Current Opinion in Pharmacology

Integrating the roles of Liver X Receptors in inflammation and infection: mechanisms and outcomes

--Manuscript Draft--

Manuscript Number:	COPHAR-D-20-00010R1
Full Title:	Integrating the roles of Liver X Receptors in inflammation and infection: mechanisms and outcomes
Article Type:	SI: 53 Immunomodulation (2020)
Short Title:	Liver X Receptors in inflammation and infection
Keywords:	LXR, infection, inflammation, bacteria, virus
Corresponding Author:	ANNABEL F VALLEDOR, PhD in Biology Universitat de Barcelona Barcelona, BARCELONA SPAIN
Corresponding Author's Institution:	Universitat de Barcelona
Corresponding Author E-Mail:	afernandezvalledor@ub.edu
First Author:	Estibaliz Glaría
Order of Authors:	Estibaliz Glaría
	Nicole A. Letelier
	ANNABEL F VALLEDOR, PhD in Biology
Abstract:	Liver X receptors (LXRs) are transcription factors from the nuclear receptor family that can be pharmacologically activated by high-affinity agonists. LXR activation exerts a combination of metabolic and anti-inflammatory actions that result in the modulation of immune responses and in the amelioration of inflammatory disorders. In addition, LXR agonists modulate the metabolism of infected cells and limit the infectivity and/or growth of several pathogens. This review gives an overview of the recent advances in understanding the complexity of the mechanisms through which the LXR pathway controls inflammation and host-cell pathogen interaction.
Author Comments:	This is a contribution for the “Immunomodulation 2020 Special Issue”. If possible, we would like to choose Dr. Tamás Röszer as handling editor. I confirm that all authors concur with the submission and that there is no conflict of interest.

Integrating the roles of Liver X Receptors in inflammation and infection: mechanisms and outcomes

Estibaliz Glaría[1,2], Nicole A. Letelier[1,2] and Annabel F. Valledor[1,2]

[1]Department of Cell Biology, Physiology and Immunology, School of Biology, University of Barcelona, 08028 Barcelona, Spain

[2]Institute of Biomedicine of the University of Barcelona (IBUB), 08028 Barcelona, Spain

Corresponding author: Dr. Annabel F. Valledor, Phone: +34-93-4039385; FAX: +34-93-4110358; e-mail address: afernandezvalledor@ub.edu

Abstract

Liver X receptors (LXRs) are transcription factors from the nuclear receptor family that can be pharmacologically activated by high-affinity agonists. LXR activation exerts a combination of metabolic and anti-inflammatory actions that result in the modulation of immune responses and in the amelioration of inflammatory disorders. In addition, LXR agonists modulate the metabolism of infected cells and limit the infectivity and/or growth of several pathogens. This review gives an overview of the recent advances in understanding the complexity of the mechanisms through which the LXR pathway controls inflammation and host-cell pathogen interaction.

Short Title: Liver X Receptors in inflammation and infection

Keywords: LXR, infection, inflammation, bacteria, virus

Declarations of interest: none

Introduction

Liver X receptors (LXRs), namely, NR1H3 (LXRα) and NR1H2 (LXRβ), are transcription factors from the nuclear receptor family (reviewed in [1]). LXRβ is ubiquitously expressed, whereas LXRα expression is more predominant in tissues that are highly involved in lipid metabolism. Within the immune system, macrophages, dendritic cells, and neutrophils express both isoforms, B lymphocytes express mainly LXRβ, and T cell populations have been reported to express either LXRβ or both isoforms [2–6]. LXRs can be activated by endogenous agonists, including specific oxysterols and intermediates of cholesterol biosynthesis, and by specific high-affinity agonists that are frequently used *in vivo* to explore the consequences of pharmacological LXR activation.

LXRs form heterodimers with retinoid X receptors (RXRs) on LXR response elements and, once activated by agonists, they positively regulate the expression of target genes. Recent studies have proposed three possible modes of action for LXRα- and LXRβ-mediated transcriptional activation [7]. Two mechanisms are based on the canonical induction of target gene expression by RXR-LXR heterodimers in a pharmacologically responsive-manner. In the absence of agonistic activation, the target genes are repressed by LXR/RXR heterodimers, which may lead to de-repression in the absence of functional LXRs [8]. A third mechanism was proposed, by which the expression of a number of transcripts depends on the presence of LXRs, but these transcripts are not upregulated upon pharmacological LXR activation [7].

Most of the targets that are positively induced in response to LXR agonists play key roles in lipid and glucose metabolism (reviewed in [1]). These include (but are not restricted to) several sterol transporters from the ATP binding cassette (ABC) family, e.g., ABCA1 and ABCG1; transcription factors sterol regulatory element-binding protein 1c

(SREBP1c) and carbohydrate regulatory element-binding protein with important lipogenic roles; the E3 ubiquitin ligase inducible degrader of the low-density lipoprotein receptor (IDOL); and several apolipoproteins involved in lipid transport.

The use of immortalized murine macrophages that express equivalent levels of FLAG-tagged LXRα or LXRβ in an LXR-deficient background has contributed in defining the specific roles of LXR isoforms in gene regulation. In addition to a signature simultaneously regulated by both isoforms, LXRα selectively regulates the expression of genes linked to the control of apoptosis and leukocyte migration, whereas LXRβ-specific functions are associated with lymphocyte differentiation and selection [7].

