At the crossroads of lipid metabolism and inflammation

The role of PPAR-γ, a lipid-activated transcription factor

Lajos Széles, Dániel Tőrocsik, László Nagy *
Department of Biochemistry and Molecular Biology, Research Center for Molecular Medicine, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary

- There is no adaptive immunity without antigen-specific T cells. Different subsets of T lymphocytes are involved in immune responses to viruses, bacteria, fungi, protozoa and worms. However effective they may be, T cells are not able to recognize antigens by themselves; they require the assistance of antigen-presenting cells (APCs) that can take up, process and present peptides and glycolipids of infectious agents. Of the antigen-presenting cells – which comprise dendritic cells (DCs), macrophages and B cells – DCs are the most effective activators of T cells. DCs are the initiators and regulators of several forms of adaptive immunity.

Signals from pathogens, tissue factors and other immune cells are all involved in defining the type and extent of immune response initiated by DCs. Lipid mediators and other lipid-soluble molecules contribute to the fine-tuning of this regulation by activating nuclear receptors. Our research group investigates the role of nuclear receptors in myeloid cells – cells derived from bone marrow – especially those in DCs. In this report, we first briefly introduce the biology and function of DCs and nuclear receptors. We then focus on the peroxisome proliferator-activated receptor-γ (PPAR-γ) and how it modifies the function of DCs. Finally, we put our findings into a broader perspective.

The biology of dendritic cells
- DCs are professional antigen-presenting cells of myeloid or lymphoid origin. In general, the development of DCs can be divided into three states: progenitors or precursors of the DCs, immature DCs and mature DCs. The first subset of DCs identified were the bone-marrow-derived Langerhans’ cells (Figure 1). Precursor Langerhans’ cells emigrate from the bone marrow, travel via the bloodstream and reach the skin’s epidermal layer as immature DCs. The precursor pool in the bone marrow is replenished by dermal CD14-expressing and other precursors from the peripheral blood. The immature Langerhans’ cells are sentinels of the epidermal layer, seeking infectious agents invading host tissues. They are well equipped to take up antigens by receptor-mediated endocytosis, phagocytosis and macropinocytosis. Antigen uptake associated with signals from surrounding tissues initiates dramatic changes in the Langerhans’ cells. Upon such signals, within a day they almost completely lose their capacity for antigen uptake. At the same time they undergo a maturation process, which enables them to fulfil their new and terminal functions: antigen presentation and T-cell activation. During maturation, Langerhans’ cells migrate to draining lymph nodes, principally due to changes in their chemokine-receptor profile, which now includes CCR7. This al-
allows Langerhans’ cells to follow CCL19 and CCL21 chemokine signals released from the lymphatic vessels\cite{6}. In addition, they start processing antigens and present them as MHC class II-peptide complexes on the cell surface. Simultaneously, Langerhans’ cells begin expression of accessory and/or co-stimulatory molecules (CD40, CD80 and CD86) and cytokines (interleukin-12, tumour necrosis factor-α, etc.)\cite{6}. In the lymph node, mature Langerhans’ cells and DCs in general are in direct contact with naive T cells and induce them to differentiate into $T_{H1}, T_{H2}$ or regulatory T cells. In-vivo mouse experiments have demonstrated that mature DCs can stay in the lymph nodes up to 2 weeks before being eliminated\cite{6}.

**Subsets and plasticity of dendritic cells**

- Based on their origin (myeloid or lymphoid), specific markers, expression profile of Toll-like receptors and localization, different subsets of DCs can be identified. Questions remain as to how much these subsets are functionally distinct and the extent of their flexibility to polarize the immune response towards tolerance or mounting an immune reaction\cite{2}. Several subtypes of DCs have been identified to date. For example, Langerhans’ cells are present in the epidermis of the skin and epithelia, whereas interstitial DCs are localized in the intestine and most peripheral tissues. A third subset, plasmacytoid DCs, circulate in the blood and can also be found in primary and secondary lymphoid tissues\cite{7}.

Although DCs are most well known for their capacity to activate naive T cells, they can also interact directly with other immune cells, such as B cells\cite{1}, natural killer T cells (NKT cells)\cite{8} and cytotoxic T lymphocytes (CTLs)\cite{9,10}. Their antigen-presentation function is also not restricted to the presentation of peptides by MHC class II molecules; they also present glycolipids in complex with CD1 molecules\cite{11} and endo- or exogenous antigens with MHC class I molecules\cite{10}.

