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Review

PPARγ – an Important Regulator of Monocyte/Macrophage Function

ANDREAS VON KNETHEN* and BERNHARD BRÜNE

Department of Cell Biology, University of Kaiserslautern, Kaiserslautern, Germany

Abstract. Regulation of monocyte/macrophage function is important in coordinating immune responses. Their contact with invading pathogens activates signaling pathways that provoke pro-inflammatory gene expression and thus causing a locally restricted inflammation. Recently, the peroxisome proliferator-activated receptor γ (PPARγ) has been identified to antagonize pro-inflammatory responses in monocytes/macrophages, causing an anti-inflammatory and/or desensitized phenotype to predominate. For PPARγ, the general mechanisms facilitating the transition from a pro- to an anti-inflammatory phenotype have been elucidated. PPARγ is a member of the nuclear receptor superfamily and is activated upon endogenous as well as exogenous agonist binding. Here we focus on its role in monocyte/macrophage biology in affecting inflammation. Summarizing current information, a model is proposed which gives rise to potential new therapeutic possibilities for the treatment of diseases presumably involving PPARγ-dependent regulatory circuits.

Key words: inflammation; monocytes/macrophages.

Monocytes/Macrophages – Important Mediators of the Innate Immunity

Monocytes/macrophages play a pivotal role in the primary phase of immune responses and predominate in regulating innate immunity[31, 41, 68, 74]. Upon the first contact with invading pathogens, macrophages respond by producing several bactericidal products, such as superoxide or nitric oxide (NO)[27, 56]. Superoxide radicals are produced upon activation of the multi-enzyme complex NADPH oxidase, which exists preformed in resting monocytes/macrophages linked to cellular vesicles and/or the plasma-membrane[7]. Superoxide radicals are generated either as a direct response to achieve killing of the triggering pathogen, i.e. microorganisms, or used indirectly to activate distinct signaling pathways that provoke expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β. In addition, macrophage activation is often associated with NO generation, which is observed in close association with inducible nitric oxide synthase (iNOS) expression[33]. Radical mediators, cytokines and chemokines are released from activated macrophages and consequently affect neighboring cells, triggering a local inflammatory reaction[49]. In parallel, phagocytosis of pathogens is initiated in order to limit aggravation of the inflammatory response[2, 3]. It is well established that monocytes/macrophages function as antigen-presenting cells (APC), delivering small peptides of the pathogen to be exposed by major histocompatibility complex class II (MHC-II) molecules on their surface. In turn, MHC-II molecules link the innate to

* Correspondence to: Andreas von Knethen, Ph.D., Department of Cell Biology, University of Kaiserslautern, Building 13/R 420A, Erwin-Schroedinger-Strasse, 67663 Kaiserslautern, Germany, tel.: +49 631 205 2885, fax: +49 631 205 2492, e-mail: aknethen@rhrk.uni-kl.de

the adaptive immune response conferred by lymphocytes[37]. T lymphocytes sense the MHC-II-presented antigen, followed by their own activation, which concomitantly accounts for the release of B lymphocyte-activating signals such as IL-4[53, 58]. Therefore, appropriate regulation of monocyte/macrophage activation/activity is of utmost importance for the immune system. Taking the fundamental role of monocyte/macrophage activation into consideration, the identification of factors or mediators that limit monocyte/macrophage activation has raised considerable interest[49]. Acknowledged anti-inflammatory mediators known to be expressed in monocytes/macrophages are transforming growth factor β (TGF-β) and IL-10. These factors have been described to modulate the profile of activating cytokines, to suppress NO production and to attenuate the respiratory burst[19, 25, 54, 66]. Recent evidence has highlighted the role of peroxisome proliferator-activated receptor γ (PPARγ) as an important new regulator of monocyte/macrophage activity.