In addition to its positive effects on gene transcription, LXRs can negatively affect the expression of inflammatory mediators through a plethora of mechanisms, which will be further revised in the following section. Agonist-bound LXRs undergo conjugation to small ubiquitin-related modifier (SUMO), a process known as SUMOylation, which is required for some of the repressive actions of these proteins [9]. Moreover, a study in astrocytes proposed different SUMOylation pathways for agonist-bound LXRα and LXRβ, mediated by separate members of the SUMO E3 ligase family [10].

In a complex scenario combining metabolic and anti-inflammatory actions, LXRs are able to modulate immune responses. These actions are particularly relevant in the management of an infection, as a number of pathogens are able to hijack host metabolic pathways for their own benefit. This review integrates the recent conceptual advances in understanding the complexity of mechanisms used by the LXR pathway to control inflammation and the response of the host to infection.

LXRs as attenuators of inflammatory disorders

Accumulated evidence indicates the importance of the LXR pathway in the negative control of inflammatory conditions. For example, pharmacological activation of LXRs reduced the extent of the inflammatory response in murine models of dermatitis [11,12], neuroinflammation [13,14], lupus [15], arthritis [16], and atherosclerosis [12], consistent with the fact that LXR-deficient mice develop an age-related lupus-like autoimmune disease [17]. Furthermore, several polymorphisms affecting the promoter region of the gene encoding LXRα were associated with susceptibility to systemic lupus erythematosus in a Korean cohort [18].

To explain the anti-inflammatory actions of pharmacologically activated LXRs, many studies have focused on the capability of high-affinity agonists to repress pro-inflammatory gene expression in macrophages and other cell types activated by the engagement of toll-like receptors (TLRs) or by endogenous inflammatory cytokines [9,12,14,19,20]. The LXR pathway impairs the transcriptional activity of nuclear factor kappa B (NF-κB) [12] and the recruitment of signal transducer and activator of transcription (STAT)1 to target gene promoters [10,14]. Putting together the pieces of evidence reported by different groups, it is apparent that several mechanisms contribute to the antagonizing actions of the LXR pathway on pro-inflammatory signaling (Figure 1). First, agonist-bound LXRs underwent SUMOylation and exerted transrepression by inhibiting the removal of nuclear receptor co-repressor (NCoR) complexes from pro-inflammatory gene promoters in response to lipopolysaccharide (LPS) [9,21,22]. In macrophages, this process involves the interaction of SUMOylated LXRs with the actin-binding protein CORONIN 2A (CORO2A). This interaction prevented actin recruitment to inflammatory gene promoters [21], in line with more recent evidence on the important roles of nuclear actin in the transcriptional control of macrophage activation [23]. In the

hepatic acute phase response in mice, the anti-inflammatory effects were selectively mediated by SUMOylated LXRβ and its interaction with the corepressor complex subunit G protein pathway suppressor 2 (GPS2) [22]. LXRβ also attenuated inflammatory cytokine production in murine mast cells stimulated with LPS or FcεRI crosslinking [24]. By contrast, both SUMOylated LXRα and LXRβ contributed in inhibiting the transcriptional response of murine macrophages and astrocytes to interferon (IFN)-γ through interference with STAT1 [10,14], which supports the notion that the relative contribution of each isoform depends on the cell type and the inflammatory trigger.

Direct repressive actions have also been proposed involving the binding of LXRs to specific sites within macrophage inflammatory gene enhancer elements and potential chromatin closure, although additional studies are required to better define this mechanism. Gene signatures affected by this repressive activity are associated with leukocyte cell-cell adhesion and neutrophil chemotaxis, in line with the inhibitory effects of LXR agonists on neutrophil infiltration in a model of zymosan-induced peritonitis in mice [25].

Other mechanisms contributing to the repression of inflammation imply the increased transcription of LXR targets in macrophages (Figure 1). First, the cholesterol and phospholipid transporter ABCA1, whose upregulation results in changes in membrane cholesterol homeostasis that are able to disrupt the recruitment of key adaptor molecules to lipid rafts, thereby antagonizing TLR signaling [20]. Second, several enzymes involved in the synthesis of fatty acids (fatty acid synthase) and in their conversion to derivatives with anti-inflammatory properties (predominantly mediated by stearoyl-CoA desaturase-2 (SCD2) and its products 9Z palmitoleic acid and oleic acid). The induction of these enzymes is exerted directly by LXRs or indirectly through the upregulation of the

transcription factor SREBP1c, depending on the type of agonist mediating LXR activation [26]. Third, MER, a receptor tyrosine kinase that recognizes the plasma protein growth arrest-specific 6 (GAS6) bound to phosphatidylserine (PtdSer) on the surface of apoptotic bodies and contributes to apoptotic cell clearance. The upregulation of MER has been proposed as a mechanism coupling the engulfment of apoptotic cells (efferocytosis) with the suppression of inflammatory pathways. Indeed, LXR deficiency resulted in an aberrant pro-inflammatory response of macrophages to apoptotic cells and in the development of autoimmune disease in mice [17]. Fourth, interferon regulatory factor (IRF)8, a transcription factor with multiple roles in myeloid cells. Through the upregulation of IRF8, the LXR pathway indirectly induced the expression of interleukin (IL)-18 binding protein (IL18BP) in the murine and human systems. IL18BP is a potent endogenous inhibitor of the pro-inflammatory cytokine IL-18 [27]. In parallel, LXR agonists also repressed IL18 transcription and blocked the processing of pro-IL-18 to its bioactive form by interfering with pro-caspase 1 expression and activation, indicating that the LXR pathway uses a combination of mechanisms to inhibit IL-18 production [27]. In addition, increased expression of IRF8 in murine macrophages overexpressing LXRα resulted in the upregulation of the anti-inflammatory enzyme arginase 1 [28].