One of the most interesting questions in DC biology is how these cells choose the appropriate strategy to induce either the most effective immune response or tolerance. What kinds of extracellular signal contribute to this decision-making process and how are these signals transmitted inside the cell? Studies have revealed details of how pattern-recognition receptors (such as Toll-like receptors) expressed on DC surfaces recognize conserved microbial molecules\cite{2,12} (Figure 2). Other important signal types come from tissue cells and immune cells affected by
pathogens; such signals include chemokines, histamine, cytokines and prostaglandin E₂. Recent data show that these tissue factors can prime Tₘ₁-, Tₘ₂- or regulatory T-cell-inducing DCs.

**DC models**
- Human DCs are difficult to investigate because of their very low levels in peripheral blood. Therefore, various isolation methods have been used to enrich these cells. Several techniques to generate DCs in vitro have been established, and these are considerably faster and yield more homogeneous cells than in-vivo enrichment techniques. Such techniques, to generate DCs in vitro from monocytes with cytokines, speed up investigations on DC immunology remarkably. We also use an in-vitro method that enables us to obtain immature or mature DCs from monocytes treated with chemokines in 5-6 days. Although there are obvious limitations, ex-vivo studies have contributed a great deal to this field.

**Immunosuppressive agents and programming dendritic cells**
- At the end of 1950s, Schwartz and Dameshek successfully introduced immunosuppressive agents to inhibit lymphocyte proliferation. Since then, organ transplantation and treatment of autoimmune diseases have utilized immunosuppressive agents to regulate the immune response. It has also become clear that DCs can be modulated by these compounds. Besides the two classical immunosuppressive molecules – corticosteroids and 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) – a series of agents has proved to be involved in controlling DCs. Rapamycin, sanglifehrin A, chloroquine, aspirin, cyclosporin A and other compounds inhibit different aspects of DCs, such as differentiation, antigen uptake and/or presentation, migration, maturation and survival. Interestingly, there are naturally occurring immunosuppressive agents, such as 1,25(OH)₂D₃, which are also in the repertoire of DCs under physiological conditions. 25-Hydroxyvitamin D₃-1α-hydroxylase – the enzyme catalysing the reaction that converts the inactive and abundant 25-hydroxyvitamin D₃ to the active 1,25(OH)₂D₃ form – is induced during the differentiation and maturation of DCs. The increasing level of active 1,25(OH)₂D₃ may be involved in controlling the extent of DC activation by a negative-feedback mechanism. Programming DCs by immunosuppressive agents or in other ways may offer alternative therapeutic methods for conditions or diseases connected with activation or dysfunction of T cells, such as transplantation, autoimmune diseases (e.g. psoriasis and rheumatoid arthritis), allergy and resistance to tumours.

These processes can be modelled in vivo. A number of ex-vivo animal experiments have proved the plasticity of DCs in several respects by specifically pushing the cells towards one or another pathway or immune strategy. DCs treated with a vitamin D analogue became tolerogenic and promoted the survival of skin grafts in vivo. In addition, treatment of DCs with oligodeoxyribonucleotides encoding nuclear factor κB (NF-κB)-binding sites was shown to increase the survival of mouse heart allografts, and applying tumour antigens to DCs and reinfusing DCs into animals protected them against tumours or reduced the size of established tumours.

**General features of nuclear receptors**
- The two classical types of immunosuppressive agent, vitamin D and corticosteroids, signal via the vitamin D receptor and glucocorticoid receptor, respectively, which are both members of the nuclear receptor superfamily.

In general, members of the nuclear receptor superfamily (48 in humans) are transcription factors with a conserved primary structure. These receptors are essential in reproduction, metabolic processes, lipid metabolism and cell homeostasis. They form...
dimers and bind directly to specific DNA sequences, thereby activating the transcription of their target genes\textsuperscript{(16)} (Figure 3). Upon ligand binding, the receptors change their conformation, resulting in recruitment of activating transcription factors (Figure 3). This leads to the transcription of target genes being switched on or off\textsuperscript{(17)}.

A key characteristic of the receptors is that their ligands are small, lipid-soluble molecules. The source of the activating ligands may be intracellular or extracellular, involving nuclear receptors in a wide range of signalling pathways. Examples of intracellular ligands include modified fatty acids that activate PPARs and oxysterols that activate the liver X receptor (LXR). These ligands may originate from metabolic processes in the same cell and thus signal locally. Extracellular ligands include the sex steroids, or glucocorticoids; these hormones circulate in the bloodstream, connecting different organs and tissues\textsuperscript{(18)} (Figure 3). Therefore, the receptors of these ligands may be classified as nuclear metabolite receptors or classical endocrine receptors.

