-CSF levels in the lavage. Rosiglitazone also had no effect on matrix metalloproteinases-9 (MMP-9) levels but dexamethasone significantly reduced MMP-9 in lavage fluid. Rosiglitazone administered post-LPS reduced tissue G-CSF. No effect was seen on KC or GM-CSF levels. Dexamethasone administered post-LPS was able to reduce all three cytokines but not tissue neutrophilia. Thus based on the finding from this animal model rosiglitazone and dexamethasone may have complementary effects on neutrophilic association lung inflammation.

In vitro studies have demonstrated reduced neutrophil chemotactic responses to IL-8 following application of troglitazone and 15d-PGJ_2 and reduced production of inflammatory cytokines (IL-12, TNF-α, IL-8) by neutrophils following stimulation with LPS [68].

Macrophages

Macrophages express PPAR-γ receptors and these are involved in monocyte/macrophage differentiation and regulation of macrophage mediated inflammation [16, 71–76]. PPAR-γ up-regulation has been demonstrated following macrophage maturation [73, 76], and activation of PPAR-γ can induce apoptosis [76], reduce oxidative burst, iNOS expression and production of the cytokines IL-12 [39, 72, 77], IL-6 [39, 72], TNF-α [39, 74], IL-1β and MMP-9 [72] and IL-1α [73]. However, Moore et al. [78] and Chawla et al. [79], using PPAR-$\gamma^{-}/^{-}$ macrophage stem cells have demonstrated that PPAR-γ is not essential for *in vitro* or *in vivo* macrophage maturation, phagocytosis and post insult inflammatory cytokine production (TNF-α and IL-6). PPAR-γ stimulation can increase the expression of CD36, which is a scavenger molecule for apoptotic neutrophils [75]. PPAR-$\gamma^{-}/^{-}$ stem cell work has demonstrated that CD36 expression does require PPAR-γ expression but other cell surface markers are normally expressed. PPAR-$\gamma^{-}/^{-}$ embryonic stem cells can be stimulated to become macrophages *in vitro* [78, 79] and PPAR-γ is not required for macrophage iNOS suppression by PPAR-γ agonists *in vitro* and *in vivo* [21].

T lymphocytes

PPAR-γ expression has been demonstrated in murine T lymphocytes [72, 80, 81] and in human resting and activated T lymphocytes [25, 35, 82] and both $CD4^{+}$ and $CD8^{+}$ T lymphocyte subsets [83].

PPAR-γ stimulation with 15d-PGJ_2 induces apoptosis in murine T cells [81] and human T cells [84], however this has not been replicated with synthetic PPAR-γ agonists [85]. PPAR-γ stimulation with thiazolidinediones promoted survival in human T lymphocytes in one series of experiments [25]. This result was also obtained using low concentrations of PPAR-γ agonists (0.5 μmol rosiglitazone), equivalent to the K_D of PPAR-γ, which stimulated PPAR-γ-dependent transcription and also facilitated T cell survival upon growth factor withdrawal. However apoptosis occurred at higher concentrations ($>5\,\mu M$ for 15d-PGJ_2 and $>80\,\mu M$ for ciglitazone).

15d-PGJ_2 and synthetic PPAR-γ agonists have been demonstrated to significantly inhibit T cell clonal proliferative response to antigen and anti-CD3 antibody stimulation, and reduce T cell IL-2 production [80, 85]. However this does not prevent their ability to respond to exogenous IL-2 [80]. Similarly reduced IL-2 production by T lymphocytes was observed following PPAR-γ stimulation by 12/15LO products (13-HODE) produced by human *ex vivo* macrophages [82]. Evidence has also been produced for a PPAR-γ mediated inhibitory effect on IL-4 production in activated T cells [28].

IFNγ expression is important for many functions of the immune system and it has a central role in the chronic phase of asthma and COPD, as well as in Th1 responses and autoimmune disease [83]. PPAR-γ stimulation has been demonstrated to suppress IFN-γ levels [29, 86]. One group has demonstrated that application of a PPAR-γ agonist decreases IFN-γ RNA levels in murine T lymphocytes whilst a PPAR-γ antagonist increases IFN-γ RNA levels [83]. Evidence exists for PPAR-γ agonist suppression of TNF-α [29], IL-2 [82] and IL-4 [28] production by stimulated T lymphocytes.