Aside from the mechanisms described above, LXR agonists also induce the expression of apoptosis inhibitory factor secreted by macrophages (AIM)/CD5L [29,30]. In the murine system, this effect is mediated specifically by LXRα [7,30]. AIM/CD5L is a soluble scavenger receptor that can also act as a pattern-recognition receptor [31]. The endogenous production of human AIM/CD5L enhanced the expression of molecules involved in the resolution of inflammation, namely, MER and CD163, increased autophagy, and promoted an anti-inflammatory profile in human monocytes, resembling

the actions of IL-10 [32], which suggests the possibility that AIM might also be involved in facilitating the resolution of inflammation in response to LXR agonists.

In contrast to predominant anti-inflammatory activities of LXR agonists in macrophages, both pro- and anti-inflammatory actions have been reported in dendritic cells. In this regard, LXR activation downregulated the expression of the actin-bundling protein fascin in human myeloid dendritic cells, suppressing T cell stimulation due to inefficient immunological synapse formation [33]. However, prolonged NF-κB activation was detected in a different study, which translated into increased pro-inflammatory and T cell stimulatory activities [34]. Moreover, LXR agonism increased the chemotaxis of murine dendritic cells to signals generated in inflammatory settings, such as chemokine (C-C motif) ligand (CCL)19 and CCL21. This action was mediated through transcriptional activation of the ectoenzyme CD38, which is capable of converting nicotinamide adenine dinucleotide (NAD) into cyclic adenosine diphosphoribose (cADPR), an important second messenger in leukocyte trafficking [6]. These contrasting observations raise the question as to whether the effects of the LXR pathway are influenced by additional factors involved in dendritic cell maturation, which requires further exploration.

In addition to the actions in myeloid cells, LXR agonists inhibited the differentiation of murine and human helper T (Th)17 cells [35], which are a subset of $CD4^+$ T cells that secrete IL-17 and contribute to the pathogeny of inflammatory diseases [36]. An indirect mechanisms was proposed, by which LXR-induced SREBP1 negatively interfered with the activity of the transcription factor aryl hydrocarbon receptor on the *Il17* promoter. The differentiation of other $CD4^+$ T cell populations was also inhibited by LXR agonists [37], consistent with the anti-proliferative actions of LXRβ in murine T cells mediated by the upregulation of ABCG1 and subsequent changes in sterol homeostasis [3]. Moreover,

LXR activation induced regulatory T cell (Treg) expansion. Although a molecular mechanism was not defined, the oral administration of an LXR agonist in mice increased the abundance of gut-associated Treg with high suppressive capacity [38], which may provide additional explanation to the protective effects of the LXR pathway against the development of autoimmune diseases.

The interplay between the metabolic actions of LXRs and their role in the modulation of adaptive immune responses was further illustrated by the observation that excessive lipid accumulation in LXRβ-deficient antigen presenting cells induced the expression of B cell activating factor (BAFF) and a proliferation inducing ligand (APRIL) that support B cell survival and differentiation [39]. This scenario triggered the expansion of auto-reactive B cells and contributed to the development of autoimmune disease. In addition, despite the fact that B cells mostly express the LXRβ isoform, the activation of LXRα repressed BAFF production in human B cell lines through interference with NF-κB, STAT1 and mothers against decapentaplegic homolog 3 (SMAD3) signaling [40].

Beyond the anti-inflammatory actions in immune cells, transcriptional activation by LXRs impairs inflammatory responses in the liver in the context of metabolic disease. In particular, lysophosphatidylcholine acyltransferase 3 (LPCAT3) is highly induced by LXR agonists in hepatic cells, where it drives the incorporation of unsaturated fatty acids into phospholipids [41]. The activity of LPCAT3 resulted in reduced membrane lipid saturation, thus inhibiting pro-inflammatory c-Src kinase activation, and in decreased availability of saturated lipids for the synthesis of inflammatory mediators.

Impact of metabolic and anti-inflammatory actions of LXRs on host cell–pathogen interaction

Despite contributing to immunopathology, inflammatory responses are crucial for the establishment of an effective immune response against infection. Based on the anti-inflammatory actions of the LXR pathway, one could expect that LXR agonism would lead to deficient immune responses against infection. However, as will be discussed in this section, several studies have shown otherwise. Notably, a number of pathogens have developed mechanisms to hijack the host immune response and establish intracellular infection, particularly in phagocytic cells, even under adverse inflammatory conditions. Metabolic reprogramming of host cells or adaptation to their metabolic status are indeed common strategies used by intracellular pathogens for survival and replication [42].

