Roszer, T., Menendez-Gutierrez, M. P., Cedenilla, M., & Ricote, M. (2013). Retinoid X receptors in macrophage biology. *Trends in Endocrinology and Metabolism, 24*(9), 460-468. doi:10.1016/j.tem.2013.04.004

Retinoid X receptors in macrophage biology

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Number of pages: 29

With 2 figures, 1 table and 2 text boxes

Abstract

Retinoid X receptors (RXRs) form a distinct and unique sub-class within the nuclear receptor superfamily of ligand-dependent transcription factors. RXRs regulate a plethora of genetic programs, including cell differentiation, immune response, and lipid and glucose metabolism. Recent advances reveal that RXRs are important regulators of macrophages, key players in inflammatory and metabolic disorders. This review outlines the versatility of RXR action in the control of macrophage gene transcription through its heterodimerization with other nuclear receptors or through RXR homodimerization. We also highlight the potential of RXR-controlled transcriptional programs as targets for the treatment of pathologies associated with altered macrophage function, such as atherosclerosis, insulin resistance, autoimmunity and neurodegeneration.

Keywords: nuclear receptors – gene transcription – macrophage – inflammation – lipid metabolism

One for all: RXRs control transcription with multiple partners

Retinoid X receptors (RXRs) are members of the nuclear receptor (NR) superfamily of ligand-dependent transcription factors [1, 2]. Three RXR isotypes exist in mammals encoded by distinct genes: RXRα (NR2B1), RXRβ (NR2B2), and RXRγ (NR2B3). Each isotype exists in several isoforms, which have specific tissue distributions and expression patterns during development [1, 2]. RXRs are highly conserved NRs, with RXR homologs identified in species from a wide range of invertebrate phyla [3] (Text Box 1).

RXRs are master regulators of gene expression and play a unique modulatory and integrative role across multiple functions through their ability to form obligate heterodimers with many other NRs (Figure 1). RXRs additionally regulate gene expression as homodimers [1, 4, 5], and even homotetramers [1, 6], generating an as yet little-explored complexity of RXR-dependent gene regulation. This versatility permits RXRs to exert pleiotropic transcriptional control over a wide range of genetic programs, including cell differentiation, immune response, and lipid and glucose metabolism [2]. Transcriptional regulation by RXRs is a complex and flexible mechanism, determined by three levels of regulation: RXR heterodimer- or homodimer-specific hormone response elements (HREs) (Table 1, Figure 1); the availability of the ligands for RXRs and their heterodimeric partners (Table 1, Figure 1); and the dynamics and recruitment of coregulator complexes [1, 2].

RXR heterodimers have been classically classified as permissive or non permissive (Figure 1). Permissive heterodimers are formed with peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), pregnane X receptor (PXR), farnesoid X receptor (FXR), Nurr1 and Nur77, and the complex can be activated by either an RXR agonist or an agonist for the heterodimeric partner. Binding by agonists for both partners could have additive or synergistic effects. Non-permissive heterodimers are formed with partners such as retinoic acid receptors (RARs), vitamin D receptor (VDR) and thyroid receptors (TRs). Unlike permissive heterodimers, non-permissive heterodimers are normally activated only by ligands specific for the partner, with the RXR acting as a "silent" partner [2]. However, several exceptions to the standard permissivity definition have been described [2]. For instance, in the case of RXR/RAR heterodimers, although RXR ligands alone cannot activate the heterodimer, binding of RAR ligands allows subsequent binding of the RXR ligand, which enhances the transcriptional potential of the RAR ligand. In addition, in hererodimers with TRs or VDR, RXRs do not always act as silent partners, and the activity of the heterodimer may depend on factors such as tissue specificity, the cellular environment, or the ability of various RXR ligands to recruit coactivator or corepressor complexes [2]. These types of RXR heterodimers have recently been termed conditional permissive heterodimers [2] (Table 1, Glossary).

RXRs are targets for drug discovery

The first identified natural RXR ligand was the vitamin-A derivative retinoid 9-cis retinoic acid (9cRA) [7], whose status as an endogenous agonist is still debated [2, 8]. Some fatty acids are also ligands for RXRs, such as docosahexaenoic acid (DHA), oleic acid, and phytanic acid [2]. Several RXR-specific synthetic ligands, called rexinoids, have also been generated [8]. One rexinoid, bexarotene, is a pan-RXR agonist already used in cancer therapy [9], and others are being tested in preclinical settings to treat insulin resistance and atherosclerosis [2]. However, treatment with rexinoids raises plasma triglyceride levels, suppresses the thyroid hormone axis and induces hepatomegaly [8]. The current challenge in drug discovery is to obtain and characterize selective RXR modulators (SRXRMs), to achieve the desired pharmacological effects of rexinoids without unwanted side effects [1, 8, 10]. SRXRMs include heterodimer- and homodimer-specific RXR agonists and antagonists, compounds that activate only a subset of the functions induced by the pan-RXR agonists or act in a cell-type specific manner [8]. Some of these SRXRMs have already been characterized [8]. For instance LG101506 selectively activates RXR/PPARγ and RXR/PPARα, antagonizes RXR/RAR signaling, and retains the desired anti-diabetic activities of pan-RXR agonists without suppressing thyroid signaling [8]. Similar anti-diabetic effects of LG100754 have been shown: this compound exhibits antagonistic activities toward RXR homodimers, but acts as an agonist for selective RXR heterodimers [1, 8, 10].

Macrophages express RXRs and RXR-partner nuclear receptors

Macrophages are effector cells of the innate immune system with primary roles in host defence against pathogens, clearance of cell debris, tissue remodeling following injury, and integration of tissue lipid metabolism [11, 12] (Text Box 2). Prolonged activation or pathological retention of macrophages in tissues can create an inflammatory microenvironment, which in turn contributes to diseases such as atherosclerosis [13], insulin resistance [12], and neurodegeneration [14].