Genomic Organization and Protein Structure of PPARγ

PPARγ is a ligand-activated transcription factor and belongs to the nuclear hormone receptor superfamily. Its original identification as a crucial factor in adipogenesis and glucose metabolism[73, 86] explains its prominent expression in adipose tissue[12]. Employing different promoters and alternative splicing, four splice variants have been acknowledged in humans[22, 23, 69, 78] giving rise to two different proteins[57]. The promoter structure of the human PPARγ gene is shown in Fig. 1. The gene is located on chromosome 3p25[32], spanning approximately 100 kb and consisting of 9 exons (A1, A2, B as well as exons 1–6)[22]. In the human system, PPARγ1, 3 and 4 encode the same protein, whereas PPARγ2 is translated into a protein which contains 28 additional amino acids at its N-terminus[21]. Exons 1 to 6 are expressed in all four isoforms. PPARγ1 contains the non-translated exons A1 and A2. PPARγ2 contains exon B[22], which in part is translated from an internal start codon, orientated in frame, thus coding for the 28 extra amino acids. PPARγ3 embeds the untranslated exon A2[23], and PPARγ4 contains exons 1 to 6 only[78]. The existence of four possible isoforms implies a highly controlled tissue-specific expression pattern. Along that line, PPARγ1 is ubiquitously expressed[57], PPARγ2 is confined to adipose tissue[67, 72], PPARγ3 seems to be restricted to macrophages, T lymphocytes, adipose tissue and colon[23, 79], whereas no data on the tissue distribution of PPARγ4 are available yet[78]. One has to keep in mind that PPARγ expression in monocytes/macrophages is predominantly initiated from the PPARγ1 and 3 promoter[69], while the protein is PPARγ1, which comprises 477 amino acids[21].

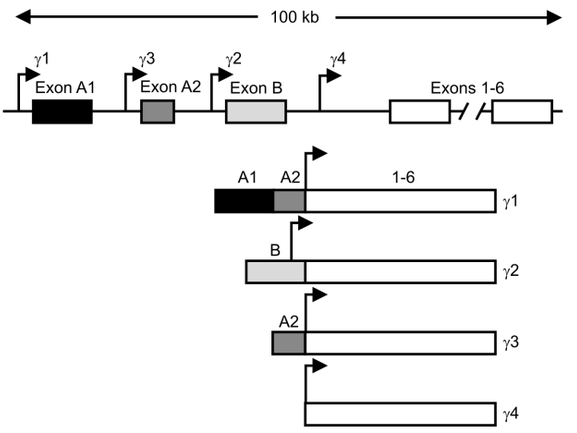

Fig. 1. Organization of the human *PPARγ* gene. For details see text

Functionally, the protein can be divided into four domains, which are schematically represented in Fig. 2. Human PPARγ1 exhibits a domain structure known for other members of the nuclear hormone receptor superfamily[6]. Its structure includes an N-terminal ligand-independent activation domain (A/B), termed activation function 1 (AF1)[85]. AF1 contains a serine residue in position 84 (S84) which has been identified as a potential phosphorylation site, leading to inhibition of PPARγ transactivation[1, 8, 34, 39] and in part negatively affecting ligand binding[75]. Following AF1, a DNA-binding domain (C) is recognized which consists of two zinc finger motifs responsible for interacting with the promoter region of responsive target genes[61]. Next, there is a hinge domain (D), which ensures flexibility of the protein with respect to the position of DNA- and ligand-binding domains. At the C-terminus there is an E/F region, which includes the ligand-binding as well as the heterodimerization domain. The carboxy terminus is also designated activation function 2 (AF2) and comprises the ligand-dependent transactivation domain.

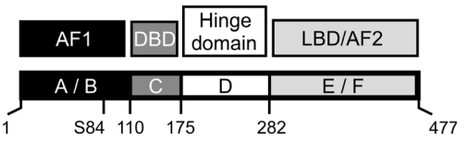

Fig. 2. Four-domain structure of human PPARγ1. AF1 – ligand-independent activation function 1, DBD – DNA-binding domain, LBD – ligand-binding domain, AF2 – ligand-dependent activation function 2, S84 – serine in position 84. For details see text

The overall PPARγ1 structure has been determined by X-ray crystallography[61].

