Arch. Immunol. Ther. Exp. (2012) 60:235–249
DOI 10.1007/s00005-012-0179-9

REVIEW

Biological Roles of Liver X Receptors in Immune Cells

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Received: 2 November 2011 / Accepted: 16 March 2012 / Published online: 14 June 2012

Abstract Liver X receptors (LXRs) are members of the nuclear receptor superfamily that are activated by specific oxysterols. LXRs heterodimerize with retinoid X receptors to regulate positively the expression of a variety of target genes, many of which are involved in lipid and glucose metabolism. In the last few years, new targets of LXR activation have been identified with roles in the modulation of immune responses. Moreover, LXRs mediate repression of inflammatory pathways through mechanisms collectively known as transrepression. Here, we revise recent findings on the impact of LXR activation on immune responses, with an emphasis on advances in the understanding of the molecular mechanisms that mediate these effects.

Keywords LXR · Transrepression · Inflammation · Gene expression

Abbreviations

ABC	ATP-binding cassette transporter
AIM	Apoptosis inhibitory protein secreted by macrophages
AP-1	Activating protein-1
Apo	Apolipoprotein
Ccl	Chemokine (C–C motif) ligand
CCR7	CC chemokine receptor-7
ChREBP	Carbohydrate response element-binding protein
CORO2A	Coronin 2A
GPS2	G protein pathway suppressor 2
GR	Glucocorticoid receptor
HDAC	Histone deacetylase
HIV	Human immunodeficiency virus
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IL	Interleukin
IRF	Interferon-regulatory factor
LDL	Low-density lipoproteins
LPS	Lipopolysaccharide
LXR	Liver X receptor
LXRE	LXR response element
Mertk	c-mer tyrosine kinase
NCoR	Nuclear receptor co-repressor
NF-κB	Nuclear factor kappa B
NOS2	Nitric oxide synthase 2
PIAS	Protein inhibitor of activated STAT
PPAR	Peroxisome proliferator-activated receptor
RXR	Retinoid X receptor
SMRT	Silencing mediator of retinoic acid and thyroid hormone receptors
SREBP-1c	Sterol regulatory element-binding protein 1c
STAT-1	Signal transducer and activator of transcription
SULT	Sulfotransferase
SUMO	Small ubiquitin-like modifier
Th	Helper T cell
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

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Introduction

Nuclear receptors are ligand-dependent transcription factors that modulate metabolism, development, proliferation and

inflammation through positive and negative regulation of gene expression (Mangelsdorf et al. 1995). Liver X receptors (LXRs) are members of the nuclear receptor superfamily that are activated by specific oxidized forms of cholesterol (oxysterols) and intermediate products of the cholesterol biosynthetic pathway (Janowski et al. 1999; Yang et al. 2006). Two LXR isoforms have been identified, namely LXRα (NR1H3) and LXRβ (NR1H2), encoded by separate genes. LXRα is expressed in liver, adipose tissue, kidney, intestine, spleen and macrophages, whereas LXRβ is ubiquitously distributed. By forming heterodimers with retinoid X receptors (RXRs), LXRs bind to LXR response elements (LXRE) that usually follow the consensus sequence (G/T/A)G(G/T)T(C/T)Annnn(C/A/T)G(G/T)(T/G)CA (Edwards et al. 2002) in the promoter or enhancer elements of LXR target genes. Activation of LXR–RXR heterodimers induces the expression of a variety of target genes, many of which are involved in lipid and glucose metabolism. In the last few years, new targets of LXR activation have been identified with roles in the modulation of immune responses. Moreover, LXRs have been shown to mediate repression of inflammatory pathways through mechanisms collectively known as transrepression. Together, these observations position LXRs at the crossroads between metabolism and immunity. In this review, we will cover the impact of LXR activation on immune responses, with an emphasis on recent advances in the understanding of the molecular mechanisms that mediate these effects.

Roads to LXR Activation

Several natural oxidized forms of cholesterol (oxysterols) have been shown to activate LXRs at physiological concentrations. These include 24(S),25-epoxycholesterol, 22(R)-, 24(S)-, 27-hydroxycholesterol and cholestenoic acid (Forman et al. 1997; Janowski et al. 1996, 1999; Lehmann et al. 1997; Song and Liao 2000). Likewise, specific intermediaries in the cholesterol biosynthetic pathway, such as desmosterol and zymosterol, are able to bind and activate LXRs (Yang et al. 2006). In addition, several compounds of plant origin, such as the plant sterol β-sitosterol (Plat et al. 2005), acanthoic acid (Jayasuriya et al. 2005) and selective acanthoic acid-related diterpenes (Traves et al. 2007), or from bacterial origin, such as the hexacyclic aromatic ketones (-)anthrabenzoxocinone and (-)bischloroanthrabenzoxocinone from *Streptomyces* sp. (Herath et al. 2005), have been also reported to activate LXRs.

Although LXRs contain a lipophilic ligand binding pocket (Williams et al. 2003), high doses of D-glucose and D-glucose-6-phosphate have been shown to bind to and activate LXRs (Mitro et al. 2007). These studies, however, did not establish how glucose and its derivatives bound to these nuclear receptors. More recent studies demonstrated that LXRs were not required for the in vivo glucose-induced expression of several genes with central roles in glucose homeostasis (Denechaud et al. 2008), thus suggesting that further work is required to clarify the physiological relevance of LXR activation by glucose.

Apart from natural products, several synthetic or semi-synthetic compounds have been developed that show agonistic properties toward LXRs, as, for example, acetyl podocarpic acid anhydride that has been developed as a semi-synthetic agonist derived from extracts of the may-apple (Singh et al. 2005). Among all the compounds developed so far that show high affinity for LXRs, two nonsteroidal synthetic agonists, namely TO901317 (Schultz et al. 2000) and GW3965 (Collins et al. 2002), have been most widely used for in vitro and in vivo studies dissecting the roles of LXRs in cell biology and physiology. These compounds, though, do not discriminate between the two LXR isoforms, α and β. Based on the interest of developing isoform-specific agonists, recent studies have identified compounds that bind more selectively to one of the two isoforms. For example, n-acylthiadiazolines and certain quinoline sulfones have been shown to activate LXRβ with selectivity over LXRα (Molteni et al. 2007; Ullrich et al. 2010). Moreover, as discussed in the following section, there is an interest for developing LXR ligands that enhance only specific functions of the LXR pathway. The identification of two novel LXR agonists with such properties, namely WAY-252623 and *N*,*N*-dimethyl-3β-hydroxy-cholenamide, has been recently reported (Kratzer et al. 2009; Quinet et al. 2009).

In physiological situations, LXRs can be activated when oxysterols are generated from accumulated cholesterol. Indeed, administration of a high cholesterol diet to mice leads to LXR activation (Peet et al. 1998). Modified low-density lipoproteins (LDL), such as acetylated or oxidized LDL is a source of LXR agonists as demonstrated in in vitro studies (Laffitte et al. 2001). During the course of infection and/or inflammation, there is the possibility that cholesterol from phagocytosed biomembranes or other sources, especially under highly oxidative conditions, may be converted into LXR-activating oxysterols. There is evidence, in fact, that the LXRα target gene Sp-α/AIM is induced during infection with *Listeria monocytogenes* in an LXR-dependent manner (Joseph et al. 2004). Likewise, prolonged infection with *Mycobacterium tuberculosis* also resulted in increased expression of several LXR target genes (Korf et al. 2009). These observations support the idea that endogenous LXR ligands are generated during the immune response to intracellular bacterial infection. Moreover, studies exploring the role of LXRs in macrophages actively involved in phagocytosis of apoptotic

thymocytes demonstrate that several LXR target genes are induced as a consequence of extensive phagocytosis (A-Gonzalez et al. 2009), although the exact nature of the ligands that mediate these effects remains elusive.

General Roles of LXRs in Regulation of Metabolic Functions

During the last decade, LXRs have been shown to be crucial mediators of cholesterol homeostasis in different tissues. For example, activation of LXRs leads to a marked increase in the expression of several sterol transporters from the ATP-binding cassette (ABC) family with important functions in reverse cholesterol transport (ABCA1 and ABCG1) (Repa et al. 2000b; Venkateswaran et al. 2000), sterol excretion into bile and feces and limiting sterol absorption in the intestine (ABCG5 and ABCG8) (Baldán et al. 2009; Lee et al. 2001; Lu et al. 2001; Repa et al. 2002; Yu et al. 2003). In general, LXRs also induce the expression of other molecules that participate in lipoprotein homeostasis (reviewed by Ricote et al. 2004). In addition to promoting reverse cholesterol transport, LXRs also reduce cellular uptake of cholesterol by inducing the expression of inducible degrader of the LDL receptor (Idol), an E3 ubiquitin ligase that targets several members of the LDL receptor family for degradation (Zelcer et al. 2009). Therefore, LXRs are considered as "sterol sensors" that coordinately regulate the expression of key molecules involved in sterol storage, efflux and elimination. LXR agonists have indeed shown therapeutic effectiveness in murine experimental models of atherosclerosis (Joseph et al. 2002; Terasaka et al. 2003).

Liver X receptors also participate in the regulation of genes involved in glucose metabolism, such as glucokinase in the liver and the insulin-sensitive glucose transporter 4 in adipose tissue (Laffitte et al. 2003). Consistent with these observations, LXR agonists have shown significant insulin-sensitizing effects in murine models of diet-induced obesity and insulin resistance (Commerford et al. 2007).