B lymphocytes

B lymphocytes express PPAR-γ mRNA and receptor protein at various stages of maturation [87]. 15d-PGJ_2 and synthetic PPAR-γ agonists have been demonstrated to induce apoptosis of a pre-B cell line [87–89] however this effect was blunted by binding of CD40 to CD40L which prevented NF-κB inhibition [88].

A heterozygous PPAR-$\gamma^{-}/^{+}$ mouse model has enhanced B cell proliferative responses compared with wild type via increased NF-κB activation, suggesting PPAR-γ stimulation plays a role in B lymphocyte proliferation *in vivo* [90].

Mast cells

Human cultured mast cells express PPAR-γ, mainly PPAR-γ_1 and increase their expression of PPAR-γ_2 on stimulation with IL-4 and anti-IgE [91]. Stimulation of mast cells with PPAR-γ agonists produce varied effects depending on the agonist used. Cyclopentenones (PGD_2, Δ^{12}-PGJ_2, 15d-PGJ_2) suppress GM-CSF production by stimulated mast cells in a dose dependent manner but troglitazone was only able to produce a reduction in GM-CSF at much higher doses (IC_{50} 33 μM) [91]. Application of cyclopentenones also reduced histamine release from activated mast cells, but troglitazone and ciglitazone were unable to produce any reduction. However leukotriene C_4 (LTC_4) release was suppressed by troglitazone, ciglitazone, and

the cyclopentenones tested. PPAR-γ stimulation using cyclopentenones has been reported to reduce FcεRI expression on basophils [92]. Ciglitazone was unable to alter FcεRI surface expression.

Research into mast cell function with relation to PPAR-γ is at an early stage when compared with the level of understanding of PPAR-γ's relationship with macrophages and eosinophil function and the evidence so far has been produced predominantly using cyclopentenone derivatives.

Stromal cells: epithelial cells, smooth muscle cells and fibroblasts

Epithelial cells release inflammatory mediators, growth factors and metalloproteinases and are involved in the airway remodelling seen in severe asthma [93]. There is epithelial damage in severe asthma, with increase in intra-epithelial goblet cells and mucosal glands. A group using a murine ovalbumin model studying epithelial PPAR-γ function [94] examined treatment with ciglitazone before and following ovalbumin challenge and demonstrated that PPAR-γ expression is markedly increased following airway sensitization. Treatment with ciglitazone was found to reduce airway hyper-responsiveness, mucus production, collagen deposition, basement membrane thickness and TGF-β synthesis suggesting an important role in controlling remodeling. PPAR-γ can modify epithelial cell cytokine expression [95]; ciglitazone substantially reduced IL-8 expression when epithelial cells were treated with IL-4.

MMPs are involved in airway remodelling and control of extracellular matrix composition and their up-regulation is seen in many inflammatory airway diseases. In asthma MMP-9 is up-regulated in epithelial cells in bronchial biopsies, and there are increased levels in BAL specimens. In a series of experiments [96], MMP-9 expression was investigated in two epithelial cell lines using rosiglitazone and pioglitazone. Reduced TNF-α and MMP-9 levels were observed following the addition of the PPAR-γ agonists in a dose dependent manner. No change occurred in levels of expression of the natural MMP inhibitor, tissue inhibitor of metalloproteinases-1 (TIMP-1).

PPAR-γ plays a role in proliferation of cultured human airway smooth muscle cells [97]. Treatment of smooth muscle cells with 15d-PGJ_2 and rosiglitazone reduced proliferation to the mitogens fibroblast growth factor-β (bFGF) and thrombin. This inhibitory function of 15d-PGJ_2 was independent of PPAR-γ, whereas rosiglitazone inhibition was via PPAR-γ stimulation. 15d-PGJ2 and ciglitazone have also been used to demonstrate suppression of GM-CSF and G-CSF expression by human smooth muscle cells *in vitro* [98]. Dexamethasone was able to inhibit GM-CSF but was unable to inhibit G-CSF as potently as the PPAR-γ agonists. Both 15d-PGJ_2 and ciglitazone inhibited smooth muscle cell proliferation in contrast to dexamethasone, which only partially inhibited proliferation.

The effects of 15d-PGJ_2, troglitazone, fluticasone and salmeterol have been examined in the context of TNF-α-induced cytokine expression in cultured human airway smooth muscle cells [32]. 15d-PGJ_2 and troglitazone both significantly inhibited TNF-α-induced eotaxin and monocyte chemotactic protein-1 (MCP-1) production. No effect was noted on IL-8 concentration in contrast to of the effects of fluticasone, which significantly inhibited production of all three.