Several recent studies point out the potential of direct modulation of RXR signaling in the treatment of macrophage-related pathologies [1, 8]. However, the role of RXRs in the regulation of macrophage functions has not been established, beyond their role as obligatory heterodimerization partners for other NRs.

RXRα is highly expressed in all human and rodent macrophage-type cells analyzed to date, whereas RXRβ is expressed at lower levels in many macrophage types, and RXRγ is not expressed. Of the 49 NRs found in rodents, 29 are expressed in macrophages [5, 14-19] and of those about 15 dimerize with RXRs [1] (Figure 1, Table 1). The function of RXRs in macrophage biology has been investigated *in vitro* and in *in vivo* studies using myeloid- [4, 20] and hematopoietic-cell-specific RXRα knockout mouse models [21].

In this review we discuss the importance of RXRs in monocyte/macrophage differentiation and macrophage specific functions, beyond their subordinate role as heterodimeric partners for other NRs. A better understanding of RXR function as a homodimer and the design of more intelligent heterodimer- or homodimer-specific modulators will offer great therapeutic potential for a variety of inflammatory diseases.

RXRs in monocyte/macrophage differentiation

Differentiation of myeloid precursors into monocytes and eventually macrophages, and the subsequent proliferation and survival of tissue macrophages, are important determinants of macrophage function. Any imbalance in these processes leads to pathological conditions, such as myeloid leukemia or atherosclerosis [22]. Although several recent studies suggest that RXRs have a role in myeloid development, they focused mostly on their partners, RARs, PPARγ, VDR, and more recently Nur77 [23].

RXRs are important players during physiological and pathological hematopoiesis

The importance of RXRs in myeloid progenitor cell fate has recently been established. RXRα down-regulation is needed for terminal neutrophil differentiation from human myeloid progenitors [24]. However, studies in mice with conditional deletion of RXRα in HSCs demonstrated that lack of RXRα was not sufficient to alter hematopoiesis [21], thus suggesting a compensatory role for RXRβ in this model. Supporting this, expression of a dominant negative form of RXRβ in myeloid cells blocked differentiation, indicating that RXRs are crucial during physiological myelopoiesis *in vivo* [24]. In addition, RXRα might be involved in the pathogenesis of myelodisplastic syndromes (MDS), since loss of functional RXRα in transgenic mouse models of myeloid leukemia impeded the development of the disease [24]. Moreover, the RXR pathway might be dysregulated in patients with advanced MDS, since several RXR target genes that are critical for maintaining a balance between self-renewal and differentiation of HSCs are differentially expressed in normal bone marrow and marrow from MDS patients [25]. Collectively, these novel results shed light on the role of RXRs in the pathogenesis of myelodisplastic diseases, and point to RXRs as potential targets for the management and treatment of myeloid leukemia.

RXRs control hematopoietic self-renewal, differentiation and apoptosis

Pharmacological studies using RXR ligands confirm the important role of RXRs in myeloid cell development at different stages of maturation. In the most primitive cells, RXRs control HSC self-renewal and differentiation through their heterodimerization with RARs and PPARγ. Indeed, allosteric inhibition of RARs by the inverse RXR/RAR agonist LG101506 sustains self-renewal capacity of human HSCs *in vitro* [26]. In contrast, activation of the permissive RXR/PPARγ heterodimers with a PPARγ agonist promoted myeloid differentiation as opposed to HSC self-renewal [26].

The role of RXRs in more differentiated cells is intriguing. In human and mouse leukemia cell lines, activation of RXRs by 9cRA inhibits clonal expansion, and induces apoptosis or differentiation toward the neutrophil lineage [23, 24]. These effects are mainly dependent on RXR/RARα heterodimers, although RAR-independent roles of RXRs have been described in some human myeloid leukemia cell lines [23, 24]. However, RXR activation induces differentiation of human leukemia cell lines into functional monocytes, through its heterodimerization with VDR [23, 27] and PPARγ [28]. RXRs might be also involved in the differentiation of mature macrophages from monocytes [29]. However, their role in this process is unclear; although RXRα expression increases during differentiation of human blood monocytes into macrophages [29], 9cRA and the rexinoid SR11237 block the differentiation of a human monocyte cell line into macrophages [30].

All these findings suggest that RXRs play a complex role in hematopoiesis, having pleiotropic effects depending on the hematopoietic target cell and the heterodimeric partners expressed in those cells. The use of SRXRMs would allow the activation of specific pathways leading to self-renewal, cellular differentiation, or cell death, which could improve the treatment of pathologies such as MDS or atherosclerosis.

Macrophage RXRs in inflammation and the immune response

NRs have been shown to regulate the immune response [31], and recent progress in the field points out the importance of RXRs in the control of macrophage immune phenotype [4, 20]. To date, PPARs and LXRs are the most extensively studied RXR partners in the context of macrophage immune functions [32], although more recently TR, RARs, VDR, PXR, FXR, Nurr1 and Nur77 have also been identified as regulators of macrophage activation [18, 19, 33-36]. Recent findings suggest that a separate RXR homodimer signaling pathway may also affect macrophage immune functions, specifically in the innate immune response [4].

Understanding RXR function in macrophages has been significantly advanced by the recent generation of macrophage RXRα-deficient mice [4, 20, 21]. Studies using this mouse model highlight the involvement of macrophage RXRs in self-immunity and the innate inflammatory response [4, 20].