Mechanisms of PPARγ Action

During activation, PPARγ binds an agonist and subsequently forms a heterodimer with the retinoic X receptor (RXR)[16]. Thereafter, binding to its recognition site, known as the peroxisome proliferator response element (PPRE), in the promoter region of responsive genes such as CD36 follows, which in turn initiates transcription[84]. Macrophages derived from mice with a conditional disrupted PPARγ gene as well as cells obtained by differentiation of PPARγ double-negative embryonic stem cells (PPARγ$^{-/-}$) confirmed the importance of PPARγ in CD36 expression[4, 10]. Apart from its impact during transcriptional regulation, which requires DNA-binding, PPARγ can also function in a DNA-binding-independent manner to trans-repress different target genes. Thus, besides gene activation PPARγ can attenuate gene expression, an activity of major importance for blocking pro-inflammatory cytokine gene expression. To date three potential mechanisms for PPARγ in attenuating gene expression and thus blocking a pro-inflammatory macrophage response have been predicted and in part experimentally proven.

First, ligand activated PPARγ-RXR heterodimers may bind coactivators which are required for gene induction by other transcription factors responsible for expressing a set of pro-inflammatory genes[38]. Without distinct cofactors, transcription factors, such as AP-1, nuclear factor κB (NF-κB), nuclear factor of activated T cells (NF-AT) or signal transducer and activator of transcription (STAT), cannot cause gene expression which, however, is characteristic for an inflammatory process. *In vitro* studies, using a mammalian two-hybrid system as well as GST pull-down assays, revealed a ligand type-specific direct interaction of PPARγ with several transcriptional coactivators, such as SRC-1, TIF2, AIB-1, CBP, p300, TRAP220, or DRIP205[46]. In lipopolysaccharide/interferon γ (LPS/IFN-γ)-treated macrophages, iNOS expression is attenuated in response to PPARγ activation. This is explained by targeting the two general co-activators CREB-binding protein (CBP) and p300 by PPARγ[52]. Binding of CBP and p300 to PPARγ has been localized to the D/E/F region, but also in a ligand-independent manner to its A/B domain structure[30].

Second, PPARγ-RXR complexes may directly bind to transcription factors to cause a functional inhibition. This type of physical interaction has been noticed for *in vitro* translated proteins of the NF-κB family, such as p50 and p65[15]. Moreover, NF-AT precipitation experiments revealed a direct contact with PPARγ in T cells, which accounts for suppression of T cell proliferation and activation by NF-AT sequestration[87].

The third mechanism which results in trans-repression may involve regulation of the mitogen-activated protein kinase (MAPK) cascade. PPARγ-RXR heterodimers may inhibit phosphorylation and activation of several MAPK family members. In PPARγ$^{+/-}$-heterozygote mice, activation of both c-Jun-N-terminal kinase (JNK) and p38 in response to 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis was significantly reduced compared with wild-type littermates[18].

PPARγ Modulation of Monocyte/Macrophage Activity during Inflammation

PPARγ shows low expression in monocytes, but is strongly induced during their differentiation to mature macrophages[14, 80]. Induction of PPARγ in monocytic THP1 cells as well as primary macrophages was established under various experimental conditions by 9-cis--retinoic acid-, phorbolester-, oxidized low-density lipoprotein (oxLDL)-, or macrophage colony-stimulating factor-(MCSF)-treatment[69, 80, 88]. The role of PPARγ in controlling monocyte/macrophage activation was first described in 1998 by two independent groups[43, 70]. Initially, it was shown that activation of PPARγ in response to synthetic oral antidiabetic agonists, known as thiazolidinediones[29], as well as several naturally occurring agents, such as fatty acids or the prostaglandin D_2 metabolite 15deoxy-$\Delta^{10, 14}$ prostaglandin J_2 (15d--PGJ_2)[11, 62], attenuated expression of pro-inflammatory cytokines. This concept has more recently been challenged by using macrophages differentiated from PPARγ$^{-/-}$ to show that PPARγ agonists attenuated pro--inflammatory cytokine production in these cells as well[10]. However, selected agonist concentrations have been very high, which may offer an explaination of these contradictory results by specific versus non-specific drug effects.