Interestingly, many studies have shown increased expression and/or activity of LXR isoforms in leukocytes infected by intracellular pathogens [30,43–46]. Although the signaling pathway/s leading to increased LXR expression during infection have not been fully characterized, muramyl dipeptide, a ligand of nucleotide-binding oligomerization domain-containing protein 2 (NOD2) that is present in many bacteria, was able to induce LXRα expression in murine macrophages [30]. In addition, type I and II IFNs and IL-36, which are produced during the immune response to infection, as well as LPS from Gram-negative bacteria, upregulated the expression of enzymes that transform free cholesterol into endogenous LXR agonists, such as 25-hydroxycholesterol (25-HC) [14,43,47,48]. However, the involvement of LXRs in the physiological actions of 25-HC is still unclear [49]. Sterile acute inflammation also increased LXR expression and activity through a mechanism requiring functional MER signaling [50], in line with the observation that efferocytosis via MER activates the LXR pathway [17].

By contrast, LXRα expression was inhibited in experimental models of sepsis [51,52] and the transcriptional control of LXR target genes was compromised in several infection/inflammatory settings [14,15,53]. In this regard, TLR3/4 ligands and IFN-γ interfered with the LXR-mediated control of cholesterol metabolism through activation of IRF3 and STAT1, respectively [14,53]. Competition for the coactivator p300/CREB-binding protein (CBP) was proposed as a mechanism for IRF3 and STAT1 to inhibit the transcriptional activity of LXRs on specific target genes.

Such divergent consequences of infection/inflammation on LXR signaling have fueled the need to explore the roles of this pathway in host-pathogen interaction (Figure 2). Initial studies in mice have defined the general role for LXRs in promoting macrophage survival after infection by different bacteria, namely *Listeria monocytogenes*, *Bacillus anthraci*), *Escherichia coli*, and *Salmonella* Typhimurium, which correlated with the upregulated expression of the anti-apoptotic molecule AIM/CD5L, a specific target of LXRα, and with the downregulation of pro-apoptotic factors [29,30]. Deficient LXR expression, particularly in bone marrow-derived cells, resulted in a higher susceptibility to infection by *L. monocytogenes*, with increased bacterial burden and neutrophilic abscesses in the liver and a lower survival rate [30]. In studies comparing the relative contribution of LXR isoforms, the lack of expression of LXRα was responsible for the increased susceptibility to *L. monocytogenes*.

Later on, a solid amount of evidence supported the involvement of LXRs in the control of the infection by *Mycobacterium tuberculosis* (*M. tuberculosis*). In human macrophages and in a murine model of mycobacterial infection, LXR agonists reduced the intracellular bacterial burden [43–45]. In line with these observations, LXR-deficient mice had higher bacterial burdens and increased granulomatous lesions in the lungs and underwent more rapid progression to systemic infection than their wild-type counterparts [45]. The

increased susceptibility of LXR-deficient mice was associated with the impaired activities of the innate and adaptive immune systems, including the infiltration of neutrophils to the lungs and the establishment of local Th1 and Th17 responses. These observations are in contrast with the general anti-inflammatory roles of the LXR pathway in non-infectious inflammatory diseases described in the previous section. Interestingly, whereas both LXRα and LXRβ participated in limiting mycobacterial infection in human macrophages *in vitro* [43], LXRα was specifically required to control the course of infection in mice [45], mirroring the selective contribution of this isoform in the protection against *L. monocytogenes* [30].

In addition, LXR agonists increased the production of antimicrobial peptides in *M. tuberculosis*-infected macrophages [43], consistent with the capability of the LXRα target AIM/CD5L to enhance this mechanism of defense and to contribute to mycobacterial clearance [54]. Therefore, it is plausible that activities regulated by AIM/CD5L beyond the control of apoptotic cell death also contribute to the protective effects of LXR agonists against bacterial infection. On the other hand, in contrast to the pro-survival actions described above, LXR agonists promoted apoptosis in human macrophages infected with *M. tuberculosis*, which may represent a mycobactericidal strategy [44]. Although the mechanisms leading to increased cell death were not determined, further investigation is required to better understand the implications of the LXR-AIM axis in different types of infection and how this pathway integrates with the other transcriptional effects of LXR agonists.

In this regard, the upregulation of the LXR targets ABCA1 and ABCG1, which mediate intracellular cholesterol efflux, may also represent an important host mechanism for inhibiting mycobacterial growth [44]. Indeed, interference with ABCA1 expression facilitated the growth of the vaccine strain Bacille Calmette–Guérin in human

macrophages [55], probably because mycobacteria have a preference for intracellular fatty acids and cholesterol as carbon sources (reviewed in [56]). The obligate intracellular bacterium *Chlamydia pneumoniae* also relies heavily on intracellular cholesterol and uses the TIR domain-containing adapter inducing IFN-β (TRIF)-IRF3 signaling pathway to promote the conversion of infected macrophages into cholesterol-loaded foam cells [57]. Although this study did not evaluate the effects on cholesterol transporters, the results are consistent with the capability of IRF3 to inhibit ABCA1 expression [53]. Interestingly, LXR activation interfered with IRF3 activity and inhibited foam cell formation during *C. pneumoniae* infection [57]. Therefore, it is possible that LXR agonists use cooperative mechanisms based on the induction of ABCA1/G1 and the repressive actions on IRF3 to limit the accumulation of cholesterol and control the infection by bacterial species that benefit from intracellular lipid storages.