Macrophage RXRs control the clearence of apoptotic cells and β-amyloid protein

Mice lacking macrophage RXRα develop an autoimmune renal disease resembling human lupus nephritis [20]. This autoimmune phenotype develops as a consequence of impaired uptake of apoptotic cells by RXRα-deficient macrophages. Deficient clearance of apoptotic cells exacerbates an autoimmune response against dying cells, and also disables the proper anti-inflammatory activation of macrophages. A similar immune phenotype has been observed in mice lacking macrophage PPARγ, PPARδ or LXRs [20, 37, 38]. The lack of RXRα impairs the transcription of genes encoding several phagocytosis-related factors, including cell surface receptors (*Cd36*, *Fcgr1*, *Mertk*, *Axl*), opsonins (*C1qa*, *C1qb*, *C1qc*) and transglutaminase-2 (*Tgm2*), which are required for particle binding and engulfment, consequently leading to a phagocytosis deficit [20]. Accordingly, 9cRA increases phagocytosis, and 9cRA and the synthetic RXR agonist LG100268 both induce the transcription of phagocytosis-related genes in wild-type but not in RXRα-deficient mouse macrophages *in vitro* [20]. RXRα controls the transcription of these genes in partnership with PPARγ, PPARδ, LXRs and RARs, as indicated by the use of specific ligands and by the identification of HREs in the promoters of these genes [37-40]. These findings show that macrophage RXRs are important constituents of immunological self-tolerance through their promotion of apoptotic cell uptake and anti-inflammatory macrophage activation. In addition, recent studies showed that bexarotene increases the clearance of β-amyloid by microglia and mitigates inflammation in a mouse model of Alzheimer's disease (AD) [41]. Similar effects are obtained by the use of LXR- and VDR-specific ligands, suggesting that RXR/LXR or RXR/VDR heterodimers might promote the capacity of macrophages to maintain phagocytosis [42, 43] (Figure 2).

Macrophage RXRs in leukocyte migration and inflammation

Another important role of RXRs in the control of macrophage immune functions is the regulation of chemokine expression, which controls leukocyte migration to inflammatory sites [4] (Figure 2). Lack of macrophage RXRα compromises the transcription of *Ccl6* and *Ccl9* chemokine genes and impairs recruitment of leukocytes to sites of inflammation. This phenotype is associated with prolonged survival in mouse models of sepsis [4]. Accordingly, 9cRA and LG100268 induce *Ccl6* and *Ccl9* expression in mouse macrophages and thus increase their chemoattractant potential *in vitro* [4]. Interestingly, the *Ccl6* and *Ccl9* expression induced by the RXR agonists can be inhibited by the selective RXR homodimer antagonist LG100754, indicating that these genes are targets of RXR homodimers [4]. This study highlights that RXRα can control gene transcription in macrophages independently of heterodimeric partners [4]. However, further studies are needed to define the *in vivo* existence and relevance of RXR homodimer mediated signaling [4].

RXRs can also control the transcription of other chemokines, such as MCP-1 in a human monocytic leukemia cell line *in vitro* [19], and in activated microglia *in vivo* [44].

RXRs and macrophage response to pathogens

Pathogen-induced macrophage responses are also affected by RXRs through their heterodimerization with LXRs. Some cellular pathogens induce macrophage apoptosis, and RXR activation can counteract this process. For example, treatment of mouse macrophages with 9cRA or LXR-specific ligands upregulates the anti-apoptotic genes *AIM/Spalpha*, *bcl-x$_L$*, and *Birc1a* [19], and inhibits the expression of pro-apoptotic factors, including caspases 1, 4/11, 7 and 12, Fas ligand, and Dnase1l3 [19]. Ligands of VDR, TRs and RARs also restrict the survival of pathogens within the macrophage phagosome [33, 36], although the role of RXRs in the underlying mechanisms is uncertain (Figure 2).

Potential clinical relevance of macrophage RXRs in immunomodulation

Clinically important beneficial effects of RXR agonists have been shown in animal models of chronic inflammatory diseases, such as insulin-resistant diabetes, atherosclerosis and neurodenegerative diseases [19]. However, the anti-inflammatory effect of RXR ligands on macrophages are still incompletely understood. 9cRA reduces the inflammatory activation of microglia, suggesting a potential medical benefit of RXR activation in neuroinflammatory disorders such as AD or multiple sclerosis [40, 45]. Similar findings with agonists for PPARα [40], LXRs [43, 45, 46] and VDR [42] suggest that RXRs can act through these NR partners in this process. Similarly, the RXR agonist Ro47-5944 inhibits the trancription of inflammatory genes in rat Kupffer cells and in the RAW264.7 mouse macrophage cell line *in vitro* [47]. However, the inflammatory phenotype of foam cells is not affected by bexarotene *in vivo*, despite the fact that RXR activation can reduce atherosclerosis in mice [1]. The anti-inflammatory role of the RXR partners PPARs and LXRs have been extensively documented under various inflammatory conditions, suggesting that these NRs can mediate the anti-inflammatory effects of RXR ligands [19]. The role of other RXR partners has recently been addressed. For example, VDR and PXR activation reduces the expression of inflammatory mediators in activated macrophages [18, 19, 48]. Similarly, FXR is also implicated in the inhibition of the inflammatory phenotype of intestinal macrophages in a mouse colitis model [17]. In activated mouse microglia, Nurr1 can reduce the inflammatory phenotype and protect against loss of neurons [14]. Conflicting results have been reported for the role of Nur77 in macrophage activation [35], indicating that increased Nur77 expression can either increase or reduce inflammatory gene transcription in mouse macrophages [49, 50]. A role of Nur77 has been described in mouse models of chronic inflammatory diseases. However, there is as yet no consensus on its role [50-52].

These findings highlight the importance of RXRs in the control of macrophage-related immune functions. Future studies will need to address the utility of selective RXR ligands to modulate these functions and treat inflammatory disorders.