In addition to their role in sterol and glucose homeostasis, LXRs promote lipogenesis through the induction of sterol regulatory element-binding protein 1c (SREBP-1c) (Repa et al. 2000a) and carbohydrate response element-binding protein (ChREBP) (Cha and Repa 2007). Both SREBP-1c and ChREBP are transcription factors that control the expression of molecules integral to fatty acid biosynthesis and esterification, and mice treated with pan-LXR agonists suffer a marked increase in plasma triglyceride levels (Schultz et al. 2000), which limit the potential therapeutic advantages of these agonists as anti-atherogenic or insulin-sensitizing drugs. Based on the fact that LXRα is more abundantly expressed than LXRβ in the liver and accounts for most of the lipogenic effects of LXR agonists in this organ, alternative strategies try to identify compounds with higher selectivity for LXRβ over LXRα to skip the hepatic lipogenic properties of general LXR agonists (Molteni et al. 2007; Ullrich et al. 2010). Also, two novel LXR agonists, WAY-252623 and *N*,*N*-dimethyl-3β-hydroxy-cholenamide, have been shown to reduce atherosclerotic lesion progression without inducing the expression of SREBP1c or activating hepatic lipogenesis, although the mechanisms involved in these selective actions have not been described (Kratzer et al. 2009; Quinet et al. 2009).

LXRs as Negative Regulators of Inflammation

Apart from their role in the control of metabolism, LXRs also exert crucial functions in the regulation of immune responses. LXR agonists downregulate the expression of selective inflammatory genes through a process known as transrepression, which is also used by other nuclear receptors to interfere with inflammatory signaling pathways. In general, transrepression is considered to involve nuclear receptor interaction with additional proteins bound to promoter regions, rather than direct interaction with DNA (reviewed by Glass and Ogawa 2006). In the last few years, several groups have described anti-inflammatory actions of LXR agonists in different mouse models of inflammatory disease (Table 1).

In macrophages, LXR agonists are able to transrepress inflammatory pathways engaged by lipopolysaccharide (LPS) (via Toll-like receptor 4, TLR4), interleukin (IL)-1β, tumor necrosis factor (TNF)-α, polyinosinic:polycytidylic acid (via TLR3 activation) or interferon (IFN)-γ (Ghisletti et al. 2007; Joseph et al. 2003; Lee et al. 2009). LXR-mediated transrepression does not affect all the genes induced by these pathways. For example, the LXR agonist GW3965 repressed several genes induced by LPS, including nitric oxide synthase 2 (NOS2), IL-6 and IL-1β, but not TNF-α (Ghisletti et al. 2007; Joseph et al. 2003; Ogawa et al. 2005). Several LXR agonists, including GW3965, were reported, however, to inhibit the induction of TNF-α by IFN-γ in astrocytes (Lee et al. 2009). In bone marrow-derived mast cells, LXR activation by 25-hydroxycholesterol or TO901317 mediated repression of IL-6 production upon engagement of the high-affinity receptor for immunoglobulin (Ig)E (Nunomura et al. 2010). In dendritic cells, LXR agonists interfered with the LPS-induced expression of IL-12 and fascin, an actin-bundling protein involved in actin polymerization in mature dendritic cells that form an immunological synapse with T lymphocytes. As a consequence, the ability of mature dendritic cells to activate T lymphocytes was impaired.

Table 1 Effects of LXR agonists on different experimental models of inflammatory disease

Model of inflammation	Agonist (administration and dose)	Anti-inflammatory effects of agonist	References
TPA-induced contact dermatitis	TO901317, GW3965 (20 µl of a 10 mM stock). Topical administration 4 h after TPA application	Decreased ear weight and thickness Reduced edema and inflammatory cell infiltration in the dermis	Joseph et al. (2003)
TPA-induced contact dermatitis	22(R)-OH-chol, 25-OH-chol, GW3965 (40 µl of 10 mM agonist). Topical administration 45 min and 4 h after TPA application	Decreased ear weight and thickness Reduced inflammatory cell infiltration (22(R)-OH-chol). Decreased expression of IL-1α and TNF-α (22(R)-OH-chol)	Fowler et al. (2003)
Oxazolone-induced allergic contact dermatitis	22(R)-hydroxycholesterol, 25-hydroxycholesterol, GW3965 (40 µl of 10 mM agonist). Topical administration 45 min and 4 h after oxazolone application	Decreased ear weight and thickness Reduced inflammatory cell infiltration (22(R)-OH-chol). Decreased expression of IL-1α and TNF-α (22(R)-OH-chol)	Fowler et al. (2003)
Ovalbumin-induced allergic response	TO901317 (4 and 20 mg/kg/day). Intraperitoneal administration every second day starting the day before the first sensitization	Reduced production of allergen-specific IgE and IgG1	Heine et al. (2009)
Collagen-induced arthritis	GW3965 (0.3 and 1 mg/kg/day). Oral administration for 40 days starting on the day of collagen treatment	Reduced incidence of arthritis and attenuated clinical and histological severity of the disease. Reduced inflammatory cell infiltration and expression of TNF-α, IL-1β, NOS2 and COX2 in joints. Decreased levels of TNF-α, IL-1β and IL-6 in serum	Park et al. (2010)
Atherosclerosis ($ApoE^{-/-}$ mice)	GW3965 (20 mg/kg/day). Oral administration daily during 3 days	Reduced expression of MMP9 in atherosclerotic aortas	Joseph et al. (2003)
Atherosclerosis ($LDLR^{-/-}$ mice)	TO901317 (3–10 mg/kg/day). Oral administration daily for 8 weeks during atherogenic diet	Reduced expression of tissue factor in atherosclerotic lesions	Terasaka et al. (2003)
Intraperitoneal injection of LPS	TO901317 (3 mg/kg) or GW3965 (30 mg/kg). Oral administration daily for 7 days before LPS injection	Reduced expression of tissue factor in kidneys and lungs	Terasaka et al. (2005)
LPS-induced hepatic acute phase response (intraperitoneal injection of LPS)	GW3965 (30 mg/kg/day). Administration for 4 days prior to LPS injection	Reduced expression of haptoglobin, SAA and CRP in the liver	Venteclef et al. (2010)
LPS and PepG-induced hepatic injury	GW3965 (0.3 mg/kg). Intravenous injection 30 min before LPS/PepG injection	Reduced infiltration of leukocytes, including mast cells, and decreased expression of chemokines eotaxin 1 and 2 in liver. Reduced levels of TNF-α and PGE2 in plasma	Wang et al. (2006)
LPS-induced lung injury (intranasal instillation with LPS)	GW3965 (20 mg/kg). Oral administration daily for 7 days prior to LPS treatment	Decreased protein, TBARS and cell numbers, including neutrophils, in BAL. Reduced neutrophil infiltration and MPO activity in lungs	Gong et al. (2009)
LPS-induced lung injury (aerosolized LPS)	TO901317 (50 mg/kg/day). Oral administration daily for 5 days prior to LPS treatment	Reduced polymorphonuclear cell and TNF-α in BAL. Reduced MPO activity in lungs	Smoak et al. (2008)
LPS-induced acute lung injury (vein infusion of LPS)	TO901317 (10 mg/kg). Oral administration daily for 1 week prior to LPS treatment	Decreased leukocytes, including neutrophils, and protein levels in BAL. Reduced levels of TNF-α, IL-1β and IL-6, and augmented IL-10 in BAL fluid. Reduced alveolar wall thickening, alveolar hemorrhage, interstitial edema and inflammatory cell infiltration in lungs	Wang et al. (2011)

Table 1 continued

Model of inflammation	Agonist (administration and dose)	Anti-inflammatory effects of agonist	References
Carrageenan-induced acute lung inflammation (injection of Carrageenan in the pleural cavity)	TO901317 (20 mg/kg). Intraperitoneal administration	Reduced edema, PMN infiltration and NOS2 expression in the lung. Reduced levels of TNF-α and IL-1β in pleural exudates and lungs. Decreased nitric oxide production in pleural exudates	Crisafulli et al. (2010b)
Alzheimer disease (Tg2576)	GW3965 (33 mg/kg/day). Oral administration daily for 4 months	Decreased number of activated plaque associated microglia and IL-6 mRNA levels in the brain	Jiang et al. (2008)
EAE	TO901317 (25 mg/kg). Intraperitoneal injection daily starting 2 days prior to EAE induction	Reduced inflammation, infiltration of $CD4^+$ T lymphocytes and macrophages, expression of IFN-γ, expression of microglial MHCII and demyelination associated with inflammation in spinal cords	Hindinger et al. (2006)
Middle cerebral artery occlusion	TO901317 and GW3965 (20 mg/kg). Intraperitoneal injection 10 min after experimental stroke	Reduced infarct volume and area. Reduced expression of NOS2, COX2, MMP9, IL-1β, IL-6, IL-12p40 and Ccl5 in the brain	Morales et al. (2008)
Middle cerebral artery occlusion	GW3965 (20 mg/kg). Intraperitoneal injection 10 min after experimental stroke	Decreased infarct volume. Reduced infiltration of monocyte/macrophages and expression of NOS2, COX2, MCP-1, Ccl5 and IL-1β in the brain	(Sironi et al. 2008)
Global cerebral Ischemia	GW3965 (20 mg/kg). Intraperitoneal injection 10 min after surgery	Reduced nuclear localization of the p65 subunit of NF-κB and decreased expression of COX2 in the brain	Cheng et al. (2010)
Spinal cord injury	TO901317 (20 mg/kg). Intraperitoneal administration 1 and 6 h after surgery	Reduced severity of spinal cord trauma. Decreased infiltration of neutrophils, reduced expression of TNF-α, IL-1β and NOS2, and decreased IκB degradation and nuclear localization of the p65 subunit of NF-κB in spinal cord tissue	Paterniti et al. (2010)
Splanchnic artery occlusion	TO901317 (20 mg/kg). Intravenous administration 15 min prior to reperfusion	Reduced tissue injury, PMN infiltration, expression of adhesion molecules ICAM-1 and P-selectin and cytokines TNF-α and IL-1β in the ileum. Decreased IκB degradation and nuclear localization of the p65 subunit of NF-κB, and reduced levels of p38, ERK and JNK activation in the ileum	Crisafulli et al. (2010a)
Ultraviolet irradiation-induced photoaging	TO901317 (50 µl of 0.1–10 mM stocks). Topical administration after each exposure to ultraviolet irradiation (5 times per week)	Reduced skin thickening and wrinkle formation	Chang et al. (2008)