Accumulated data support that alterations in the membrane cholesterol as a consequence of increased ABCA1 expression may also affect other critical steps in the infection cycle of several pathogens. Lipid rafts are membrane microdomains enriched in cholesterol and glycosphingolipids that concentrate molecules specifically targeted by a number of microorganisms for host cell binding, invasion, or dissemination, as well as receptors that initiate signaling pathways in host cells in response to environmental stimuli [58]. Indeed, a number of pathogens disrupt cellular cholesterol homeostasis either to promote lipid raft formation and gain entry into host cells or to hijack host cell signaling pathways that facilitate intracellular survival/replication [59]. For example, human immunodeficiency virus (HIV)-1, via its protein Nef, diminished cholesterol efflux from macrophages by modulating the post-transcriptional expression of ABCA1 and its redistribution, thus facilitating viral infectivity [60]. Reciprocally, the activation of the LXR-ABCA1 axis resulted in antiviral effects against HIV-1, including inhibitory effects on viral entry into

human $CD4^+$ T cells [61], on virus production and the fusion activity of the virions [62], and on the capability of human dendritic cells to capture HIV-1 and trans-infect T cells [63]. Furthermore, pharmacological treatment with an LXR agonist reduced the viral load in humanized models of HIV infection in mice [62,64]. The antiviral effects were not exclusive for HIV infection, as the control of cholesterol homeostasis by the LXR-ABCA1 pathway also impacted the capability of hepatitis C virus (HCV) to establish virus-host cell fusions and consequently enter the liver cells [65], as well as both the entry and replication capacity of Newcastle disease virus (NDV) [66].

In addition to mechanisms for cholesterol efflux, LXRs control cholesterol uptake through the transcriptional upregulation of IDOL, an E3 ubiquitin ligase that triggers the ubiquitination and degradation of several members of the low-density lipoprotein receptor (LDLR) family [67]. Therefore, the role of IDOL in lowering intracellular cholesterol could help, in combination with the activity of ABCA1/G1, reduce the infectivity and/or growth of some pathogens. Moreover, HCV associates with lipoproteins and benefits from the surface expression of the LDLR to infect hepatocytes (reviewed in [68]). As the overexpression of IDOL inhibited the infection of human hepatocytes with HCV [69], it is plausible that a reduction in the LDLR levels represents an additional mechanism mediating the inhibitory actions of LXR agonists on HCV entry into host cells.

Most studies exploring the role of synthetic LXR agonists in viral infection have not addressed the exact contribution of LXR isoforms. However, the expression of at least LXRα (in the absence of pharmacological treatment) was required to restrict the reactivation of gammaherpesvirus in chronically infected mice [70]. LXRα-deficiency resulted in viral reactivation in peritoneal cells, but not in splenocytes, despite intact virus-specific T cell responses.

The recent discovery of the multifunctional protein CD38 as an additional LXR transcriptional target provided new insights to the way LXR agonists control bacterial infection [6,71]. Indeed, CD38 exerts multiple roles in the regulation of the immune response to pathogens [72]. Its expression in cells originating at the bone marrow was required for LXR agonists to ameliorate the clinical severity of *S.* Typhimurium infection in mice [71]. These effects were consistent with the reduced internalization of *S.* Typhimurium by macrophages [71] and may be influenced by an enhanced migratory potential of dendritic cells [6] upon activation of the LXR-CD38 axis. CD38 displays strong NADase activity, being able to modulate cellular NAD^+ homeostasis while generating calcium-mobilizing second messengers [73]. It also exerts important receptorial and accessory functions in immune cells. Interestingly, the effects of LXR agonists on bacterial cell internalization were overcome with exogenous supplementation of NAD^+ [71], highlighting the potential significance of intracellular NAD^+ levels in host cell-pathogen interaction. Whether the effects in NAD^+ metabolism cooperate with other LXR-mediated metabolic changes in the control of infection has not been determined. In addition, the contribution of the LXR-CD38 axis in controlling the progression of other types of infection requires investigation.

The LXR pathway can also impact the course of infection through mechanisms based on transcriptional repression. As an example, LXR agonists repressed the basal transcription of HIV-1 in infected macrophages and counteracted HIV-1 replication in response to TLR signaling. These effects were mediated by preventing the release of the corepressor NCoR and inhibiting the recruitment of NF-κB, AP1 components, and CBP to the proviral DNA [74]. Additionally, the repression of pro-inflammatory genes was also proposed as a potential mechanism to downregulate the activation of HIV-1 expression in infected cells.

In line with anti-inflammatory effects in the context of endotoxemia [75], LXR agonists reduced organ dysfunction and mortality associated with sepsis in rodent models [51,52]. The functional expression of silent mating type information regulation 2 homolog (Sirt)-1 was required for the protective effects of LXR agonists on myocardial function in septic mice, which coincided with a reduction in NF-κB activity, oxidative stress, and myocardial cell apoptosis, although the mechanism leading to increased Sirt-1 transcription/activation was not defined [51]. In addition, evidence was provided for a selective role of LXRα, but not of LXRβ, in the protection against liver injury during sepsis [52], which contrasts with the role of LXRβ in ameliorating the hepatic acute response [22]. In general, these observations argue that the LXR pathway plays a role in limiting exacerbated tissue damage due to infection. However, in a different study, LXR agonism increased sepsis-induced mortality in mice due to an impairment of neutrophil infiltration to the infection site [5], raising the possibility that the outcome of LXR activation in sepsis depends on additional factors, which warrants further investigation.