RXRs in macrophage lipid metabolism

Macrophages are important regulators of lipid metabolism under both homeostatic and pathological conditions [53]. Macrophage lipid-handling mechanisms involve lipid uptake and storage in lipid droplets, β-oxidation, and cholesterol efflux [54]. A precise balance between these processes is crucial for the maintenance of cellular lipid homeostasis and prevention of disease. Several NRs, including RXRs, have been proposed to control macrophage lipid homeostasis by regulating the expression of gene networks involved in lipid metabolism, transport, storage and elimination. The best known of these are PPARs and LXRs [19, 32]. However, recent studies point to other RXR heterodimer partners, PXR, FXR, RARs, Nurr1 and Nur77, as key players in these processes. The importance of RXRs in macrophage lipid metabolism has been addressed by a clinical study in patients with advanced carotid atherosclerotic lesions. In this study, low macrophage expression of RXRα in these lesions was associated with more pronounced disease progression [55]. Activation of RXRs by specific ligands such as bexarotene might have potential in the treatment of atherosclerosis [56] and also in other disorders in which macrophage lipid handling is important, such [57] obesity-associated insulin resistance and metabolic syndrome [12], and neurodegenerative diseases [58].

RXRs control macrophage cholesterol uptake, efflux and storage

The mechanism underlying the regulatory effects of RXR activation on macrophage lipid metabolism involves the modulation of scavenger receptors, which mediate uptake of modified lipoproteins. 9cRA and the rexinoids PA024 and HX630 upregulate the expression of *CD36* in human macrophage cell lines or mouse peritoneal macrophages [20, 59]. However, activation of RXRs with these ligands also decreases the activity of another scavenger receptor (SRA-I/II) [53], and downregulates the expression of the receptor for ApoB48 [60], the overall effect being a reduction in lipid accumulation and storage. Similar findings have been obtained in *in vitro* studies with PPARγ and PPARα agonists [19], indicating that these effects on lipid uptake are mediated by permissive RXR/PPAR heterodimers. Recent studies suggest that other RXR heterodimeric partners regulate the expression of specific scavenger receptors in human and mouse macrophages. Thus, *CD36* is regulated by RXR/RAR heterodimers [19], and possibly by RXR/PXR [61, 62] and RXR/FXR [63] heterodimers. However, in the case of PXR and FXR more studies need to be performed since it is not clear whether these receptors are expressed in mouse macrophages [16, 61]. In addition, SR-A might be regulated by permissive RXR/Nurr1 and RXR/Nur77 heterodimers, since Nurr1 and Nur77, such as RXR, negatively regulate its expression and activity [64].

Another function of RXRs in macrophage lipid metabolism is the stimulation of cholesterol efflux through regulation of different ABC transporters. Ligand-dependent induction of RXRs with 9cRA and the rexinoids bexaroten, PA024, HX630, and LG100268 promotes *ABCA1* and *ABCG1* expression in human macrophage cell lines [53, 59], mouse primary macrophages [4, 20] and mouse microglia [41]. Ligand-activated RXRs regulate the expression of other proteins involved in cholesterol efflux, such as ADP-ribosylation factor-like7, a protein implicated in cholesterol transport to the membrane [65], and CYP27A1, an important enzyme in the sterol elimination pathway [66]. Gene expression of these factors is activated by specific ligands for PPARγ, PPARα, and/or LXRs [53, 65, 67, 68], which indicates that the control of cholesterol efflux by RXRs is mainly mediated by its permissive heterodimerization with these NRs. RXR/FXR and RXR/RARγ heterodimers might also be implicated in this process, since ligand activation of FXR and RARγ increases the expression of *Abca1* in mouse peritoneal macrophages [34, 69].

RXR activation also modulates the expression of molecules involved in macrophage lipid processing and storage. For example, rexinoid-mediated activation of RXR in human and mouse macrophages [70] and primary mouse microglia [41] induces the expression of apoE, which promotes efflux of lipids to apolipoproteins. RXR activation also increases the expression of Srebp1, a key transcriptional regulator of genes involved in cholesterol biosynthesis and uptake [71], and target gene of the RXR/LXR heterodimers [71]. Finally, 9cRA and LG100268 activation in mouse primary macrophages induces the expression of adipose differentiation-related protein (ADRP) [4, 20], a molecule that contributes to storage of triglycerides and cholesterol in macrophages, and that is a target gene of the RXR/PPARδ and RXR/PPARγ heterodimers [20, 53] (Figure 1).

RXR ligands in atherosclerosis and other lipid-handling-related diseases

Most data supporting a role of RXRs in atherosclerosis are based on the use of ligands specific for RXR or its heterodimer partners. Rexinoid-mediated activation of RXRs significantly reduced the development of atherosclerosis in two mouse models of dislypemia [63]. In both studies, rexinoids were able to enhance the lipid efflux capacity of macrophages. Similar anti-atherogenic effects have been reported in different *in vivo* studies with agonists of PPARγ, PPARδ and LXR [19], suggesting that RXRs exert their atheroprotective effects by forming permissive heterodimers with these partners. The role of other RXR partners in atherosclerosis is less certain. PPARα and FXR agonists have been shown to exert antiatherogenic or proatherogenic effects in animal models of dislypemia [19, 40], and macrophage expression of Nur77 is reported to either prevent or have no effect on the development of atherosclerosis in mice [50-52].

Recent research furthermore indicates that modulation of lipid handling by RXRs in specialized macrophages has beneficial effects in the treatment of AD. Activation of RXRs by rexinoids in primary microglia enhances secretion of ApoE HDL particles, which facilitates the degradation of soluble β-amyloid from the brain and reverses β-amyloid-induced symptoms in a mouse model of AD [41].

