Prostaglandin and PPAR control of immune cell function

DINO ROTONDO & JILLIAN DAVIDSON *Department of Immunology, University of Strathclyde, Glasgow G4 ONR, UK*

Peroxisome proliferator-activated receptors (PPARs) belong to a superfamily of intracellular ligand-activated receptors. These receptors are also transcription factors exerting their regulatory functions directly at the gene level.[1] PPARs appear to act as messengers responsible for translating a wide variety of stimuli into changes in gene expression and cellular differentiation pathways. They were originally identified as molecular targets for compounds that induce an increase in the size and number of hepatic and renal peroxisomes. Peroxisomes are single-membrane organelles present in nearly all eukaryotic cells and carry out metabolic peroxidation reactions, primarily the β-oxidation of fatty acids. Peroxisome proliferators increase the capacity of peroxisomes to metabolize fatty acids via increased expression of the enzymes required for β-oxidation.[2,3] Currently three subtypes of mammalian PPARs have been identified, termed α, δ (originally termed β) and γ, encoded by different genes and showing distinct tissue distributions. Both PPAR-α and PPAR-γ act as regulators of lipid metabolism; PPAR-α plays a crucial role in hepatic liver metabolism and PPAR-γ participates in adipocyte differentiation and function. No specific function has yet been identified for PPAR-δ, although it has a very wide distribution, can be activated by prostacyclin, may be involved in the control of cellular development and is potentially involved in the pathogenesis of tumours such as in colorectal cancer.[4]

PPARs are activated by a group of structurally diverse compounds including fatty acids, eicosanoids and drugs such as the hypolipideamic fibrates.[5] It was also fortuitously discovered that they can be activated by the thiazolidinediones class of antidiabetic drugs (oral hypoglycaemics), thus providing a useful tool with which to characterize their distribution and the spectrum of actions that they modulate. Specific thiazolidinediones such as troglitazone and rosiglitazone have been used to elucidate the role of PPARγ-activated pathways and these compounds have been shown to bind directly to the PPARs.[6] The discovery that PPARs were activated by direct ligand binding led to a search for the physiological ligand regulator of PPARs, especially PPAR-γ. A potential candidate, on the basis of binding studies and the ability to functionally activate PPAR-γ, was 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2). It is now generally accepted that the naturally occurring activating ligand for PPAR-γ is 15d-PGJ_2, which can be derived as a metabolite from prostaglandin D_2 (PGD_2), and is predominantly found in adipose tissue.[7] PGD_2 metabolites, however, have not been identified in adipocytes but are major products of arachidonic metabolism in macrophages, and PGD_2 synthase, which is required for 15d-PGJ_2 synthesis, is predominantly expressed in macrophages and other antigen-presenting cells.[8] Although much of the early work surrounding PPARs was directed at understanding mechanisms of lipid metabolism and adipose tissue function in obesity, this raised the possibility that PPARs, specifically PPAR-γ, were important regulators of immune function.

Correspondence: Dr Dino Rotondo, Department of Immunology, University of Strathclyde, Strathclyde Institute for Biomedical Sciences, 27 Taylor Street, Glasgow G4 ONR, UK. E-mail: D.Rotondo@strath.ac.uk

PPAR-γ potentially possessed an important role in regulating inflammatory responses by modulating the activity of macrophages/monocytes. Several studies on the role of PPAR-γ in macrophage functions confirmed that activation of PPAR-γ did indeed modulate their activity. Ricote *et al.*[9] showed that resting murine bone-marrow-derived macrophages expressed low levels of PPAR-γ mRNA, whereas activated murine peritoneal macrophages expressed high levels of PPAR-γ mRNA and in the presence of either 15d-PGJ_2 or the thiazolidinedione BRL 49653 (rosiglitazone), a specific PPAR-γ agonist, IFNγ-activated macrophages retained the morphological characteristics of resting cells. This indicates that PPAR-γ pathways are capable of halting macrophage activation. Ricote *et al.*[9] also examined two other markers of macrophage activation: (a) expression of inducible nitric oxide (iNOS) (cytotoxic for microorganisms) and (b) up-regulation of expression of gelatinase B (a matrix metalloproteinase associated with tissue damage during inflammation). 15d-PGJ_2 and synthetic PPAR-γ ligands inhibited IFNγ-stimulated nitric oxide (NO) production and the induction of iNOS mRNA. 15d-PGJ_2 also inhibited the induction of gelatinase B mRNA. These observations suggested that PPAR-γ can inhibit the expression of genes which are up-regulated during macrophage activation and differentiation.