BAL bronchoalveolar lavage, *COX2* cyclooxygenase 2, *CRP* C-reactive protein, *EAE* experimental autoimmune encephalomyelitis, *ERK* extracellularly-regulated kinase, *ICAM*-1 intercellular adhesion molecule-1, *JNK* c-Jun N-terminal kinase, *LDLR* LDL receptor, *MCP* monocyte chemoattractant protein, *MHCII* major histocompatibility complex type II, *MMP9* matrix metalloprotease 9, *MPO* myeloperoxidase, *OH-chol* hydroxycholesterol, *PGE2* prostaglandin E2, *PMN* polymorphonuclear cells, *SAA* serum amyloid A, *TBARS* thiobarbituric acid reactive substances, *TPA* 12-O-tetradecanoylphorbol-13-acetate

Interestingly, the inhibitory effects of LXR agonists were not observed when dendritic cells were activated through the CD40 ligand (Geyeregger et al. 2007).

The mechanisms underlying these promoter, stimulus and perhaps cell type-specific effects are still subject of study. Initial studies in murine macrophages described that LXR-mediated transrepression of the LPS pathway targeted nuclear factor (NF)-κB signaling, but not activating protein (AP)-1 (Joseph et al. 2003). This effect contrasted with the actions mediated by some other members of the nuclear receptor superfamily, such as the glucocorticoid receptor (GR), which interfered with both NF-κB and AP-1 to downregulate a broad range of inflammatory genes (Galon et al. 2002), or the all-trans-retinoic acid receptor, which repressed AP-1 regulated genes by altering the composition of fos- and jun-related proteins within the AP-1 heterodimer (Benkoussa et al. 2002).

In the absence of proinflammatory stimuli, several inflammatory genes are actively repressed by a co-repressor complex that acts on their promoters (Lee et al. 2000; Ogawa et al. 2004). Induction of transcription of these genes in response to a pro-inflammatory signal requires an initial de-repression step in which the co-repressor complex is removed from the gene promoter and at least some of its components are subjected to ubiquitylation and proteasome-dependent degradation (Hoberg et al. 2004). Nuclear receptor co-repressor (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptors (SMRT) were initially identified as co-repressor proteins involved in the process by which several nuclear receptors actively repress the expression of their own target genes in the absence of an agonistic ligand (Chen and Evans 1995; Hörlein et al. 1995). Later on, NCoR and SMRT were also shown to be part of the co-repressor complexes that mediate basal repression of pro-inflammatory genes in the absence of the pro-inflammatory stimulus (Lee et al. 2000; Ogawa et al. 2004). Interestingly, interference with the release of co-repressor proteins from inflammatory gene promoters has been reported as the mechanism how several nuclear receptors, including LXRs and peroxisome proliferator-activated receptors (PPARs), inhibit the expression of LPS-induced genes in macrophages (Ghisletti et al. 2007; Pascual et al. 2005). The expression of functional NCoR is required for nearly all the repressive effects of LXR on LPS target genes. For a subset of these genes, SMRT was also necessary to establish a stable co-repressor complex (Ghisletti et al. 2007, 2009). LXR-mediated inhibition of the acute phase response in the liver is also based on prevention of co-repressor complex removal from inflammatory gene promoters (Venteclef et al. 2010). In contrast, NCoR knockdown in astrocytes did not affect LXR-mediated transpression of IFN-γ induced genes (Lee et al. 2009), which suggests that either SMRT can replace the absence of NCoR or a different mechanism independent of retention of these co-repressor complexes mediates the repressive effects of LXR agonists in that system. In fact, interaction with co-repressor complexes is not a prerequisite for the inhibition of inflammatory gene expression by all nuclear receptors. As an example, several models for GR-mediated transrepression have been proposed which interfere with selective NF-κB-dependent genes in a co-repressor-independent manner (reviewed by Glass and Ogawa 2006).

Studies on the mechanisms involved in nuclear receptor action led to the discovery of the association between nuclear receptors and members of the small ubiquitin-like modifier (SUMO) family (Poukka et al. 2000; Tian et al. 2002). SUMOylation is a reversible post-translational modification consisting in the conjugation of a SUMO to a substrate protein. In mammals, there are three members in the SUMO family, namely SUMO-1, -2 and -3, which can be conjugated to target proteins. SUMOylation requires first the activation of a SUMO by a heterodimeric SUMO-1 activating enzyme (E1 enzyme), which cleaves a C-terminal peptide from SUMO in an ATP-dependent manner. Then, activated SUMO is temporarily transferred to a conjugating enzyme (E2 enzyme), generally ubiquitin carrier protein 9, which subsequently mediates the conjugation to the target protein in cooperation with SUMO E3 ligases (reviewed by Treuter and Venteclef 2011). Members of the protein inhibitor of activated STAT (PIAS) family are the most characteristic SUMO E3 ligases (Palvimo 2007), although other proteins including histone deacetylase (HDAC)2 and HDAC4/5 can also act as E3 ligases (reviewed by Treuter and Venteclef 2011). In general, nuclear receptor SUMOylation is associated with transcriptional repression. However, SUMOylation of retinoid-related orphan receptor-alpha is an exception, as it potentiates transcriptional activation (Hwang et al. 2009).

Liver X receptor-mediated transrepression has been reported as a SUMO-dependent process. In murine macrophages, GW3965-activated LXRs underwent SUMOylation, which prevented the clearance of co-repressor complexes from the promoter of specific inflammatory genes (Ghisletti et al. 2007). The proteins implicated in LXR SUMOylation seem to vary between cell types and perhaps the inflammatory environment. In macrophages stimulated with LPS, GW3965-activated LXRβ was SUMOylated by SUMO-2/3 in a process that involved HDAC4 as the E3 ligase (Ghisletti et al. 2007). More recent studies in astrocytes stimulated with IFN-γ proposed the use of different SUMOylation mechanisms for LXRα and β in response to LXR ligands: LXRα being SUMOylated by SUMO2/3 using HDAC4 as the E3 ligase, and LXRβ being SUMOylated by SUMO1, with PIAS1 acting as the E3 ligase (Fig. 1). PIAS1 has been also

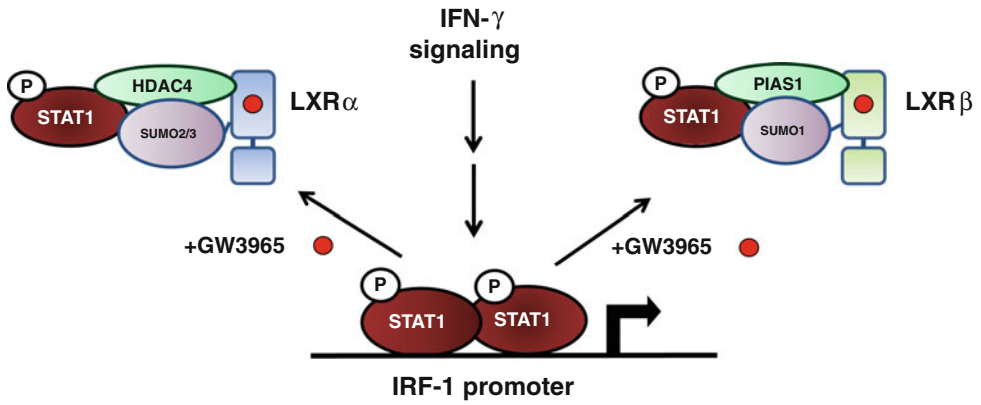

Fig. 1 Model that describes parallel mechanisms used by LXRα and *β* to inhibit IFN-γ-induced expression of IRF-1. Activation of astrocytes with IFN-γ leads to STAT-1 recruitment to the IRF-1 promoter. In the presence of the LXR agonist GW3965, LXRα becomes SUMOylated by SUMO2/3 using HDAC4 as an E3 ligase, whereas LXR*β* is SUMOylated by SUMO1, with PIAS1 acting as an E3 ligase. SUMOylated LXRs form a complex with STAT1 resulting in inhibited recruitment of STAT1 to the IRF-1 promoter (Lee et al. 2009)

implicated in PPAR-γ SUMOylation (Pascual et al. 2005). SUMOylated LXRs formed a complex with STAT1 in astrocytes resulting in inhibited recruitment of STAT1 to the interferon-regulatory factor (IRF)-1 promoter, without affecting STAT1 phosphorylation or its nuclear translocation (Lee et al. 2009). An independent report, however, did observe reduced levels of IFN-γ-induced STAT1 serine and tyrosine phosphorylation in human THP-1 macrophages in response to 22 (R)-hydroxycholesterol (Li et al. 2011).