These results underpin the importance of macrophage RXR heterodimers in the control of lipid metabolism and in the development of metabolic diseases. However, a question that needs to be addressed is whether impairment of RXR expression or activity in macrophages affects lipid homeostasis and thus whether modulation of RXR signaling in macrophages might have a clinical impact on metabolic diseases.

Concluding remarks

Despite the growing body of literature on RXR biology in macrophages, there is still no consensus on the place that RXRs occupy in the transcriptional control of macrophage function. Since the discovery of RXRs in 1990 by Mangelsdorf and Evans [7], they have been mainly studied as subordinate partners of other NRs. It is now clear that RXRs have the ability to modulate other NRs in a ligand-dependent manner *in vivo*, making RXRs important pharmacological targets for the control of gene transcription. Moreover, the discovery of RXR homodimer-mediated gene regulation raises the intriguing possibility that RXR homodimers and heterodimers might act through separate signaling pathways. However, it is still uncertain whether RXR homodimers can function as biologically relevant transcription units (Outstanding Questions). There are still several roadblocks to overcome to address the *in vivo* functions of RXR homodimers. RXRs form homodimers with relatively low affinity compared with RXR heterodimers *in vitro* [19], and a similar scenario is feasible *in vivo*. To overcome this limitation, an animal model is needed to allow RXR homodimerization and heterodimerization to be separated. Progress will also come from *in vivo* studies with the use of the RXR homodimer antagonist LG100754 and the design of a new generation of RXR modulators which allow the selective activation or inhibition of RXR homodimers, providing valuable tools the decipher RXR homodimer functions [8].

RXRs play multifaceted roles in macrophage immune functions, and also occupy an important place in the control of macrophage lipid metabolism (Figure 2). This versatility of macrophage RXRs points to the potential medical utility of RXR ligands. However, the medical use of the currently available pan-RXR modulators is limited by the pleiotropic effects of RXR activation. This brings urgency to efforts to design SRXRMs, which can achieve cell- and dimer-specific effects. There are already examples of tissue-specific delivery of NR activators [72], opening a new direction in the future design of RXR modulators. For instance, the design and delivery of SRXRMs to macrophages might improve the treatment of macrophage-associated diseases and reduce unwanted side effects of systemic RXR activation (Outstanding Questions). Another strategy to make RXR ligands therapeutically more viable is the use of selective RXR hetero- or homodimer modulators. Currently available dimer-selective modulators, such as LG101506 and LG100754, are able to achieve the antidiabetic effects of pan-RXR agonists without side effects [8]. In addition, RXR-isotype selective modulators are being developed [8]. The use of these ligands might help to answer whether RXR isotypes have distinct pharmacolocigal profiles (Outstanding Questions). However, the highly conserved ligand-binding pocket structure of the three RXRs makes it difficult to achieve isotype-specific activation [8].

Recent studies combining crystallographic and fluorescence anisotropy approaches show the correlation between the pharmacological activity of SRXRMs and their impact on the structural dynamics of specific RXR-heterodimers [8]. Understanding these ligand-dependent structural changes of RXRs can aid the design of SRXRMs [8]. Advances in the development of SRXRMs and the macrophage-specific delivery of these ligands can overcome the current limitations of RXR targeting in macrophage-related pathologies, such as insulin-resistant diabetes, atherosclerosis and neurodenegerative diseases.

Acknowledgements

The work performed in the authors' laboratory was funded by awards from the Spanish Ministry of Economy and Competitiveness (SAF2012-31483) to M. Ricote, a European Foundation for the Study of Diabetes – Lilly Fellowship Program to T. Rőszer, and a Spanish Ministry of Science, and Innovation (FPU, AP2008-00508) grant to M. Cedenilla. The CNIC is supported by the Spanish Ministry of Economy and Competitiveness and the Pro-CNIC Foundation. Dr. Simon Bartlett (CNIC) provided English language editing. We apologize to our many colleagues for not being able to cite all relevant references owing to space limitations.

Text Box 1:

RXR biology: an evolutionary outlook

RXRs are highly conserved NRs [3], and have been identified in species representing a wide range of invertebrate phyla, including sponges, flatworms, arthropods, molluscs, echinoderms and chordates. To date the insect RXR homolog ultraspiracle protein is the best characterized invertebrate RXR. Ultraspiracle protein is a heterodimeric partner of the ecdysone receptor and regulates gene transcriptional changes associated with development, metamorphosis, reproduction and behavioral plasticity in insects [73-75]. In general, invertebrate RXRs can bind and be activated by vertebrate RXR ligands; however, flatworm and arthropod RXRs and one chordate RXR lack 9cRA- and bexarotene-dependent transactivation activity [3]. It is likely that RXRs in some invertebrate species can be activated by specific, as yet unidentified endogenous ligands [3, 76]. The vertebrate RXRs (RXRα, RXRβ and RXRγ) arose after the divergence of the non-vertebrate chordate and the vertebrate clades, and have evolved by multiple gene duplication events. Functional divergence of RXRα and RXRβ was followed by a further separation of RXRγ from RXRα [75]. The structure of the three extant vertebrate RXRs is highly conserved, particularly the helix involved in dimerization and the DNA-binding domain [77]. Heterodimerization is a general property of RXRs in evolutionarily distinct species. However, transcriptional control by RXR homodimers has also been reported in a mollusc species [78] and in mouse [4], suggesting that RXR homodimerization might be a more general and evolutionarily conserved mechanism than is considered today.

Text Box 2:

Macrophages: a diverse and plastic cell population

Macrophages are a highly heterogeneous cell population. Subsets of specialized resident macrophages include brain microglia, liver Kupffer cells, bone osteoclasts, lung alveolar macrophages, splenic macrophages, intestinal macrophages, peritoneal macrophages, adipose tissue macrophages, and atherosclerotic plaque foam cells [79].