In contrast to the predominant protective effects of the LXR pathway on bacterial and viral infections, the anti-inflammatory environment potentiated by LXR agonists may be a favorable scenario for certain pathogens. In this regard, LXR deficiency conferred resistance to the parasite *Leishmania chagasi/infantum* [76], despite the fact that *Leishmania* spp. are NAD^+ auxotrophs and highly sensitive to the host cell membrane cholesterol for infection [77]. Resistance to infection was associated with increased production of nitric oxide and IL-1β and augmented parasite killing by LXR-deficient macrophages [76]. Similarly, LXR agonists enhanced mortality during *Klebsiella pneumoniae* infection in mice, which correlated with the changes in the course of infiltration of neutrophils to the infected lungs [78]. The inhibition of chemokine-induced RhoA activation was proposed as a potential underlying mechanism.

Putting together all of the pieces of evidence obtained from the different models of infection, the modulation of inflammatory and metabolic responses by LXRs has different consequences depending on the pathogen. Therefore, targeting the LXR pathway as a strategy against infection must take into account the multiple mechanisms contributing to the effects of LXRs in host cell-pathogen interaction.

Conclusions and future perspectives

Due to the emergence of antimicrobial resistances and the absence of effective vaccines for a large number of pathogens, one of the major necessities in public health is the development of innovative host-directed therapies (HDTs) against infection. LXRs, by virtue of their condition as druggable targets and their multiple roles at the intersection between metabolism and inflammation, are promising candidates for HDT.

As summarized in this review, LXR activation exerts a protective role in many pre-clinical models of viral and bacterial infection. Different studies have focused on at least one molecular mechanism to explain these protective effects, but it is likely that several mechanisms cooperate simultaneously to reduce the capacity of infection of pathogens and the inflammatory response. As discussed here, some commonalities exist in relation to the metabolic resources hijacked by different pathogens. Accumulated evidence points toward the LXR pathway as part of the host response to modulate the metabolism of the infected cell and limit the infectivity and/or growth of intracellular pathogens, a role that can be boosted upon pharmacological LXR activation. In this regard, cholesterol metabolism is targeted by LXR agonists in a manner that is beneficial to limiting the infection, at least in animal and *in vitro* studies. Reciprocally, pathogens that are able to interfere with the capacity of LXRs to alter the host cell metabolism may benefit from a

more favorable environment. In fact, there is significant evidence of the LXR pathway itself being modulated at the level of both expression and activity by signals derived from pathogen recognition or from cytokines produced at the infection site.

In addition, excessive tissue damage due to an exacerbated immune response is a common feature in infection and in inflammatory disorders. Beyond its role in limiting the extent of infection, activated LXRs trigger mechanisms to keep the inflammatory response under control and to avoid excessive organ injury in pre-clinical studies.

Given their role at the intersection of lipid metabolism and immune responses, the effects of LXR activation in the context of infection have been studied in depth in macrophages. Indeed, despite their relevance in microbial killing and in the recruitment of immune cells to the site of infection, macrophages are commonly targeted by intracellular pathogens for their replication and dissemination [79]. Therefore, LXRs limit the extent of infection and restrict excessive inflammatory responses in a cell type that represents a selective niche for intracellular infection and, at the same time, is crucial for the preservation of tissue integrity. Despite the importance of LXRs in macrophage biology, this review also integrates data showing the beneficial effects of LXR agonists in other host cells that are targets of the infection, especially in the context of viral infection.

A major limitation in the use of LXR agonists is their adverse effects in pre-clinical models of disease due to the activation of a lipogenic program [80]. Based on hepatic LXRα as the main isoform involved in agonist-induced lipogenesis, attempts have been made to develop LXRβ-specific ligands to circumvent this problem (reviewed in [1]). However, this kind of approach would probably have limitations as a HDT against infection. Whereas the anti-inflammatory effects of LXR agonists depend on LXRβ in a number of disease models in mice, LXRα activity is essential for the development of

protective immune responses against several types of infection (Table I). Therefore, the development of more sophisticated agonists that are capable of promoting selective LXR functions while inhibiting specific targets [81] and/or new routes of administration targeting specific immune compartments [82] deserves further attention in the context of infection.

Acknowledgments

This work was supported by grants from the Spanish Ministry of Economy and Competitivity (MINECO) to AFV (SAF2017-89510-R) and to the NuRCaMeIn network (SAF2017-90604REDT), and from Fundació La Marató de TV3 to AFV (201605-31). EG received a fellowship from the University of Barcelona (APIF). NAL received a fellowship from CONICYT (Comisión Nacional de Investigación Científica y Tecnología) (PFCHA/DOCTORADO BECAS CHILE/2016-72170639), Chilean Ministry of Education.

Author contributions

Estibaliz Glaría: Conceptualization, Writing - original draft, revision & editing. Nicole A. Letelier: Conceptualization, Writing - original draft & revision. Annabel F. Valledor: Conceptualization, Writing - original draft, revision & editing, Supervision, Funding acquisition.