Two proteins previously identified within co-repressor complexes have been recently shown to directly interact with SUMOylated LXRs. In the liver, transrepression of the acute phase response by GW3965 was selectively mediated by LXR*β*. In the presence of GW3965, LXR*β* underwent SUMOylation by SUMO-2/3 and was recruited to a co-repressor complex on the C-reactive protein and haptoglobin gene promoters through the interaction with G protein pathway suppressor (GPS)2 (Fig. 2) (Venteclef et al. 2010). Interestingly, the interaction between LXR and GPS2 may also play an important role in LXR-mediated transactivation, as it facilitated recruitment of GW3965-activated LXR to at least an ABCG1-specific promoter/enhancer unit and subsequent histone demethylation of that genomic region. GPS2 was capable of binding both LXRα

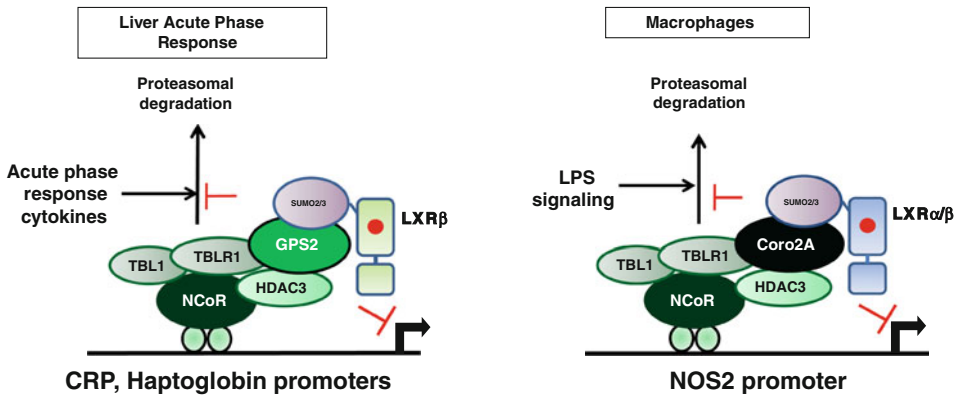

Fig. 2 Models that describe the mechanisms used by LXRs to inhibit transcriptional responses to LPS in different cell types. In the absence of proinflammatory stimuli, co-repressor complexes, typically containing NCoR or SMRT, transducin (beta)-like 1 X-linked (TBL1) and TBL1-related protein 1 (TBLR1), bind to inflammatory gene promoters to keep their basal expression in a repressed state. In response to LPS stimulation, key components within co-repressor complexes are subject to proteasomal degradation, thus releasing repression from inflammatory gene promoters. In the liver (*left side*), transrepression of the LPS-induced acute phase response by GW3965 is selectively mediated by LXR*β*. SUMOylation of LXR*β* by SUMO-2/3 in response to GW3965 activation results in recruitment of LXR*β* to a co-repressor complex on the *Crp* and *Haptoglobin* gene promoters through the interaction with GPS2, thus preventing co-repressor complex removal from those promoters (Venteclef et al. 2010). In macrophages (*right side*), LXR mediated transrepression of the *Nos2* promoter involved conjugation of LXR*β* to SUMO2/3 and the interaction of SUMOylated LXR*β* with CORO2A rather than GPS2, preventing actin recruitment and NCoR turnover on the *Nos2* gene promoter (Huang et al. 2011)

and LXRβ, most probably through a surface that is distinct from the co-activator-interaction motif in the LXR molecule (Jakobsson et al. 2009). In macrophages, LXR-mediated transrepression involved interaction of SUMOylated LXRs with coronin 2A (CORO2A) rather than GPS2. CORO2A is an actin-binding protein that contains a conserved SUMO2/3-interaction motif and is expressed mainly in the nucleus at least in primary macrophages (Huang et al. 2011). CORO2A localized on several NCoR-dependent promoters in resting macrophages and participated in TLR-induced NCoR turnover from the *Nos2* promoter through the interaction with oligomeric nuclear actin, although the exact role of actin in this process remains enigmatic. The model proposed by Huang et al. (2011) suggests that SUMOylated LXRs bind to the SUMO2/3-interaction motif in CORO2A, thus putatively preventing actin recruitment and NCoR turnover on the *Nos2* promoter (Fig. 2).

Interestingly, serine phosphorylation of LXRs by signals that activate calcium/calmodulin-dependent protein kinase IIγ, such as the TLR1/TLR2 ligand Pam_3CSK, resulted in the loss of the LXR-mediated transrepression potential. Such a phosphorylation step occurred on serine 427 and led to LXRβ deSUMOylation by sentrin/SUMO-specific protease 3 and its subsequent release from CORO2A (Huang et al. 2011). These observations raise the possibility that signal-induced negative regulation of LXR-mediated transrepression may contribute to chronic inflammatory processes.

Molecular modeling suggests that the ligand binding domain of human LXRβ contains a putative SUMO acceptor lysine in close proximity to the area used for heterodimerization with RXR (Treuter and Venteclef 2011). The requirement of LXR–RXR heterodimerization has been well established for LXR-mediated transactivation (Willy et al. 1995). However, whether or not this heterodimerization is required for LXR-mediated transrepression remains uncertain. So far, no reports demonstrate RXR recruitment to LXR-transrepressed loci. In fact, Treuter and Venteclef (2011) observed no recruitment of RXR to SUMO-dependent loci, whereas RXR was recruited in an LXR-dependent manner to classical positively regulated LXR/RXR target genes (Wagner et al. 2003). Based on the model proposed by Jakobsson et al. (2009), GPS2 binds LXRs probably once they have formed heterodimers with RXR on the LXRE of the ABCG1 promoter, which raises the question of whether a complex between GPS2 and the LXR–RXR heterodimer is also necessary for the transrepressive effects described for the GPS2–LXR axis. More studies focused on the role of RXR heterodimerization will be necessary to clarify these issues.

Apart from LXR-mediated transrepression, indirect effects of LXRs also contribute to relevant negative effects on immune responses. As an example, activation of LXRs by TO901317 reduced secreted IgE in activated B lymphocytes, without affecting IgE class switch recombination or its transcription. LXR activation resulted in reduced phosphorylation of c-Jun N-terminal kinase 2 and increased membrane expression of CD23, the low-affinity receptor for IgE, which helps reduce IgE secretion as a negative feedback loop (Heine et al. 2009).

LXRs Regulate Positively the Expression of Genes with Specific Roles in Immune Responses

Based on the anti-inflammatory actions of LXR agonists, one could predict that LXR activation would impact on the capability of immune cells to establish an aggressive response against pathogens. In fact, LXR-deficient mice have been shown to be markedly resistant to systemic infection with *Leishmania chagasi/infantum*. Primary macrophages derived from mice lacking LXRs generated higher levels of nitric oxide and IL-1β and were able to kill parasites in the presence of IFN-γ more efficiently than wild-type macrophages (Bruhn et al. 2010). However, in other models of infection, LXR deficiency leads to opposite effects. For example, LXR-deficient mice were more susceptible to infection by *M. tuberculosis*, in correlation with the fact that these mice were not capable of mounting an effective early neutrophilic airway response to infection and helper T cell (Th)1 and Th17 responses were abrogated in the lungs of these animals (Korf et al. 2009). Likewise, mice deficient in LXRs were more susceptible to infection by the intracellular bacteria *L. monocytogenes* and reconstitution of irradiated wild-type mice with bone marrows from LXR-deficient mice conferred susceptibility toward these bacteria (Joseph et al. 2004). These observations suggest that the LXR pathway exerts complex regulatory actions on immune responses that extend beyond transrepression of proinflammatory responses and may lead to different outcomes depending on the invading pathogen.

Liver X receptor agonists have been shown to regulate positively the expression of the anti-inflammatory enzyme arginase II (Marathe et al. 2006). Arginase activity catalyzes the conversion of L-arginine into L-ornithine and urea, which are precursors for the synthesis of polyamines and collagen. The consumption of L-arginine for polyamine production results in decreased availability of this amino acid for nitric oxide synthesis mediated by NOS2, although arginase activity may also inhibit nitric oxide production by several other means, including repression of the translation and stability of the NOS2 protein, uncoupling or inhibition of NOS activity by end products of arginase activity, and sensitization of NOS to the endogenous inhibitor asymmetric dimethyl-L-arginine (Durante et al.