These subpopulations have been historically defined according to anatomic location and surface marker profiles. More recently, exhaustive gene expression profiling has revealed the existence of unique molecular signatures among macrophages from different tissues [79]. The ontogeny of tissue macrophages is also subject of debate. In contrast to the prevalent concept that monocytes are precursors of tissue macrophages [80], recent work demonstrates that some macrophage subpopulations arise from primitive hematopoietic progenitors independently of the monocyte lineage [80]. Moreover, in adulthood, the maintenance of tissue macrophages involves local proliferation independently of monocytes and definitive hematopoiesis [81]. Macrophages are moreover highly plastic cells that can rapidly adjust their immune phenotype in response to injury, infection and the surrounding microenvironment. The immune phenotype of macrophages can be broadly classified into classically activated M1 macrophages or alternatively activated M2 macrophages. M1 macrophages express proinflammatory cytokines, chemokines, and effector molecules, which increase their pathogen killing activity. In contrast, M2 macrophages are low cytokine producers with prominent functions in tissue turnover and renewal, parasite clearance and immune modulation. Alterations in the M1/M2 balance are associated with autoimmunity, atherosclerosis, insulin resistance, tumor progression and neuroinflammation [12-14].

Outstanding Questions

1. Do macrophage-expressed RXRs have potential as targets in the treatment of leukemia, inflammatory and metabolic diseases?

2. Can we achieve macrophage-specific RXR modulation?

3. Is there a separate RXR-ligand-mediated signaling pathway or do RXRs act only as partners for other NRs?

4. Do RXRα and RXRβ have distinct or overlapping functions in macrophages?

5. Do RXR homodimers function as biologically relevant transcription units?

Glossary

Bexarotene (LG100269): a synthetic rexinoid **(**4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl2-naphthalenyl)ethenyl] benzoic acid) that acts as a pan-RXR agonist. It has been approved by the U.S. Food and Drug Administration (FDA) as an antineoplastic agent for the oral treatment of cutaneous T cell lymphoma (marketed name Targretin®).

LG101506: a synthetic rexinoid ((2*E*,4*E*,6*Z*)-7-[2-(2,2-Difluoroethoxy)-3,5-*bis*(1,1-dimethylethyl)phenyl]-3-methyl-2,4,6-octatrienoic acid) that selectively activates RXR/PPARγ and RXR/PPARα, and antagonizes RXR/RAR signaling by an allosteric event that results in inhibition of RAR within the RXR/RAR heterodimer.

LG100268: a synthetic rexinoid (2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)cyclopropyl]pyridine-5-carboxylic acid) that acts as a pan-RXR agonist.

LG100754: a synthetic rexinoid ((2E,4E,6Z)-3-Methyl-7-(5,6,7,8-tetr-ahydro-5,5,8,8-tetramethyl-3-propoxy-3-naphthaleny-l)-2,4,6-octatrienoic acid) that selectively activates RXR/PPARγ and RXR/PPARα heterodimers and antagonizes RXR homodimers.

Retinoids: a class of naturally found compounds chemically related to vitamin A, which can bind to RARs and RXRs. Retinoids play muliple roles in cell physiology;

they regulate epithelial cell growth, cell proliferation and differentiation, immune function, as well as vision.

Rexinoids: a class of synthetic compounds that selectively bind to and activate RXRs, They are currently being tested for the treatment of metabolic syndrome due to their glucose-lowering, insulin-sensitizing, and antiobesity effects in animal models of insulin resistance and type 2 diabetes. However, some of them have been linked to side effects such as hypertriglyceridemia and suppression of the thyroid hormone axis.

Selective RXR Modulators (SRXRMs): a class of synthetic compounds which include heterodimer and homodimer specific RXR agonists and antagonists; compounds that activate only a subset of the functions induced by the pan-RXR agonists; or act in a cell-type specific manner.

Hormone-response element (HRE): a short sequence of DNA within the promoter of a gene that allows for binding a specific NR complex, leading to transciptional activation. RXR may bind to direct repeats (DR), inverted repeats (IR), or everted repeats (ER) of the hexameric sequence AGGTCA separated by 1 to 5 bases, depending on the specific RXR heterodimeric partner.

Permissive heterodimer: RXR permissive heterodimers, formed with PPARs, LXRs, PXR, FXR, Nurr1 and Nur77 can be activated by either an RXR ligand or a ligand for the heterodimeric partner. Binding by both agonists could have additive or synergistic effects.

Non-permissive heterodimer: this concept has been classically used to define heterodimers formed by RXRs and RARs, VDR and TRs, which are normally activated only by ligands specific for the partner and not by RXR ligands. It this scenario RXR acts as a "silent" partner.

Conditionally permissive heterodimer: current concept used to define heterodimers formed between RXR and RARs, VDR and TRs, which are conditionally activated by RXR ligands only in the presence of the partner agonist.