As to a possible mechanism, Ricote *et al.*[9] provided data from transcriptional assays in cultured macrophage-like cell lines which demonstrated that activation of PPAR-γ

© 2002 Blackwell Science Ltd

by either BRL 49653 or 15d-PGJ_2 antagonizes the effects of three other different classes of transcription factor, AP-1, STAT 1 and NF-κB, which play general roles in mediating inflammatory responses in many cell types including macrophages. A major primary activation response of monocytes/macrophages is the release of proinflammatory cytokines in response to a wide range of stimuli, including bacterial lipopolysaccharide and phorbol esters. PPAR-γ agonists have been shown to inhibit the production of monocyte (Mø) inflammatory cytokines at concentrations similar to those found to promote adipogenesis. Using freshly isolated human peripheral blood mononuclear cells, Jiang *et al.*[10] demonstrated that PMA-induced tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 synthesis could be potently inhibited by PGJ_2 and 15d-PGJ_2 whereas A, B and E series prostaglandins had little or no effect at similar concentrations. The structurally dissimilar PPAR-γ agonist troglitazone also inhibited PMA-induced TNF-α production whereas the PPAR-α ligands leukotriene B_4, eicosatetraynoic acid and 8[S]-hydroxyeicosatetraenoic acid had no effect. Using RNA blot analysis, the study by Jiang *et al.*[10] clearly showed that PMA-induced TNF-α mRNA accumulation in Mø was inhibited by 15d-PGJ_2 and troglitazone, indicating that PPAR-γ agonists inhibit cytokine synthesis pretranslationally. Interestingly, several non-steroidal anti-inflammatory drugs are known to have PPAR-γ agonist activity at high concentrations,[11] and Jiang *et al.*[10] also showed that at relatively high concentrations indomethacin, ibuprofen and fenoprofen suppressed TNF-α mRNA accumulation.

More recently, the potential immunoregulatory control by PPARs and 15d-PGJ_2 has been investigated in lymphocytes. PPAR-γ ligands and 15d-PGJ_2 were shown to display dose-dependent antiproliferative and cytotoxic effects on normal and malignant murine B cells.[12] The cells were shown to express PPAR-γ mRNA and the 67-kDa PPAR-γ protein and the cytotoxic actions of PPAR-γ activation on the B cells occurred as a result of apoptosis.[12] Similar observations have been reported for murine T lymphocytes by Harris & Phipps.[13] A dramatic inhibition of both naive and antigen-activated T-cell proliferation was induced by troglitazone and 15d-PGJ_2, which was accompanied by apoptosis of the cells and expression of PPAR-γ mRNA and protein.[13] This clearly indicates that lymphocytes can also be controlled via PPAR activation and also revisits the question of prostaglandins as endogenous, but not necessarily physiological, possibly pathophysiological regulators of immune function via PPARs. Classically, prostaglandins have always been associated with immune/inflammatory responses as they are the end mediators which culminate in the symptomatic manifestations of inflammation. In addition, anti-inflammatory therapy has traditionally been directed towards the inhibition of prostaglandin biosynthesis with the use of non-steroidal anti-inflammatory agents such as aspirin, indomethacin and ibuprofen.