2007). Based on these effects, LXR agonists have the potential to interfere with nitric oxide production both by inhibiting NOS2 expression and upregulating arginase II expression and therefore indirectly affecting NOS activity. These effects may especially compromise macrophage responses during infections by *Leishmania*, as the pathogen can benefit both from reduced nitric oxide production and from polyamines derived from arginase activity (Kropf et al. 2005), although Bruhn et al. (2010) were not able to correlate changes in arginase expression with resistance to *L. chagasi/infantum* in LXR-deficient mice. Recently, LXR agonists have been also shown to exert indirect positive regulation of arginase I expression via upregulation of IRF-8 (Pourcet et al. 2011). In those studies, IRF-8 was identified as a direct target of LXRα in Raw264.7 macrophages that overexpress human LXRα. IRF-8 is a transcription factor whose expression is restricted to immune cells and it plays important roles in regulation of gene expression in response to type I and II IFNs (Kanno et al. 2005). The model proposed by Pourcet et al. described the association of IRF-8 with the transcription factor PU.1 on a composite element in the promoter region of the arginase I gene. Occupancy of this element by IRF-8/PU.1 was increased by the LXR agonist TO901317, consistent with the induction of IRF-8 expression in response to LXR activation (Pourcet et al. 2011). It would be interesting to know whether the LXRα/IRF-8/PU.1 axis exerts other regulatory effects on additional genes.

Several intracellular pathogens, e.g., *Chlamydia pneumoniae* and *M. tuberculosis*, have developed mechanisms to convert host macrophages into foam cells (lipid-laden macrophages). Somehow, these pathogens meet nutritional advantages by residing within cells that accumulate lipids (Cao et al. 2007; Peyron et al. 2008). As an example, Raw 264.7 macrophages infected with *C. pneumoniae* became foam cells with increased contents of cholesteryl esters when co-cultured in the presence of LDL (Cao et al. 2007), in line with the idea that infections with *C. pneumoniae* accelerate atherosclerosis (Moazed et al. 1999). The process by which certain pathogens convert macrophages into foam cells is not completely understood. *C. pneumoniae*-induced acceleration of atherosclerosis in apoE-deficient mice was further enhanced in animals deficient in both apoE and LXRα (Naiki et al. 2008), suggesting that interference with LXR-induced mechanisms for cholesterol efflux may provide a strategy for pathogen-induced lipid accumulation. Interestingly, infection by other microorganisms, such as *Escherichia coli* or RNA virus influenza A, blocked the induction of LXR target genes, including ABCA1, ABCG1 and apoE, in macrophages. Activation of TLR3 and TLR4 by microbial ligands also mimicked these effects via activation of IRF-3, leading to downregulation of cholesterol efflux from macrophages (Castrillo et al. 2003), which suggests that pathogenic pathways signaling via IRF-3 have the capability to interfere with LXR-mediated cholesterol homeostasis. Conversely, independent groups have shown that treatment with the LXR agonist GW3965 leads to reduction of foam cell formation during infection by *C. pneumoniae* (Cao et al. 2007; Chen et al. 2008), which probably occurs by virtue of the upregulation of ABCA1 and other molecules with key roles in cholesterol efflux.

Several years ago, LXR activation was shown to promote macrophage survival during infection by several bacteria, including *L. monocytogenes* (Joseph et al. 2004), *Bacillus anthracis*, *E. coli* and *Salmonella typhimurium* and in response to several forms of cellular stress, such as growth factor deprivation (Valledor et al. 2004). These effects correlated with downregulated expression of several members of the caspase family and other pro-apoptotic factors (Valledor et al. 2004) and with induction of an apoptosis inhibitory protein secreted by macrophages (AIM; also known as Sp-α) (Joseph et al. 2004; Valledor et al. 2004), which was shown to be a specific target of LXRα (Joseph et al. 2004). Induction of AIM was also attributed a role in prevention of macrophage apoptosis within atherosclerotic lesions (Arai et al. 2005), although the mechanism used by AIM to promote macrophage survival to date remains elusive.

Macrophage-secreted AIM has been recently shown to be endocytosed into adipocytes via the scavenger receptor CD36. Within adipocytes, AIM bound to and inhibited the activity of cytosolic fatty acid synthase, therefore reducing the size of lipid droplets and stimulating the efflux of free fatty acids and glycerol from adipocytes (Kurokawa et al. 2010). In particular, palmitic and stearic acids released upon AIM-dependent adipocyte lipolysis were able to engage TLR4 signaling and stimulate the production of the chemokines monocyte chemoattractant protein-1, -2 and -3, and chemokine (C–C motif) ligand 5 (Ccl5)/Rantes in adipocytes. Chemokines released by adipocytes are important for monocyte/macrophage recruitment to adipose tissue. Possibly for this reason, mice deficient in AIM showed reduced obesity-associated infiltration of inflammatory macrophages and production of inflammatory cytokines in the adipose tissue (Kurokawa et al. 2011). However, these studies did not evaluate whether AIM deficiency additionally resulted in decreased macrophage survival in the adipose tissue. The pro-inflammatory actions of AIM contrast with the general role of LXRs as negative regulators of inflammation. It would be interesting to know whether the lipolysis-mediated proinflammatory activities of AIM are also observed under conditions that simultaneously stimulate LXR-mediated transrepression.

Liver X receptor α can be phosphorylated at serine 198 by casein kinase II. Phosphorylation of LXRα on this site seems to exert repressive effects on AIM expression, as overexpression of LXRα with a mutation at serine 198

resulted in increased induction of AIM in response to the LXR agonist TO901317. These effects were specific toward selective LXR targets, also including lipoprotein lipase, but not ABCA-1 and SREBP-1c (Torra et al. 2008). Whether this selective modulation of target genes also occurs in vivo deserves to be uncovered in the future.

Recent studies have highlighted a role for the LXR pathway in the positive regulation of phagocytosis of apoptotic cells. Several years ago, ABCA1 was reported to participate in the regulation of phagocytosis. ABCA1 expression favored engulfment by inducing local modifications of the membrane composition in phospholipids (Hamon et al. 2000), which can determine lateral mobility or clustering of receptors at intercellular contact sites and the recruitment of dynamin to forming phagosomes. Recently, c-mer tyrosine kinase (Mertk) has been also shown to be a direct target for LXR (A-Gonzalez et al. 2009). Mertk is a member of the Axl/Mer/Tyro3 receptor tyrosine kinase family that also participates in macrophage-mediated engulfment and clearance of apoptotic cells. Mertk functions as a receptor for Gas6, a protein that binds to phosphatidylserine exposed as an "eat-me" signal in the external side of the plasma membrane of dying cells (Scott et al. 2001). As mentioned earlier in this review, phagocytosis of apoptotic cells resulted in LXR activation (A-Gonzalez et al. 2009). Therefore, apoptotic cells are able to promote their own clearance by providing a mechanism to activate LXR and induce Mertk and ABCA1 in phagocytic cells. ABCA1 may also play an extra role in this process by facilitating the efflux of excess cholesterol derived from extensive phagocytosis of dead cells. Interestingly, functional expression of LXRs was required for inhibition of the expression of several proinflammatory mediators and for transcriptional induction of the deactivating cytokines tissue growth factor β and IL-10 in macrophages that had engulfed apoptotic cells. These observations suggest that the LXR pathway serves an important role also in macrophage deactivation in response to phagocytosis of apoptotic cells. In fact, animals deficient in LXRs exhibited both a defect in phagocytosis of apoptotic cells and a breakdown in self-tolerance with development of autoantibodies against nuclear proteins and double-stranded DNA and development of autoimmune glomerulonephritis (A-Gonzalez et al. 2009). In line with these observations, administration of GW3965 ameliorated the progression of autoimmune disease in a murine model of systemic lupus erythematosus (A-Gonzalez et al. 2009).

Macrophages also exert an important angiogenic activity in the context of wound healing, chronic inflammation and cancer. Vascular endothelial growth factor (VEGF), a cytokine produced by macrophages that promotes angiogenesis and vasculogenesis, was shown several years ago to be a direct target of positive transcriptional regulation by LXRs. Induction of VEGF expression by LXR agonists was independent of the activation of hypoxia-inducible factor-1α (Walczak et al. 2004). These observations suggested a putative role for LXRs in the regulation of angiogenesis.