References

1. Schulman IG: **Liver X receptors link lipid metabolism and inflammation**. *FEBS Lett* 2017, **591**:2978–2991.

2. Walcher D, Kümmel A, Kehrle B, Bach H, Grüb M, Durst R, Hombach V, Marx N: **LXR activation reduces proinflammatory cytokine expression in human CD4-positive lymphocytes**. *Arterioscler Thromb Vasc Biol* 2006, **26**:1022–1028.

3. Bensinger SJ, Bradley MN, Joseph SB, Zelcer N, Janssen EM, Hausner MA, Shih R, Parks JS, Edwards PA, Jamieson BD, et al.: **LXR signaling couples sterol metabolism to proliferation in the acquired immune response**. *Cell* 2008, **134**:97–111.

4. Diehl CJ, Barish GD, Downes M, Chou MY, Heinz S, Glass CK, Evans RM, Witztum JL: **Research resource: Comparative nuclear receptor atlas: Basal and activated peritoneal B-1 and B-2 cells**. *Mol Endocrinol* 2011, **25**:529–545.

5. Souto FO, Castanheira FVS, Trevelin SC, Lima BHF, Cebinelli GCM, Turato WM, Auxiliadora-Martins M, Basile-Filho A, Alves-Filho JC, Cunha FQ: **Liver X Receptor Activation Impairs Neutrophil Functions and Aggravates Sepsis**. *J Infect Dis* 2020, **221**:1542–1553.

6. Beceiro S, Pap A, Czimmerer Z, Sallam T, Guillén JA, Gallardo G, Hong C, A-Gonzalez N, Tabraue C, Diaz M, et al.: **Liver X Receptor Nuclear Receptors Are Transcriptional Regulators of Dendritic Cell Chemotaxis**. *Mol Cell Biol* 2018, **38**:e00534-17. *Using gain- and loss-of-function models, this work shows that dendritic cells depend on functional LXRs to migrate *in vivo*. The study also defines CD38 as the molecular mechanism by which LXR agonists enhance

dendritic cell chemotaxis.

7. Ramón-Vázquez A, de la Rosa JV, Tabraue C, Lopez F, Díaz-Chico BN, Bosca L, Tontonoz P, Alemany S, Castrillo A: **Common and Differential Transcriptional Actions of Nuclear Receptors Liver X Receptors α and β in Macrophages**. *Mol Cell Biol* 2019, **39**:e00376-18. *Through the design of immortalized macrophages expressing equivalent amounts of tagged LXRα and LXRβ in an LXR-deficient background, the authors characterize the genomic distribution and transcriptional capacity of each isoform. The study shows that LXRα and LXRβ control the transcription of receptor-exclusive sets of genes.

8. Wagner BL, Valledor AF, Shao G, Daige CL, Bischoff ED, Petrowski M, Jepsen K, Baek SH, Heyman RA, Rosenfeld MG, et al.: **Promoter-Specific Roles for Liver X Receptor/Corepressor Complexes in the Regulation of ABCA1 and SREBP1 Gene Expression**. *Mol Cell Biol* 2003, **23**:5780–5789.

9. Ghisletti S, Huang W, Ogawa S, Pascual G, Lin M-E, Willson TM, Rosenfeld MG, Glass CK: **Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma**. *Mol Cell* 2007, **25**:57–70.

10. Lee JH, Park SM, Kim OS, Lee CS, Woo JH, Park SJ, Joe E, Jou I: **Differential SUMOylation of LXRalpha and LXRbeta mediates transrepression of STAT1 inflammatory signaling in IFN-gamma-stimulated brain astrocytes**. *Mol Cell* 2009, **35**:806–817.

11. Fowler AJ, Sheu MY, Schmuth M, Kao J, Fluhr JW, Rhein L, Collins JL, Willson TM, Mangelsdorf DJ, Elias PM, et al.: **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* 2003, **120**:246–255.

12. Joseph SB, Castrillo A, Laffitte BA, Mangelsdorf DJ, Tontonoz P: **Reciprocal regulation of inflammation and lipid metabolism by liver X receptors**. *Nat Med* 2003, **9**:213–219.

13. Hindinger C, Hinton DR, Kirwin SJ, Atkinson RD, Burnett ME, Bergmann CC, Stohlman SA: **Liver X Receptor Activation Decreases the Severity of Experimental Autoimmune Encephalomyelitis**. *J Neurosci Res* 2006, **1234**:1225–1234.

14. Pascual-García M, Rué L, León T, Julve J, Carbó JM, Matalonga J, Auer H, Celada A, Escolà-Gil JC, Steffensen KR, et al.: **Reciprocal Negative Cross-Talk between Liver X Receptors (LXRs) and STAT1: Effects on IFN-γ–Induced Inflammatory Responses and LXR-Dependent Gene Expression**. *J Immunol* 2013, **190**:6520–6532.

15. Han S, Zhuang H, Shumyak S, Wu J, Xie C, Li H, Yang LJ, Reeves WH: **Liver X receptor agonist therapy prevents diffuse alveolar hemorrhage in murine lupus by repolarizing macrophages**. *Front Immunol* 2018, **9**:135.

16. Park M-C, Kwon Y-J, Chung S-J, Park Y-B, Lee S-K: **Liver X receptor agonist prevents the evolution of collagen-induced arthritis in mice.** *Rheumatology (Oxford)* 2010, **49**:882–890.