The major arachidonic acid metabolite produced by activated immune cells, especially monocytes/macrophages, is PGE_2. PGE_2 is not only produced in response to cytokine stimulation, primarily IL-1 and TNF-α, but also provides feedback regulation by inhibiting the further release of these cytokines, thereby limiting the response.[14] Thus, PGE_2 is generally an inhibitory end signal for both monocytes/macrophages and T lymphocytes and appears to mediate this effect via EP2/EP4 receptors which are G-protein coupled to the activation of adenylate cyclase. PGD_2 is also reportedly an inhibitory prostanoid and acts via its respective receptor, the DP receptor, which is also a cyclic AMP coupled receptor. In T cells, PGE_2 can inhibit mitogen and cytokine-amplified proliferation[15] in the nanomolar concentration range and this appears to occur via a cyclic AMP-dependent mechanism,[16] indicating a role for EP2/EP4 receptors in this response. In this respect, it is highly unlikely that either PGD_2 or PGE_2 acts via PPAR-γ as they can stimulate elevations in cyclic AMP levels at lower concentrations than those required for ligand activation of PPAR-γ. Also, it is much more likely that in this context the responses would be mediated via cyclic AMP response elements (CREs) which can act independently of PPARs; however, it is thought that PPARs may modulate the action of cyclic AMP via binding to the CRE binding protein.[17]

It has now been shown by Harris & Phipps (in this issue of *Immunology*) that PPAR-γ agonists and 15d-PGJ_2 can also induce apoptosis in human transformed T cells, as they previously showed for murine T cells.[13] However, they have also demonstrated other particularly novel effects on human T cells, primarily that PGD_2 can also induce identical responses, albeit at 5-fold higher concentrations. In addition, they showed that this was restricted to transformed T cells, with normal anti-CD3/anti-CD28-activated T cells being unaffected. The EC_{50} concentration of 15d-PGJ_2 is less than 1 μM (in the 0·5–1·0 μM range), but the EC_{50} for PGD_2 is 5- to 10-fold greater, possibly supporting the view that PGD_2 requires to be converted to its 15d-PGJ_2 metabolite either within cells or extracellularly in order to exert its action.

Harris & Phipps (in this issue of *Immunology*) also examined the possibility that PGD_2 may be acting through the classical PGD_2 (DP) receptor, as they were able to show that the transformed cells, which responded to PGD_2, also expressed mRNA for the DP receptor. However, they also demonstrated that a potent DP receptor agonist was not able to mimic the actions of PGD_2, and they also showed that a DP receptor antagonist was unable to inhibit the actions of PGD_2. The study did not show whether authentic PGD_2 itself was binding directly to PPAR-γ or whether in their cell system the PGD_2 could be converted to 15d-PGJ_2; however, this has been confirmed in other systems and would most likely have occurred under the similar incubation conditions in the study by Harris & Phipps. It has been shown that mast cells *in vitro* can rapidly convert both exogenous added and endogenously produced PGD_2 to PGJ2.[18] It has also been observed that human T cells can synthesize PGD_2.[19]

The study of Harris & Phipps would tend to suggest that the regulatory effects of PGD_2 on transformed human T cells do not occur through the known DP receptor, although from a pharmacological perspective it is possible that the data point to a new form of the DP receptor.

Similar observations on the inability of various PGE_2 agonists and antagonists to modulate PGE_2 functions led to the discovery that there are four forms of the prostaglandin E receptor (EP1–EP4).

CAUTIONARY OBSERVATIONS

The development of new PPAR-γ ligands, including novel prostaglandin analogues, may be of potential therapeutic use in a variety of diseases, such as chronic inflammatory disorders where activated macrophages play a pivotal role or in the control of T-cell leukaemia as suggested by the study of Harris & Phipps (see above). Highly selective ligands for the different PPAR forms have recently been described.[20] However, although many studies have employed PPAR ligands such as the thiazolidinediones to elucidate the potential role of PPARs in specific functions, it would appear that some of the actions of thiazolidinediones are not always mediated via PPARs. It has been shown that the inhibition of cell proliferation and tumour growth by thiazolidinediones is independent of PPARs. Instead they appear to inhibit translation initiation by rendering eIF2 inactive.[21] Similarly, macrophage inflammatory actions can be suppressed by 15d-PGJ_2 and thiazolidinediones that are independent of PPAR-γ even although PPAR-γ is required for the control of lipid metabolism in macrophages.[22]

REFERENCES

1 Schoonjans K, Staels B, Auwerx J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. J Lipid Res 1996; **37:**907–25.