Nuclear metabolite receptors were first identified as orphan receptors (not having ligands) and later adopted by the identification of endogenous activators. This group includes PPARs (\(\alpha, \beta/\delta, \gamma\)), liver X receptors \(\alpha\) and \(\beta\) and the bile acid receptor (FXR/BAR)\textsuperscript{(19)}. Interestingly, in most cases, intermediary metabolites (fatty acids, cholesterol or bile acids) have been identified as ligands for these receptors, qualifying them as metabolic sensors. Exploring the roles of these receptors in different biological systems can thus be expected to lead to the identification of novel metabolic signalling mechanisms in (patho)physiological processes. We found that at least 20 receptors of the 48 members of the nuclear receptor superfamily are expressed in human DCs and macrophages at appreciable levels (Figure 4). The glucocorticoid receptor, retinoid acid receptor-\(\alpha\) and retinoid X receptor-\(\alpha\) are expressed constitutively at high levels. PPAR-\(\delta\) and PPAR-\(\gamma\), the vitamin D receptor and the liver X receptors are robustly induced during cell differentiation. We therefore use cell types expressing these receptors as our model system to study the activity of nuclear receptors. We chose PPAR-\(\gamma\) for further analysis because it is only expressed at very low levels in monocytes, the precursors of DCs, and is robustly induced to high levels during the first day of differentiation.

**PPAR-\(\gamma\) plays many roles**

- PPAR-\(\gamma\) was first identified as a regulator of fat-cell – or adipocyte – differentiation and lipid accumulation during adipogenesis\textsuperscript{(20)}. In addition, it plays a well documented role in inflammation by the blocking of NF-\(\kappa\)B and mitogen-activated protein (MAP) kinase pathways in inflammatory macrophages\textsuperscript{(20)}. We found it intriguing that PPAR-\(\gamma\) could influence both processes and sought to determine why one receptor can be important in various, sometimes seemingly disparate, biological processes. To this end, we aimed to identify the components of these pathways and the crosstalk between them, if they existed\textsuperscript{(20,21,23,24)}. We decided to carry out global gene-expression profiling using microarrays to identify PPAR-\(\gamma\)-regulated genes, gene networks and biological processes.

**The role of PPAR-\(\gamma\) in macrophage biology: the lipid connection**

- PPAR-\(\gamma\) is also expressed in the macrophages present in atherosclerotic plaques. In these cells, its activation is at least partially responsible for the accumulation of lipid, leading to the formation of so-called foam cells. These two findings supported the hypothesis that PPAR-\(\gamma\) regulates not only inflammation but also lipid homeostasis in macrophages. A key event in foam-cell formation is the uptake of oxidized lipoproteins via scavenger receptors. Using gene expression and promoter analyses, we showed that PPAR-\(\gamma\) directly regulates the scavenger receptor CD36 and that this regulation is responsible for at least some of the uptake of oxidized low-density lipoprotein (oxLDL)\textsuperscript{(25)}. Further characterization by us and others of PPAR-\(\gamma\)’s activity in macrophages revealed another of its target genes, encoding the liver X receptor-\(\alpha\). This receptor is regulated by oxysterols and, importantly, controls the expression of membrane transporters such as ATP-binding cassette A1 (ABCA1), which is a known contributor to lipid efflux\textsuperscript{(26)}. Two key processes, cholesterol uptake and efflux, are controlled by two nuclear
hormone receptor-mediated pathways (PPAR-γ and liver X receptor). Furthermore, CYP27, a cytochrome P450 enzyme that converts cholesterol into a water-soluble, oxidized form, 27-hydroxycholesterol, was shown by us to be regulated by PPAR-γ. This result provided an additional link between the two receptor systems, the liver X receptor-α and PPAR-γ-mediated pathways. Induction of CYP27 by PPAR-γ leads to the production of 27-hydroxycholesterol, which is an oxysterol metabolite capable of activating LXR[27]. LXR then induces ABCA1 expression and induces cholesterol efflux. This mechanism allows co-ordinate regulation of cholesterol uptake and efflux by external activation of PPAR-γ alone in atherosclerotic-plaque macrophages.

**The role of PPAR-γ in DC biology: the immune-response connection**

- Another antigen-presenting cell type, DCs, has also proved to be a very useful model for the identification of PPAR-γ-regulated processes. PPAR-γ expression was first detected in DCs derived from murine spleen. This finding was further substantiated in both cultured monocyte-derived DCs and in peripheral lymphoid organs, in a subset of S100-positive antigen-presenting cells of human tonsils[28] (Figure 5; S100 is a marker for antigen-presenting cells). Later, high levels of PPAR-γ expression were also found in Langerhans’ cells and in bone-marrow-derived murine DCs[28–30]. We used human monocyte-derived DCs as our model in vitro, in which we induced differentiation by adding granulocyte-macrophage colony-stimulating factor and interleukin-4. In-vitro activation of PPAR-γ in such DCs leads to induction of its target genes, showing that the receptor is not just present but can also be activated during DC differentiation[28–30]. Before embarking on a large-scale analysis of gene expression, we first characterized the functions of control cells and DCs treated with the PPAR-γ ligand.