Table 1: RXR and heterodimeric partners expressed in human and/or rodent monocyte/macrophages [1, 5, 14-19, 50, 52]

NR	Isotypes	Expression in rodents	Expression in humans	Natural ligands	Synthetic ligands	Dimer	DR	REF
RXR	α (NR2B1)	PEM, BMDM, M, KC	Mon, DC	9cRA DHA Honokiol Phytanic acid Oleic acid	Rexinoids: LG100268, Bexarotene (LG100269) LG101506 LG100754	Homo- Hetero-	DR-1 *	[5, 14, 15, 19]
	β (NR2B2)	KC, OC, BMDM, M	Mon, DC					[5, 14, 15, 19]
PPAR	α (NR1C1)	Low levels of KC	Mon, MDM	Polyunsaturated and oxidized fatty acids	α: GW7647 β/δ: GW0742 γ: TZD	P	DR-1	[15]
	β/δ (NR1C2)	PEM, BMDM, OC, KC, M	Mon, DC					[5, 15, 19]
	γ (NR1C3)	PEM, BMDM, KC, M, AM	DC, MDM					[5, 14, 15, 19]
RAR	α (NR1B1)	BMDM, KC, OC	Mon, DC, MDM	Retinoids	AM580 TTNPB	CP	DR-2 DR-5	[5, 15, 19]
	β (NR1B2)	KC						[15]
	γ (NR1B3)	BMDM, KC, M	Mon					[14, 15]
LXR	α (NR1H1)	PEM, BMDM, KC, M	Mon, DC	Oxysterols	GW3965 T0901317	P	DR-4	[5, 14, 15, 19]
	β (NR1H2)	PEM, BMDM, KC, M	Mon, DC					[5, 14, 15, 19]
TR	α (NR1A1)	BMDM	OC	Thyroid hormones	GC-1 KB141 GC-24	CP	DR-4	[15]
	β (NR1A2)	BMDM	OC					[15]
VDR	(NR1I1)	KC, BMDM, M	Mon, DC	$1,25(OH)_2VD_3$	MC903	CP	DR-3	[5, 14, 15, 19]
FXR	(NR1H4)	IM, SM	MDM	Farnesol and its metabolites	Fexaramine 6E-CDCA GW406	P	IR-1	[17]
PXR	(NR1I2)	PEM		Xenobiotics Sterols and its metabolites	Rifampicin Ritonavir Carbamazipine	P	DR-3-5 IR-6 ER-6,8	[18]
Nur77	(NR4A1)	BMDM, PEM, Mon, M	Mon, FC, PM	Not known	DIMs, Citosporone B	P	DR-5	[14, 35, 50, 52]
Nurr1	(NR4A2)	BMDM, PEM, Mon, M	Mon, FC, PM	Not known	DIMs, XCT0139508	P	DR-5	[14, 50]

The information about expression is based on documented studies in rodents and human. **PEM:** peritoneal-elicited macrophages; **BMDM:** bone marrow-derived macrophages; **Mon:** blood monocytes; **DC:** dendritic cells; **KC:** Kupffer cells; **OC:** osteoclasts; **IM:** intestinal macrophages; **SM:** splenic macrophages; **MDM:** blood monocyte-derived macrophages; **M:** microglia; **FC:** foam cells; **PM:** blood primary macrophages; **AM:** alveolar macrophages; **DIMs:** diindolylmethanes; **P:** permissive heterodimer; **CP:** conditionaly permissive heterodimer ; * Different six-base pair repetitions depending on RXR-heterodimeric partner; **DR:** direct repeat; **IR:** inverted repeat; **ER:** everted repeat

Figure 1. RXRs form homodimers or heterodimers with other nuclear receptors

RXRs are active integrators of distinct nuclear receptor signaling pathways, regulating gene transcription by forming permissive heterodimers with PPARs, LXRs, PXR, FXR, Nurr1 and Nur77, and non-permissive heterodimers with VDR, TR and RARs. RXRs can also control gene transcription as homodimers. RXRs are indicated in green and their ligands in red. DR: direct repeat, ER: everted repeat, IR: inverse repeat.

Figure 2. Complex roles of RXRs in macrophages

Macrophages express RXRα and RXRβ. RXRs play roles in the integration of macrophage immune functions and lipid metabolism by controlling apoptotic cell uptake, β-amyloid clearance, inflammation, pathogen killing, cholesterol transport and lipid handling. Alterations in these RXR-mediated processes cause diseases such as atherosclerosis, neurodegeneration, autoimmunity and disorders of the immune response.

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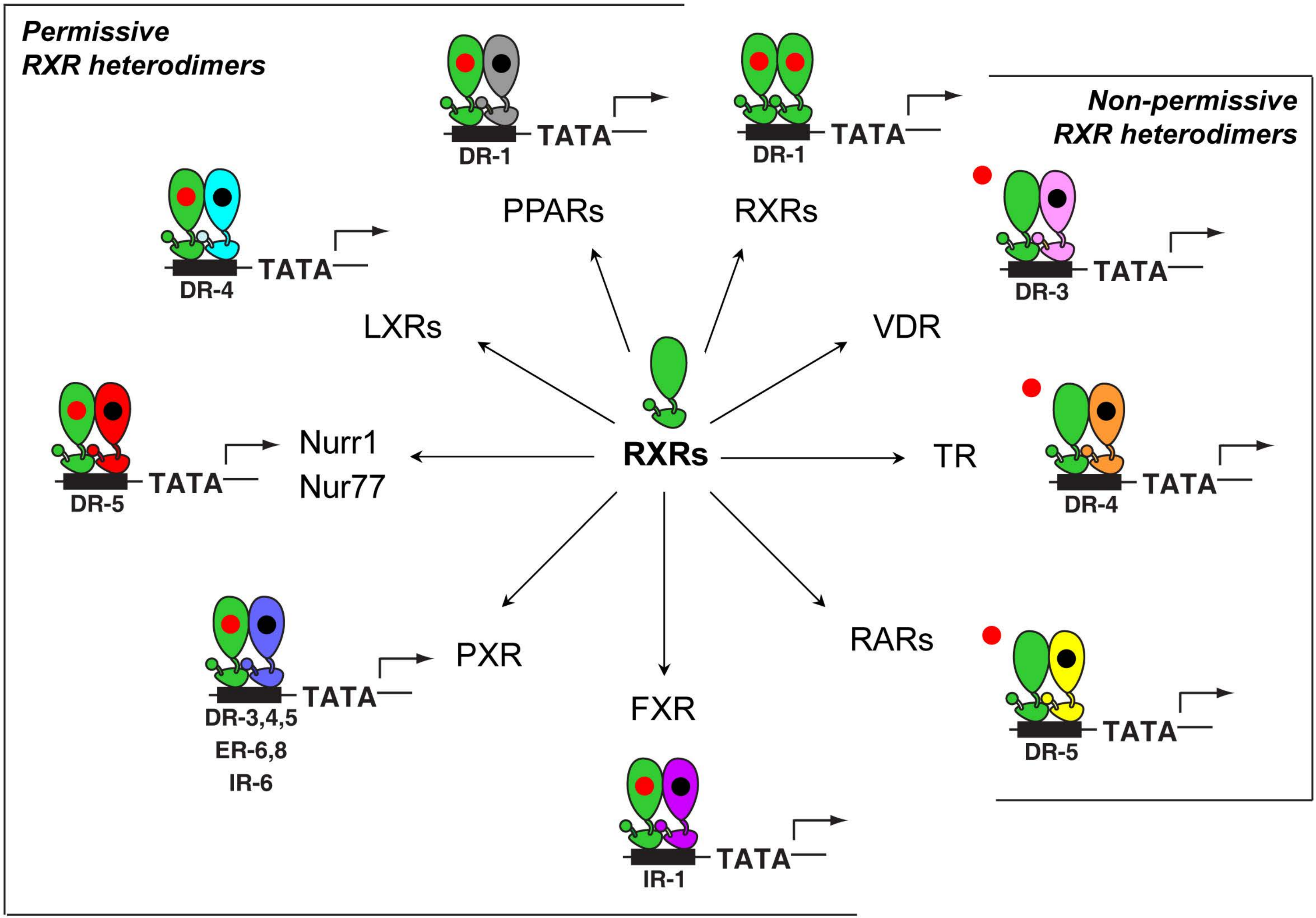