In dendritic cells, LXR agonists have been shown to increase the expression of CC chemokine receptor-7 (CCR7) (Feig et al. 2010), which is a receptor for the chemokines Ccl19 and Ccl21 (Ricart et al. 2011). In atherosclerotic apoE-deficient mice, treatment with the LXR agonist TO901317 resulted in a CCR7-dependent decrease in $CD68^+$ cells (putatively macrophages and dendritic cells) within atherosclerotic plaques, indicative of atherosclerosis regression. In apoE-deficient mice transplanted with aortic arches from apoE-deficient mice with a bone marrow deficiency of either LXRα or LXRβ, the expression of CCR7, the emigration of $CD68^+$ cells and the regression of atherosclerotic plaques were reduced. The CCR7 gene promoter was indeed shown to contain a functional LXRE. These observations suggest that LXRs potentiate CCR7-mediated monocyte-derived cell egress during atherosclerosis regression in mice (Feig et al. 2010). These observations contrast, however, with results obtained in other animal models. In particular, tumors have been shown to produce putative LXR agonists that inhibit the expression of CCR7 in dendritic cells and therefore interfere with CCR7-mediated dendritic cell migration to secondary lymphoid organs (Villablanca et al. 2010). Interestingly, tumors expressing the enzyme sulfotransferase (SULT)1B1b, an enzyme that transfers sulfate groups to oxysterols leading to their inactivation as LXR agonists, were not able to inhibit dendritic cell migration to tumor-draining lymph nodes. Moreover, inflammatory responses developed more efficiently within these tumors. These observations suggest that inhibition of CCR7 by LXR agonists that are generated by tumor cells represents a strategy for immunoescaping (Villablanca et al. 2010). Inhibition of the migration of dendritic cells by LXR agonists was also reported in the context of infection by human immunodeficiency virus (HIV). Migration of HIV-1-carrying dendritic cells from the mucosa to draining lymph nodes contributes to virus dissemination toward $CD4^+$ T lymphocytes, a process known as trans-infection, which is enhanced by simultaneous TLR-induced maturation of dendritic cells. In the model studied by Hanley et al. (2010), LXR agonists interfered with dendritic cell maturation and their production of proinflammatory cytokines. LXR agonists prevented TLR-upregulation of CCR7, which correlated with decreased dendritic cell migration in response to the chemokine Ccl21. In addition to these observations, LXR activation inhibited the capture of HIV-1 by dendritic cells. Intracellular cholesterol has been shown to be required for the capture of HIV-1 by dendritic

cells. The inhibitory effects of LXR agonists on HIV capture were based on increased ABCA1-mediated cholesterol efflux in those cells (Hanley et al. 2010). In contrast to the inhibitory role of LXR agonists on dendritic cell maturation, administration of LXR agonists resulted in increased expression of maturation markers, such as CD80 and CD86, in human monocyte-derived dendritic cells. In mature dendritic cells, LXR activation augmented the production of inflammatory cytokines, including IL-12, TNF-α, IL-6 and IL-8, and increased the capacity of these cells to activate $CD4^+$ T cell proliferation upon stimulation with TLR4 or TLR3 ligands, with prolonged NF-κB signaling observed under these conditions (Töröcsik et al. 2010). Molecular studies aimed at identifying the mechanisms involved in LXR-mediated regulation of dendritic cell biology may help clarify the discrepancies between activating and repressing effects of LXR agonists on this cell type (e.g., on CCR7 expression) in different experimental models.

LXRs as Regulators of Immune Cell Proliferation

Several groups have reported the capability of LXR agonists to exert anti-proliferative actions in a number of cell types (Blaschke et al. 2004; Chuu et al. 2007; Meng et al. 2009; Vedin et al. 2009), including T lymphocytes and macrophages (Bensinger et al. 2008; Geyeregger et al. 2009; Kim et al. 2010; Pascual-García et al. 2011). In T lymphocytes, LXR agonists inhibited mitogen- and antigen-driven proliferation through a pathway involving functional expression of ABCG1, which suggests that reduction of cellular cholesterol contents due to increased cholesterol efflux represents a mechanism for the LXR-mediated anti-proliferative effects in these cells. Indeed, these studies revealed that the LXR pathway was negatively regulated during T cell proliferation. T cell expansion in response to anti-CD3 antibodies correlated with enhanced expression of the LXR ligand-inactivating enzyme SULT2B1. As a consequence, reduced expression of the LXR target genes ABCA1 and ABCG1 was observed in proliferating T cells. Simultaneously to these effects, the SREBP-2 pathway for cholesterol synthesis was upregulated. Reduced expression of ABCG1 was proposed to affect the intracellular distribution of cholesterol and represent a strategy for T cells to ensure adequate progression through the cell cycle. Conversely, lymphocytes deficient in LXR showed higher levels of proliferation during antigen-driven responses (Bensinger et al. 2008). The ability of LXR agonists to limit the expansion of T cell populations reveals, therefore, the capability of the LXR pathway to interfere with the onset of adaptive immune responses.

Liver X receptor agonists also inhibited the proliferation of macrophages in an in vivo model of concanavalin A-induced peritonitis. In vitro experiments revealed that the anti-proliferative actions of LXR agonists in primary bone marrow-derived macrophages were independent of functional expression of ABCG1, ABCA1 or apoE (Pascual-García et al. 2011), which, in comparison with the observations obtained from T cells, suggests that different mechanisms account for the anti-proliferative effects of LXR agonists in distinct cell types. In macrophages, LXR activation resulted in changes in the protein expression of several members of the cyclin and cyclin-dependent kinase family. LXR activation did not lead to a reduction in the expression of S-phase kinase-associated protein 2, a molecule involved in ubiquitin-mediated degradation of the cell cycle negative regulator p27Kip1 (Carrano et al. 1999), in contrast to what had been observed in some other cellular systems (Blaschke et al. 2004; Chuu et al. 2007). In the same line of evidence, expression of p27Kip1 was not required for the LXR anti-proliferative effects in macrophages (Pascual-García et al. 2011). More work will have to be developed to know whether distinct anti-proliferative mechanisms reported in different cell types derive from common originating effects.

Future Perspectives

In the last decade, the identification of positive and negative effects of LXR agonists on target genes with key roles in the immune response has opened the door to the consideration of pharmacological strategies based on LXR activation for treating disorders highly dependent on inflammatory or other type of immune responses. Based on the identification of binding sites through recent genome-wide profiling studies (Boergesen et al. 2012), novel genes regulated by LXRs are expected to be reported in the subsequent years, which will help understand global effects in the organism mediated by these nuclear receptors. Development of agonists or combined treatments that selectively mediate the transrepressing or transactivating actions of LXRs may be of interest to impede human disease. The roles of posttranscriptional modifications of LXRs deserve further attention in this context. As described early in this review, several natural compounds from bacterial or plant origin have been shown to exert LXR agonistic activity. In vivo studies directed at the understanding of the impact of such agonists on positive/negative regulation of LXR-dependent gene expression will help define whether the development of preventive strategies based on the use of natural agonists through the diet may help reduce the incidence of long-term chronic diseases with either an inflammatory or an autoimmunity component.

Acknowledgments This work was supported by grants from the Spanish Ministry of Science and Innovation (SAF2010-14989) and the Fundació Marató de TV3 (080930) (to A. F. Valledor.). M. Pascual-García is recipient of a fellowship from the Spanish Ministry of Education (FPU program).

References

A-Gonzalez N, Bensinger SJ, Hong C et al (2009) Apoptotic cells promote their own clearance and immune tolerance through activation of the nuclear receptor LXR. Immunity 31:245–258

Arai S, Shelton JM, Chen M et al (2005) A role for the apoptosis inhibitory factor AIM/Spalpha/Api6 in atherosclerosis development. Cell Metab 1:201–213

Baldán Á, Bojanic DD, Edwards PA (2009) The ABCs of sterol transport. J Lipid Res 50(Suppl):S80–S85

Benkoussa M, Brand C, Delmotte M-H et al (2002) Retinoic acid receptors inhibit AP1 activation by regulating extracellular signal-regulated kinase and CBP recruitment to an AP1-responsive promoter. Mol Cell Biol 22:4522–4534

Bensinger SJ, Bradley MN, Joseph SB et al (2008) LXR signaling couples sterol metabolism to proliferation in the acquired immune response. Cell 134:97–111

Blaschke F, Leppanen O, Takata Y et al (2004) Liver X receptor agonists suppress vascular smooth muscle cell proliferation and inhibit neointima formation in balloon-injured rat carotid arteries. Circ Res 95:e110–e123

Boergesen M, Pedersen TA, Gross B et al (2012) Genome-wide profiling of LXR, RXR and PPARα in mouse liver reveals extensive sharing of binding sites. Mol Cell Biol 32:852–867

Bruhn KW, Marathe C, Maretti-Mira AC et al (2010) LXR deficiency confers increased protection against visceral *Leishmania* infection in mice. PLoS Negl Trop Dis 4:e886

Cao F, Castrillo A, Tontonoz P et al (2007) *Chlamydia pneumoniae*-induced macrophage foam cell formation is mediated by Toll-like receptor 2. Infect Immun 75:753–759

Carrano AC, Eytan E, Hershko A et al (1999) SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. Nat Cell Biol 1:193–199

Castrillo A, Joseph SB, Vaidya SA et al (2003) Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism. Mol Cell 12:805–816

Cha JY, Repa JJ (2007) The liver X receptor (LXR) and hepatic lipogenesis. The carbohydrate-response element-binding protein is a target gene of LXR. J Biol Chem 282:743–751

Chang KCN, Shen Q, Oh IG et al (2008) Liver X receptor is a therapeutic target for photoaging and chronological skin aging. Mol Endocrinol 22:2407–2419

Chen JD, Evans RM (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 377:454–457

Chen S, Sorrentino R, Shimada K et al (2008) *Chlamydia pneumoniae*-induced foam cell formation requires MyD88-dependent and -independent signaling and is reciprocally modulated by liver X receptor activation. J Immunol 181:7186–7193

Cheng O, Ostrowski RP, Liu W et al (2010) Activation of liver X receptor reduces global ischemic brain injury by reduction of nuclear factor-kappaB. Neuroscience 166:1101–1109

Chuu CP, Kokontis JM, Hiipakka RA et al (2007) Modulation of liver X receptor signaling as novel therapy for prostate cancer. J Biomed Sci 14:543–553

Collins JL, Fivush AM, Watson MA et al (2002) Identification of a nonsteroidal liver X receptor agonist through parallel array synthesis of tertiary amines. J Med Chem 45:1963–1966