PPARγ is not only involved in down-regulation of pro-inflammatory cytokine expression, but also confers monocyte/macrophage desensitization. We have shown that stimulation of monocytes/macrophages with a combination of LPS, a component of the cell wall of Gram-negative bacteria, and IFN-γ, a cytokine released e.g. by T cells[71], elicits desensitization. Macrophage inactivation is characterized by an attenuated oxidative burst in response to the phorbolester, a known NADPH oxidase activator[82]. The role of PPARγ in causing

LPS/IFN-γ-evoked macrophage desensitization has been demonstrated by using specific PPARγ agonists such as ciglitazone. To definitely exclude a PPARγ-independent effect, we performed a PPRE-decoy approach to verify the impact of PPARγ in mediating macrophage desensitization. In order to link reduced superoxide formation with possible genes mediating this effect, $p47^{phox}$, a component of the NADPH oxidase system was analyzed[83]. We hypothesized that any inference with the expression of NADPH oxidase components such as $p47^{phox}$ may eradicate superoxide formation[7]. Experiments have shown that mRNA of $p47^{phox}$ decreased under the impact of PPARγ agonists. Therefore, a mechanism of transcriptional regulation of $p47^{phox}$ may account for PPARγ-dependent macrophage deactivation. Interestingly, in this study NO, endogenously generated by iNOS or exogenously delivered by NO-donors, was able to activate PPARγ and to desensitize macrophages as described[83].

The expression of PPARγ in mature macrophages and the formation of endogenous PPARγ ligands such as lipid mediators or NO in pro-inflammatory activated cells implies a regulatory role of PPARγ during a late stage of inflammation. The formation of physiological PPARγ agonists, such as 15d-PGJ_2 and 9- or 13-hydroxyoctadienoic acid as well as NO, may initiate a negative autocrine or paracrine feedback-loop via PPARγ activation followed by macrophage desensitization[28, 40]. It can be proposed that an anti-inflammatory cellular phenotype is initiated by attenuating pro-inflammatory gene expression and/or provoking expression of anti-inflammatory cytokines, exemplified by TGF-β. This cytokine is expressed at a low level in unstimulated monocytes/macrophages, but is strongly upregulated in activated, i.e. LPS-treated cells and known to inhibit monocyte/macrophage activation. Interestingly, recent studies have provided evidence for TGF-β-induced PPARγ expression which gives rise to an autocrine feedback loop promoting anti-inflammatory properties of TGF-β via PPARγ[17, 45, 50]. The interplay of these signaling cascades may boost an autocrine feedback loop to limit activation of macrophages.

Mechanisms of macrophage desensitization coincide with some pathophysiological aspects associated with sepsis. Although monocyte/macrophage expression of pro-inflammatory cytokines in response to invading pathogens is important for the efficient control of their dissemination, overproduction of pro-inflammatory cytokines is harmful for the host, causing the serious disease pattern of septic shock, accompanied by multi-organ dysfunction syndrome, frequently with lethal outcome[63]. It has been shown that in a significant subgroup of septic shock survivors, monocytes/macrophages show a deactivated phenotype, provoking an inadequate answer to secondary infections concomitantly elevating the risk of delayed mortality[9, 20, 44]. Of note, IFN-γ can at least in part antagonize this state of macrophage un-responsiveness[20]. Mechanistically, IFN-γ may target PPARγ for ubiquitin-proteasome degradation by increasing extracellular signal-regulates kinase-mediated serine phosphorylation of PPARγ, which limits its synthesis and/or increases its degradation[5, 26, 36].

PPARγ and Transplantation

It is well known that monocytes/macrophages function as APCs, thus connecting the innate with the adaptive immune system. Foreign antigens are presented by MHC-II molecules on the cell surface, causing $CD4^+$ T cell activation and concomitantly initiating the acquired immune response[81]. Therefore, tightly regulated MHC-II molecule expression is very important to guarantee an appropriate immune reaction[64]. In macrophages, MHC-II expression is increased in the presence of pro-inflammatory cytokines such as IFN-γ by the induction of the class II transcriptional activator[77]. Recently, it has been shown that ligands for PPARγ attenuate induction of MHC-II molecule expression in response to IFN-γ treatment as well as their constitutive expression in monocytic THP1 cells and primary macrophages[48]. These data indicate a possible mechanism of PPARγ in negatively affecting antigen presentation of monocytes/macrophages and, thereby, attenuating associated T cell activation. Therefore, the knowledge of molecular mechanisms regulating MHC-II expression in monocytes/macrophages will open new possibilities to prevent rejection after organ transplantation by interfering with the onset of adaptive immunity.