**Enhanced phagocytosis**

- As antigen uptake is a major function of DCs, we tested the uptake ability of DCs treated with PPAR-γ ligand (rosiglitazone). We analysed two uptake pathways: mannose-receptor-mediated endocytosis by monitoring the internalization of fluorescein isothiocyanate-labelled dextran and phagocytosis by engulfment of latex beads. We and others detected increased phagocytic activity in ligand-treated DCs[28–30]. In immature DCs, a number of surface receptors are involved in antigen uptake. CD36 is known to mediate the uptake of oxidized low-density lipoprotein and is also involved in the phagocytosis of other molecules. Although CD36 is increased upon PPAR-γ activation, detailed analysis of increased phagocytosis and CD36 expression revealed that the increased uptake capacity is independent of CD36 protein expression[28–30]. Therefore, the mechanism of the antigen-uptake phenotype of DCs remains elusive for now.

**Reprogrammed lipid-antigen-presentation capacity and increased iNKT cell activation**

- The most surprising finding in our expression profiling was that the activation of PPAR-γ changed the CD1 profile of the DCs. CD1 molecules of group I (a, b, c, e) and group II (d) are important in lipid-antigen presentation of DCs, forming complexes with glycolipids and presenting them to lymphocytes. CD1d differs in both structure and function from other CD1 molecules by binding and presenting special glycolipids and by being a potent activator of invariant natural killer T (iNKT) cells. iNKT cells are a subset of T lymphocytes that are present at low levels in lymphocyte populations. In animal models, their absence enhances autoimmunity[30]. Hence, iNKT cells are also capable of producing large amounts of cytokines, such as interferon-γ and interleukin-4.
During monocyte-derived DC differentiation, CD1a is highly upregulated, but CD1d is not. When DC differentiation is induced in the presence of a ligand-activated PPAR-γ, the DCs lack the group I CD1s and, moreover, the CD1d level is increased\(^{[28,30]}\). The switch in the expression pattern of CD1 could influence antigen-presentation capacity and/or activation of the immune system towards immune response or tolerance. Further experiments showed that PPAR-γ programming of DCs increased cells’ potency to induce iNKT cell proliferation, causing a significant increase in the number of iNKT cells in the lymph node (Figure 6). This finding proves that increased CD1d levels in DCs regulated by PPAR-γ are functional and can play a central role in the immune-modulatory effects of the activated cells\(^{[28]}\).

The discovery of this pathway has several implications. First, it shows that DCs can alter their gene expression and immune phenotype in very specific ways in response to extracellular stimuli and that they use intracellular hormones or metabolite receptors in doing so. Second, this pathway could also provide an entry point for intervention during physiological or pathological responses by reprogramming DCs. It is particularly relevant in DC-based therapy where ex vivo-differentiated DCs are loaded with tumour antigens and used to initiate anti-tumour immunotherapy. In this case, a higher phagocytic capacity and an increased ability to induce anti-tumour iNKT cells might prove useful. Finally, one may speculate on the biological consequences of increased CD1d expression and iNKT cell activation. For example, in non-obese diabetic (NOD) mice, the expansion and differentiation of autoimmune T cells leads to the destruction of β-cells and the onset of diabetes. This is linked to both the CD1d locus and iNKT cells\(^{[28,33]}\), suggesting that modulation of these could potentially alter the course of the disease.

**Conclusion**

- PPAR-γ appears to be a versatile molecule with many different faces. It is a well-established regulator of adipogenesis, lipid metabolism, and glucose homeostasis. In addition, there is increasing evidence to suggest that it has previously unpredicted roles in immune regulation (Figure 7). It appears that this transcription factor has different, but related, functions in various cell types and under different physiological and pathological conditions. Much work needs to be done to clarify how these two processes – lipid metabolism and inflammation control – are linked via this transcription factor and how it might be used to fight diseases such as chronic inflammation and autoimmunity.

**Acknowledgement**

- L.N. is an International Scholar of the Howard Hughes Medical Institute and holds a Wellcome Trust Senior Research Fellowship in Biomedical Sciences in Central Europe. In 1998 he received the Boehringer Ingelheim Fonds research award for postdoctoral fellows, enabling him to start his own research group in Debrecen, Hungary.

**References**


![FIG. 6: The role of PPAR-γ in dendritic cell (DC) function. DCs activated by PPAR-γ ligand (rosiglitazone; RSG), if cocultured with lymphocytes, induce the proliferation of iNKT cells, characterized by the expression of vβ11 and vα24.](image6)

![FIG. 7: Summary of the different, but overlapping roles of PPARγ, from lipid metabolism to immunomodulation and inflammation.](image7)