Commerford SR, Vargas L, Dorfman SE et al (2007) Dissection of the insulin-sensitizing effect of liver X receptor ligands. Mol Endocrinol 21:3002–3012

Crisafulli C, Di PR, Mazzon E et al (2010a) Liver X receptor agonist treatment reduced splanchnic ischemia and reperfusion injury. J Leukoc Biol 87:309–321

Crisafulli C, Mazzon E, Paterniti I et al (2010b) Effects of liver X receptor agonist treatment on signal transduction pathways in acute lung inflammation. Respir Res 11:19

Denechaud PD, Bossard P, Lobaccaro JM et al (2008) ChREBP, but not LXRs, is required for the induction of glucose-regulated genes in mouse liver. J Clin Invest 118:956–964

Durante W, Johnson FK, Johnson RA (2007) Arginase: a critical regulator of nitric oxide synthesis and vascular function. Clin Exp Pharmacol Physiol 34:906–911

Edwards PA, Kast HR, Anisfeld AM (2002) BAREing it all: the adoption of LXR and FXR and their roles in lipid homeostasis. J Lipid Res 43:2–12

Feig JE, Pineda-Torra I, Sanson M et al (2010) LXR promotes the maximal egress of monocyte-derived cells from mouse aortic plaques during atherosclerosis regression. J Clin Invest 120:4415–4424

Forman BM, Ruan B, Chen J et al (1997) The orphan nuclear receptor LXRalpha is positively and negatively regulated by distinct products of mevalonate metabolism. Proc Natl Acad Sci USA 94:10588–10593

Fowler AJ, Sheu MY, Schmuth M et al (2003) Liver X receptor activators display anti-inflammatory activity in irritant and allergic contact dermatitis models: liver-X-receptor-specific inhibition of inflammation and primary cytokine production. J Invest Dermatol 120:246–255

Galon J, Franchimont D, Hiroi N et al (2002) Gene profiling reveals unknown enhancing and suppressive actions of glucocorticoids on immune cells. FASEB J 16:61–71

Geyeregger R, Zeyda M, Bauer W et al (2007) Liver X receptors regulate dendritic cell phenotype and function through blocked induction of the actin-bundling protein fascin. Blood 109:4288–4295

Geyeregger R, Shehata M, Zeyda M et al (2009) Liver X receptors interfere with cytokine-induced proliferation and cell survival in normal and leukemic lymphocytes. J Leukoc Biol 86:1039–1048

Ghisletti S, Huang W, Ogawa S et al (2007) Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma. Mol Cell 25:57–70

Ghisletti S, Huang W, Jepsen K et al (2009) Cooperative NCoR/SMRT interactions establish a corepressor-based strategy for integration of inflammatory and anti-inflammatory signaling pathways. Genes Dev 23:681–693

Glass CK, Ogawa S (2006) Combinatorial roles of nuclear receptors in inflammation and immunity. Nat Rev Immunol 6:44–55

Gong H, He J, Lee JH et al (2009) Activation of the liver X receptor prevents lipopolysaccharide-induced lung injury. J Biol Chem 284:30113–30121

Hamon Y, Broccardo C, Chambenoit O et al (2000) ABC1 promotes engulfment of apoptotic cells and transbilayer redistribution of phosphatidylserine. Nat Cell Biol 2:399–406

Hanley TM, Blay Puryear W, Gummuluru S et al (2010) PPARgamma and LXR signaling inhibit dendritic cell-mediated HIV-1 capture and trans-infection. PLoS Pathog 6:e1000981

Heine G, Dahten A, Hilt K et al (2009) Liver X receptors control IgE expression in B cells. J Immunol 182:5276–5282

Herath KB, Jayasuriya H, Guan Z et al (2005) Anthrabenzoxocinones from *Streptomyces* sp. as liver X receptor ligands and antibacterial agents. J Nat Prod 68:1437–1440

Hindinger C, Hinton DR, Kirwin SJ et al (2006) Liver X receptor activation decreases the severity of experimental autoimmune encephalomyelitis. J Neurosci Res 84:1225–1234

Hoberg JE, Yeung F, Mayo MW (2004) SMRT derepression by the IkappaB kinase alpha: a prerequisite to NF-kappaB transcription and survival. Mol Cell 16:245–255

Hörlein AJ, Näär AM, Heinzel T et al (1995) Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 377:397–404

Huang W, Ghisletti S, Saijo K et al (2011) Coronin 2A mediates actin-dependent de-repression of inflammatory response genes. Nature 470:414–418

Hwang EJ, Lee JM, Jeong J et al (2009) SUMOylation of RORalpha potentiates transcriptional activation function. Biochem Biophys Res Commun 378:513–517

Jakobsson T, Venteclef N, Toresson G et al (2009) GPS2 is required for cholesterol efflux by triggering histone demethylation, LXR recruitment, and coregulator assembly at the ABCG1 locus. Mol Cell 34:510–518

Janowski BA, Willy PJ, Devi TR et al (1996) An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. Nature 383:728–731

Janowski BA, Grogan MJ, Jones SA et al (1999) Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. Proc Natl Acad Sci USA 96:266–271

Jayasuriya H, Herath KB, Ondeyka JG et al (2005) Diterpenoid, steroid, and triterpenoid agonists of liver X receptors from diversified terrestrial plants and marine sources. J Nat Prod 68:1247–1252

Jiang Q, Lee CYD, Mandrekar S et al (2008) ApoE promotes the proteolytic degradation of Abeta. Neuron 58:681–693

Joseph SB, McKilligin E, Pei L et al (2002) Synthetic LXR ligand inhibits the development of atherosclerosis in mice. Proc Natl Acad Sci USA 99:7604–7609

Joseph SB, Castrillo A, Laffitte BA et al (2003) Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. Nat Med 9:213–219

Joseph SB, Bradley MN, Castrillo A et al (2004) LXR-dependent gene expression is important for macrophage survival and the innate immune response. Cell 119:299–309

Kanno Y, Levi BZ, Tamura T et al (2005) Immune cell-specific amplification of interferon signaling by the IRF-4/8-PU.1 complex. J Interf Cytokine Res 25:770–779

Kim KH, Lee GY, Kim JI et al (2010) Inhibitory effect of LXR activation on cell proliferation and cell cycle progression through lipogenic activity. J Lipid Res 51:3425–3433

Korf H, Vander Beken S, Romano M et al (2009) Liver X receptors contribute to the protective immune response against *Mycobacterium tuberculosis* in mice. J Clin Invest 119:1626–1637

Kratzer A, Buchebner M, Pfeifer T et al (2009) Synthetic LXR agonist attenuates plaque formation in apoE-/- mice without inducing liver steatosis and hypertriglyceridemia. J Lipid Res 50:312–326

Kropf P, Fuentes JM, Fähnrich E et al (2005) Arginase and polyamine synthesis are key factors in the regulation of experimental leishmaniasis in vivo. FASEB J 19:1000–1002

Kurokawa J, Arai S, Nakashima K et al (2010) Macrophage-derived AIM is endocytosed into adipocytes and decreases lipid droplets via inhibition of fatty acid synthase activity. Cell Metab 11:479–492

Kurokawa J, Nagano H, Ohara O et al (2011) Apoptosis inhibitor of macrophage (AIM) is required for obesity-associated recruitment of inflammatory macrophages into adipose tissue. Proc Natl Acad Sci USA 108:12072–12077

Laffitte BA, Joseph SB, Walczak R et al (2001) Autoregulation of the human liver X receptor alpha promoter. Mol Cell Biol 21:7558–7568

Laffitte BA, Chao LC, Li J et al (2003) Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. Proc Natl Acad Sci USA 100:5419–5424

Lee SK, Kim JH, Lee YC et al (2000) Silencing mediator of retinoic acid and thyroid hormone receptors, as a novel transcriptional corepressor molecule of activating protein-1, nuclear factor-kappaB, and serum response factor. J Biol Chem 275:12470–12474

Lee MH, Lu K, Hazard S et al (2001) Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nat Genet 27:79–83

Lee JH, Park SM, Kim OS et al (2009) Differential SUMOylation of LXRalpha and LXRbeta mediates transrepression of STAT1 inflammatory signaling in IFN-gamma-stimulated brain astrocytes. Mol Cell 35:806–817

Lehmann JM, Kliewer SA, Moore LB et al (1997) Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. J Biol Chem 272:3137–3140

Li N, Salter RC, Ramji DP (2011) Molecular mechanisms underlying the inhibition of IFN-γ-induced, STAT1-mediated gene transcription in human macrophages by simvastatin and agonists of PPARs and LXRs. J Cell Biochem 112:675–683

Lu K, Lee MH, Hazard S et al (2001) Two genes that map to the STSL locus cause sitosterolemia: genomic structure and spectrum of mutations involving sterolin-1 and sterolin-2, encoded by ABCG5 and ABCG8, respectively. Am J Hum Genet 69:278–290

Mangelsdorf DJ, Thummel C, Beato M et al (1995) The nuclear receptor superfamily: the second decade. Cell 83:835–839

Marathe C, Bradley MN, Hong C et al (2006) The arginase II gene is an anti-inflammatory target of liver X receptor in macrophages. J Biol Chem 281:32197–32206

Meng ZX, Nie J, Ling JJ et al (2009) Activation of liver X receptors inhibits pancreatic islet beta cell proliferation through cell cycle arrest. Diabetologia 52:125–135