PPAR-γ expression has been studied in bronchial biopsies from human asthmatic airways [17]. Corticosteroid naive asthmatic patients demonstrated increased PPAR-γ expression compared with control subjects. This increased expression was evident in all epithelial compartments (epithelial cells, smooth muscle cells and bronchial mucosal cells). Patients treated with inhaled corticosteroids had reduced levels of PPAR-γ expression. Cell specific expression was localized to eosinophils, macrophages and fibroblast-like cells.

Fibroblasts play an important role in the development of fixed airway obstruction in COPD [12] and asthma [93]. PPAR-γ is expressed in human lung fibroblasts and PPAR-γ agonists have been demonstrated to inhibit fibroblast differentiation into myofibroblasts and reduce collagen production following TGF-β stimulation *in vitro* [99].

Potential clinical impact of peroxisome proliferator–activated receptor-γ agonists in asthma and other inflammatory airway diseases

Many components of the immune system are activated in both asthma and COPD. Therefore a new therapy that can exert control over many pathways simultaneously in a fashion analogous to, but different from corticosteroids is required for severe asthma and COPD. Based on *in vitro* and animal studies, PPAR-γ agonists have properties that suggest that this group of drugs may have a role in the treatment of inflammatory airway diseases.

PPAR-γ agonists exert anti-inflammatory effects on multiple cell types and in animal models of asthma. Eosinophilic inflammation in murine models of asthma appears sensitive to treatment with PPAR-γ agonists as are dendritic cells and T lymphocytes. Macrophages become activated by PPAR-γ agonists and appear to become ready to increase phagocytosis and smooth muscle proliferation can be suppressed by PPAR-γ agonists. Established neutrophilic infiltration of the lungs of experimental animals is also susceptible to PPAR-γ agonists. Less impressive is the impact on mast cells.

Taken as a whole these findings suggest that PPAR-γ agonists have anti-inflammatory actions which could be of relevance to asthma and COPD. However some *in vitro* research has used large doses of thiazolidinediones to

produce their anti-inflammatory effects leading to questions on the relevance of this work to PPAR-γ stimulation and *in vivo* research. As we have discussed these large doses may enable thiazolidinediones to act via non-PPAR-γ related mechanisms but may require larger doses than those currently used clinically. This coupled to a number of lessons learned from the genesis of currently available treatments should be borne in mind in examining the results of *in vitro* work using thiazolidinediones.

Long acting β agonists (LABAs) have proven useful in the treatment of patients with asthma when prescribed in combination with inhaled corticosteroids. During their development LABAs were reported to have multiple *in vitro* anti-inflammatory properties in many cell types. However research *in vivo* has not been able to consistently replicate previous findings. For example LABAs abilities to inhibit inflammatory mediator release from mast cells *in vitro* [100] are not consistently found *in vivo* and treatment of mild asthmatics with LABAs does not alter mast cell numbers [101] or tryptase or histamine levels in BAL [102]. Similar apparent inconsistencies exist between *in vitro* and *in vivo* results from research examining LABA effects on T lymphocytes, eosinophils and neutrophils. Interestingly the LABA salmeterol, when combined with the inhaled corticosteroid fluticasone, has been shown to reduce bronchial inflammation in COPD [103].

PDE_4 inhibitors provide another cautionary example. *In vitro* work with many compounds selective for PDE_4 has demonstrated that inhibition of this enzyme is able to reduce inflammatory cell activation and inflammatory response in murine models of asthma. However despite some promising *in vivo* results in phase III trials no PDE_4 inhibitors are available for clinical use at the present time and concerns exist over their potential role [104].

Large well-designed clinical trials are the true test of findings from *in vitro* and animal research and this is certainly the case for this class of medication. Thiazolidinediones are ideally placed for human research given their long record of safe use in the treatment of type 2 diabetes. However to date no human study has been reported in either asthma or COPD utilizing a PPAR-γ agonist. A clinical trial examining the effect of rosiglitazone on lung function in comparison with low dose inhaled corticosteroids in steroid naïve smokers with asthma is currently underway [105], and further proof of concept clinical trials may be warranted in other inflammatory lung diseases.

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