PPARγ and Arteriosclerosis

PPARγ-dependent desensitization of macrophages may additionally account for problems associated with morbidity as a result of oxLDL exposition. Macrophages not only account for innate immune responses, but also scavenge oxLDL, thereby transforming into foam cells, an ongoing process during development of arteriosclerosis[42, 55, 60]. In the body, oxLDL is generated as a result of cell-mediated LDL oxidation[47]. Oxidized LDL is a well-established regulator of cellular signaling pathways, inducing among others the expression of adhesion molecules in endothelial cells or pro-inflam-

matory cytokines and growth factors in vessels[13]. Circulating monocytes are chemotactically attracted by oxLDL. This results in accumulation of monocytes in the vessel wall followed by monocyte differentiation into lipid-laden foam cells. Foam cells exhibit an anti-inflammatory phenotype, thus resembling a desensitized cell. Therefore, foam cells are a major determinant of arteriosclerosis[51]. In cell culture, a short-time exposure of monocytes/macrophages to oxLDL produces superoxide radicals, whereas a long-lasting supplementation with non-toxic doses of oxLDL attenuates the oxidative burst in macrophages. Considering the ability of oxLDL to attenuate the oxidative burst in macrophages and taking into account that oxLDL is a pathophysiological but natural PPARγ agonist[15, 35, 59, 80], one may conclude that the nuclear hormone receptor directly participates in desensitizing macrophages upon long-lasting oxLDL exposure[24].

Perspectives

The fundamental role of PPARγ in regulating monocyte/macrophage activity is complex. A proposed scheme of action is given in Fig. 3, summarizing essential considerations of how PPARγ affects monocyte/macrophage biology. Understanding PPARγ-dependent signalling mechanisms, i.e. inhibition of pro-inflammatory cytokine production and desensitization of macrophages, will hopefully open new avenues for therapeutical approaches to using PPARγ agonists instead of glucocorticoids for treatment of chronic inflammatory diseases[65]. Moreover, repression of MHC-II gene expression might attenuate antigen presentation of monoctyes/macrophages and concomitantly interfere with initiation of adaptive immunity. Therefore, PPARγ may serve as an alternative to well-established immunosuppressive drugs such as cyclosporin A and tacrolimus, known to cause severe side effects[76].

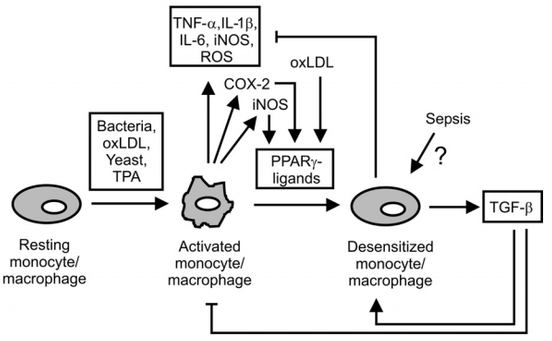

Fig. 3. Anti-inflammatory and/or desensitizing effect of PPARγ in monocytes/macrophages. Resting monocytes/macrophages are activated in response to invading pathogens, natural ligands such as oxLDL, or synthetic agonists (TPA). This initiates the expression of pro-inflammatory genes (TNF-α, IL-1β, IL-6, iNOS, ROS, COX-2), leading to pro-inflammatory cytokine synthesis, superoxide and NO generation as well as prostaglandin production. These mediators act in an autocrine and/or paracrine fashion provoking a local inflammatory situation. In turn, factors such as PPARγ and TGF-β known to attenuate inflammation are produced/activated, thus allowing an anti-inflammatory response to predominate. This reaction might be enhanced by TGF-β in a negative feedback loop, possibly involving a PPARγ-dependent mechanism, causing an anti-inflammatory, desensitized monocyte/macrophage phenotype. For details see text

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