Mitro N, Mak PA, Vargas L et al (2007) The nuclear receptor LXR is a glucose sensor. Nature 445:219–223

Moazed TC, Campbell LA, Rosenfeld ME et al (1999) *Chlamydia pneumoniae* infection accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. J Infect Dis 180:238–241

Molteni V, Li X, Nabakka J et al (2007) *N*-Acylthiadiazolines, a new class of liver X receptor agonists with selectivity for LXRbeta. J Med Chem 50:4255–4259

Morales JR, Ballesteros I, Deniz JM et al (2008) Activation of liver X receptors promotes neuroprotection and reduces brain inflammation in experimental stroke. Circulation 118:1450–1459

Naiki Y, Sorrentino R, Wong MH et al (2008) TLR/MyD88 and liver X receptor alpha signaling pathways reciprocally control *Chlamydia pneumoniae*-induced acceleration of atherosclerosis. J Immunol 181:7176–7185

Nunomura S, Endo K, Makishima M et al (2010) Oxysterol represses high-affinity IgE receptor-stimulated mast cell activation in liver X receptor-dependent and -independent manners. FEBS Lett 584:1143–1148

Ogawa S, Lozach J, Jepsen K et al (2004) A nuclear receptor corepressor transcriptional checkpoint controlling activator protein 1-dependent gene networks required for macrophage activation. Proc Natl Acad Sci USA 101:14461–14466

Ogawa S, Lozach J, Benner C et al (2005) Molecular determinants of crosstalk between nuclear receptors and toll-like receptors. Cell 122:707–721

Palvimo JJ (2007) PIAS proteins as regulators of small ubiquitin-related modifier (SUMO) modifications and transcription. Biochem Soc Trans 35(Pt 6):1405–1408

Park MC, Kwon YJ, Chung SJ et al (2010) Liver X receptor agonist prevents the evolution of collagen-induced arthritis in mice. Rheumatology 49:882–890

Pascual G, Fong AL, Ogawa S et al (2005) A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. Nature 437:759–763

Pascual-García M, Carbó JM, León T et al (2011) Liver X receptors inhibit macrophage proliferation through downregulation of cyclins D1 and B1 and cyclin-dependent kinases 2 and 4. J Immunol 186:4656–4667

Paterniti I, Genovese T, Mazzon E et al (2010) Liver X receptor agonist treatment regulates inflammatory response after spinal cord trauma. J Neurochem 112:611–624

Peet DJ, Turley SD, Ma W et al (1998) Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. Cell 93:693–704

Peyron P, Vaubourgeix J, Poquet Y et al (2008) Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for *M. tuberculosis* persistence. PLoS Pathog 4:e1000204

Plat J, Nichols JA, Mensink RP (2005) Plant sterols and stanols: effects on mixed micellar composition and LXR (target gene) activation. J Lipid Res 46:2468–2476

Poukka H, Karvonen U, Janne OA et al (2000) Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). Proc Natl Acad Sci USA 97:14145–14150

Pourcet B, Feig JE, Vengrenyuk Y et al (2011) LXRα regulates macrophage arginase 1 through PU.1 and interferon regulatory factor 8. Circ Res 109:492–501

Quinet EM, Basso MD, Halpern AR et al (2009) LXR ligand lowers LDL cholesterol in primates, is lipid neutral in hamster, and reduces atherosclerosis in mouse. J Lipid Res 50:2358–2370

Repa JJ, Liang G, Ou J et al (2000a) Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. Genes Dev 14:2819–2830

Repa JJ, Turley SD, Lobaccaro JA et al (2000b) Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. Science 289:1524–1529

Repa JJ, Berge KE, Pomajzl C et al (2002) Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta. J Biol Chem 277:18793–18800

Ricart BG, John B, Lee D et al (2011) Dendritic cells distinguish individual chemokine signals through CCR7 and CXCR4. J Immunol 186:53–61

Ricote M, Valledor AF, Glass CK (2004) Decoding transcriptional programs regulated by PPARs and LXRs in the macrophage: effects on lipid homeostasis, inflammation, and atherosclerosis. Arterioscler Thromb Vasc Biol 24:230–239

Schultz JR, Tu H, Luk A et al (2000) Role of LXRs in control of lipogenesis. Genes Dev 14:2831–2838

Scott RS, McMahon EJ, Pop SM et al (2001) Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature 411:207–211

Singh SB, Ondeyka JG, Liu W et al (2005) Discovery and development of dimeric podocarpic acid leads as potent agonists of liver X receptor with HDL cholesterol raising activity in mice and hamsters. Bioorg Med Chem Lett 15:2824–2828

Sironi L, Mitro N, Cimino M et al (2008) Treatment with LXR agonists after focal cerebral ischemia prevents brain damage. FEBS Lett 582:3396–3400

Smoak K, Madenspacher J, Jeyaseelan S et al (2008) Effects of liver X receptor agonist treatment on pulmonary inflammation and host defense. J Immunol 180:3305–3312

Song C, Liao S (2000) Cholestenoic acid is a naturally occurring ligand for liver X receptor alpha. Endocrinology 141:4180–4184

Terasaka N, Hiroshima A, Koieyama T et al (2003) T-0901317, a synthetic liver X receptor ligand, inhibits development of atherosclerosis in LDL receptor-deficient mice. FEBS Lett 536:6–11

Terasaka N, Hiroshima A, Ariga A et al (2005) Liver X receptor agonists inhibit tissue factor expression in macrophages. FEBS J 272:1546–1556

Tian S, Poukka H, Palvimo JJ et al (2002) Small ubiquitin-related modifier-1 (SUMO-1) modification of the glucocorticoid receptor. Biochem J 367(Pt 3):907–911

Töröcsik D, Baráth M, Benko S et al (2010) Activation of liver X receptor sensitizes human dendritic cells to inflammatory stimuli. J Immunol 184:5456–5465

Torra IP, Ismaili N, Feig JE et al (2008) Phosphorylation of liver X receptor alpha selectively regulates target gene expression in macrophages. Mol Cell Biol 28:2626–2636

Traves PG, Hortelano S, Zeini M et al (2007) Selective activation of liver X receptors by acanthoic acid-related diterpenes. Mol Pharmacol 71:1545–1553

Treuter E, Venteclef N (2011) Transcriptional control of metabolic and inflammatory pathways by nuclear receptor SUMOylation. Biochim Biophys Acta 1812:909–918

Ullrich JW, Morris R, Bernotas RC et al (2010) Synthesis of 4-(3-biaryl)quinoline sulfones as potent liver X receptor agonists. Bioorg Med Chem Lett 20:2903–2907

Valledor AF, Hsu LC, Ogawa S et al (2004) Activation of liver X receptors and retinoid X receptors prevents bacterial-induced macrophage apoptosis. Proc Natl Acad Sci USA 101:17813–17818

Vedin LL, Lewandowski SA, Parini P et al (2009) The oxysterol receptor LXR inhibits proliferation of human breast cancer cells. Carcinogenesis 30:575–579

Venkateswaran A, Repa JJ, Lobaccaro JM et al (2000) Human white/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages. A transcriptional role for specific oxysterols. J Biol Chem 275:14700–14707

Venteclef N, Jakobsson T, Ehrlund A et al (2010) GPS2-dependent corepressor/SUMO pathways govern anti-inflammatory actions of LRH-1 and LXRbeta in the hepatic acute phase response. Genes Dev 24:381–395

Villablanca EJ, Raccost L, Zhou D et al (2010) Tumor-mediated liver X receptor-alpha activation inhibits CC chemokine receptor-7 expression on dendritic cells and dampens antitumor responses. Nat Med 16:98–105

Wagner BL, Valledor AF, Shao G et al (2003) Promoter-specific roles for liver X receptor/corepressor complexes in the regulation of ABCA1 and SREBP1 gene expression. Mol Cell Biol 23:5780–5789

Walczak R, Joseph SB, Laffitte BA et al (2004) Transcription of the vascular endothelial growth factor gene in macrophages is regulated by liver X receptors. J Biol Chem 279:9905–9911

Wang YY, Dahle MK, Agren J et al (2006) Activation of the liver X receptor protects against hepatic injury in endotoxemia by suppressing Kupffer cell activation. Shock 25:141–146

Wang D, Liu M, Wang Y et al (2011) Synthetic LXR agonist TO901317 attenuates lipopolysaccharide-induced acute lung injury in rats. Int Immunopharmacol 11:2098–2103

Williams S, Bledsoe RK, Collins JL et al (2003) X-ray crystal structure of the liver X receptor beta ligand binding domain: regulation by a histidine-tryptophan switch. J Biol Chem 278:27138–27143

Willy PJ, Umesono K, Ong ES et al (1995) LXR, a nuclear receptor that defines a distinct retinoid response pathway. Genes Dev 9:1033–1045

Yang C, McDonald JG, Patel A et al (2006) Sterol intermediates from cholesterol biosynthetic pathway as liver X receptor ligands. J Biol Chem 281:27816–27826

Yu L, York J, von Bergmann K et al (2003) Stimulation of cholesterol excretion by the liver X receptor agonist requires ATP-binding cassette transporters G5 and G8. J Biol Chem 278:15565–15570

Zelcer N, Hong C, Boyadjian R et al (2009) LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. Science 325:100–104