2 Lemberger T, Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. Ann Rev Cell Dev Biol 1996; **12:**335–45.

3 Schoonjans K, Martin G, Staels B, Auwerx J. Peroxisome proliferator-activated receptor, orphans with ligands and functions. Curr Opin Lipidol 1997; **8:**159–66.

4 Gupta RA, Tan J, Krause WF, Geraci MW, Willson TM, Dey SK, DuBois RN. Prostacyclin-mediated activation of peroxisome proliferator-activated receptor delta in colorectal cancer. Proc Natl Acad Sci USA 2000; **97:**13275–80.

5 Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Whali W, Willson TM, Lenhard JM, Lehmann JM. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. Proc Natl Acad Sci USA 1997; **94:**4318–23.

6 Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPARgamma). J Biol Chem 1995; **270:**12953–6.

7 Kliewer SA, Lenhard JM, Wilson JM, Patel I, Morris DC, Lehmann JM. A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. Cell 1995; **83:**813–9.

8 Urade Y, Ujihara M, Horiguchi Y, Ikai K, Hayaishi O. The major source of endogenous prostaglandin D_2 production is likely antigen-presenting cells. J Immunol 1989; **143:**2982–9.

9 Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. Nature 1998; **391:**79–82.

10 Jiang C, Ting AT, Seed B. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. Nature 1998; **391:**82–6.

11 Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA. Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem 1997; **272:**3406–10.

12 Padilla J, Kaur K, Cao HJ, Smith TJ, Phipps RP. Peroxisome proliferator activator receptor-gamma agonists and 15-deoxy-Delta12,14-PGJ2 induce apoptosis in normal and malignant B-lineage cells. J Immunol 2000; **165:**6941–8.

13 Harris SG, Phipps RP. The nuclear receptor PPAR gamma is expressed by mouse T lymphocytes and PPAR gamma agonists induce apoptosis. Eur J Immunol 2001; **31:**1098–105.

14 Rotondo D. Review: Fatty acid modulation of cell responsiveness. Biochem Soc Trans 1995; **23:**291–6.

15 Rotondo D, Earl CRA, Laing K, Kaimakamis D. Inhibition of cytokine-stimulated thymic lymphocyte proliferation by fatty acids: The role of eicosanoids. Biochim Biophys Acta 1994; **1223:**185–95.

16 Davidson J, Smith F, Rotondo D. Prostaglandin E_2-and fatty acid suppression of cytokine-stimulated thymic lymphocyte proliferation: the role of cyclic AMP. Br J Pharmacol 1995; **116:**354.

17 Gelman L, Zhou G, Fajas L, Raspe E, Fruchart JC, Auwerx J. p300 interacts with the N- and C-terminal part of PPARγ2 in a ligand-independent and -dependent manner, respectively. J Biol Chem 1999; **274:**7681–8.

18 Haberl C, Hultner L, Flugel A, Falk M, Geuenich S, Wilmanns W, Denzlinger C. Release of prostaglandin D_2 by murine mast cells: Importance of metabolite formation for antiproliferative activity. Med Inflammation 1998; **7:**79–84.

19 Tanaka K, Ogawa K, Sugamura K, Nakamura M, Takano S, Nagata K. Cutting edge: Differential production of prostaglandin D_2 by human helper T cell subsets. J Immunol 2000; **164:**2277–80.

20 Liu KG, Smith JS, Ayscue AH, Henke BR, Lambert MH, Leesnitzer LM, Plunket KD, Willson TM, Sternbach DD. Identification of a series of oxadiazole-substituted [alpha]-isopropoxy-phenylpropanoic acids with activity on PPAR[alpha], PPAR[gamma], and PPAR[delta]. Bioorganic Med Chem Lett 2001; **11:**2385–8.

21 Palakurthi SS, Aktas H, Grubissich LM, Mortensen RM, Halperin JA. Anticancer effects of thiazolidinediones are independent of peroxisome proliferator-activated receptor-γ and mediated by inhibition of translation initiation. Cancer Res 2001; **61:**6213–8.

22 Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM. PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. Nature Med 2001; **7:**48–52.