Figure 1
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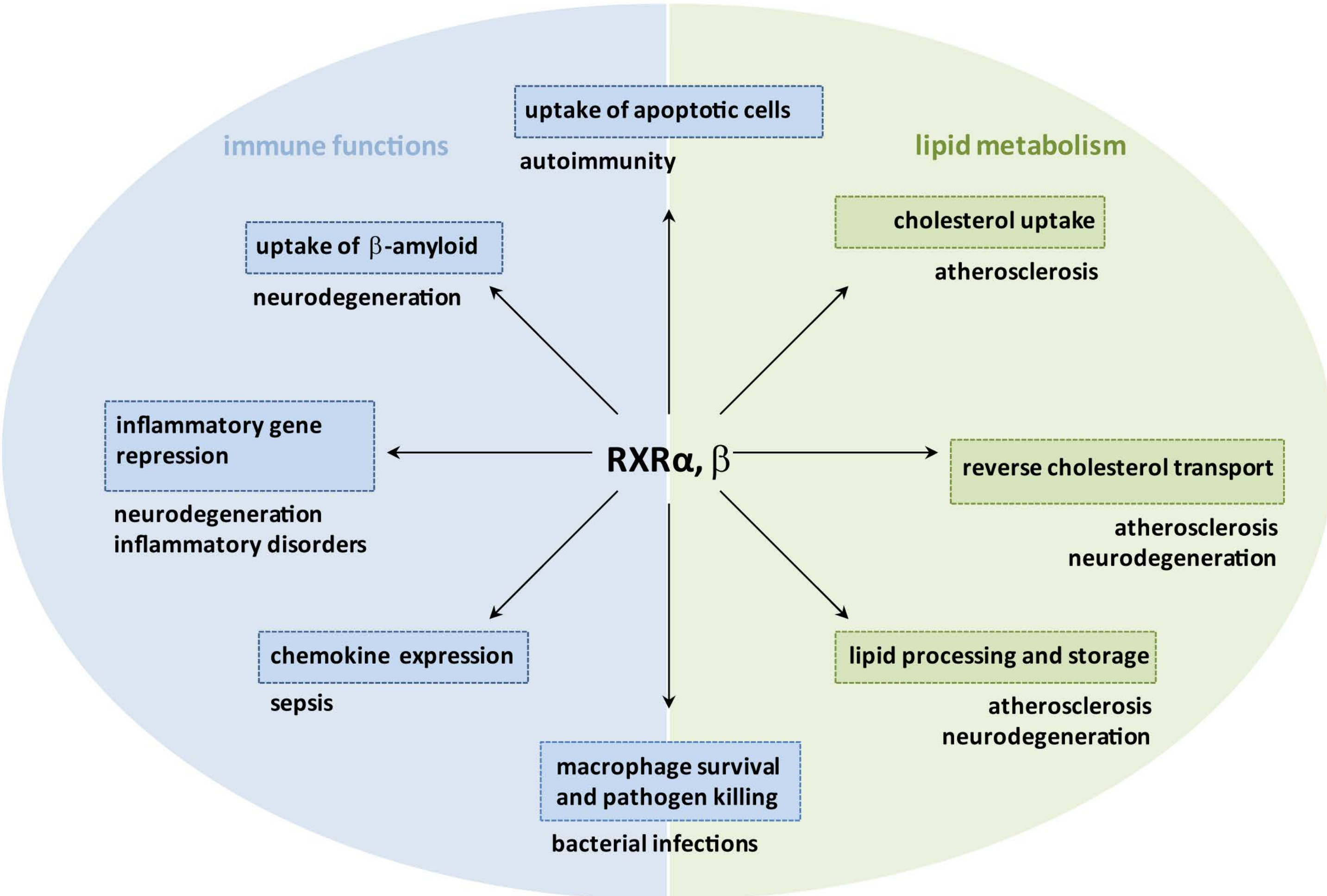

Figure 2
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