17. A-Gonzalez N, Bensinger SJ, Hong C, Beceiro S, Bradley MN, Zelcer N, Deniz J, Ramirez C, Díaz M, Gallardo G, et al.: **Apoptotic cells promote their own clearance and immune tolerance through activation of the nuclear receptor LXR**. *Immunity* 2009, **31**:245–258.

18. Jeon JY, Nam JY, Kim HA, Park YB, Bae SC, Suh CH: **Liver X receptors alpha gene (NR1H3) promoter polymorphisms are associated with systemic lupus erythematosus in Koreans**. *Arthritis Res Ther* 2014, **16**:R112.

19. Endo-Umeda K, Nakashima H, Komine-Aizawa S, Umeda N, Seki S, Makishima M: **Liver X receptors regulate hepatic F4/80 + CD11b+ Kupffer cells/macrophages and innate immune responses in mice**. *Sci Rep* 2018, **8**:9281.

20. Ito A, Hong C, Rong X, Zhu X, Tarling EJ, Hedde PN, Gratton E, Parks J, Tontonoz P: **LXRs link metabolism to inflammation through Abca1-dependent regulation of membrane composition and TLR signaling.** *Elife* 2015, **4**:e08009.

21. Huang W, Ghisletti S, Saijo K, Gandhi M, Aouadi M, Tesz GJ, Zhang DX, Yao J, Czech MP, Goode BL, et al.: **Coronin 2A mediates actin-dependent de-repression of inflammatory response genes.** *Nature* 2011, **470**:414–418.

22. Venteclef N, Jakobsson T, Ehrlund A, Damdimopoulos A, Mikkonen L, Ellis E, Nilsson L-M, Parini P, Jänne O a, Gustafsson J-A, et al.: **GPS2-dependent corepressor/SUMO pathways govern anti-inflammatory actions of LRH-1 and LXRbeta in the hepatic acute phase response.** *Genes Dev* 2010, **24**:381–395.

23. Misu S, Takebayashi M, Miyamoto K: **Nuclear actin in development and transcriptional reprogramming**. *Front Genet* 2017, **8**:27.

24. Nunomura S, Okayama Y, Matsumoto K, Hashimoto N, Endo-Umeda K, Terui T, Makishima M, Ra C: **Activation of LXRs using the synthetic agonist GW3965 represses the production of pro-inflammatory cytokines by murine**

mast cells. *Allergol Int* 2015, **64 Suppl**:S11-7.

25. Thomas DG, Doran AC, Fotakis P, Westerterp M, Antonson P, Jiang H, Jiang XC, Gustafsson JÅ, Tabas I, Tall AR: **LXR Suppresses Inflammatory Gene Expression and Neutrophil Migration through cis-Repression and Cholesterol Efflux**. *Cell Rep* 2018, **25**:3774-3785.e4.

26. Spann NJ, Garmire LX, McDonald JG, Myers DS, Milne SB, Shibata N, Reichart D, Fox JN, Shaked I, Heudobler D, et al.: **Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses.** *Cell* 2012, **151**:138–152.

27. Pourcet B, Gage MC, Leon TE, Waddington KE, Pello OM, Steffensen KR, Castrillo A, Valledor AF, Pineda-Torra I: **The nuclear receptor LXR modulates interleukin-18 levels in macrophages through multiple mechanisms**. *Sci Rep* 2016, **6**:25481.

28. Pourcet B, Feig JE, Vengrenyuk Y, Hobbs A, Kepka-Lenhart D, Garabedian M, Morris SM, Fisher E a, Pineda-Torra I: **LXRα regulates macrophage arginase 1 through PU.1 and interferon regulatory factor 8.** *Circ Res* 2011, **109**:492–501.

29. Valledor AF, Hsu LC, Ogawa S, Sawka-Verhelle D, Karin M, Glass CK: **Activation of liver X receptors and retinoid X receptors prevents bacterial-induced macrophage apoptosis**. *Proc Natl Acad Sci U S A* 2004, **101**:17813–17818.

30. Joseph SB, Bradley MN, Castrillo A, Bruhn KW, Mak PA, Pei L, Hogenesch J, O'connell RM, Cheng G, Saez E, et al.: **LXR-dependent gene expression is important for macrophage survival and the innate immune response**. *Cell*

2004, **119**:299–309.

31. Sarrias MR, Roselló S, Sánchez-Barbero F, Sierra JM, Vila J, Yélamos J, Vives J, Casals C, Lozano F: **A role for human SPα as a pattern recognition receptor**. *J Biol Chem* 2005, **280**:35391–35398.

32. Sanjurjo L, Aran G, Téllez É, Amézaga N, Armengol C, López D, Prats C, Sarrias MR: **CD5L promotes M2 macrophage polarization through autophagy-mediated upregulation of ID3**. *Front Immunol* 2018, **9**:480. *This paper establishes the relevance of CD5L as a potential target in therapeutic strategies aiming at modulating human macrophage polarization.

33. Geyeregger R, Zeyda M, Bauer W, Kriehuber E, Säemann MD, Zlabinger GJ, Maurer D, Stulnig TM: **Liver X receptors regulate dendritic cell phenotype and function through blocked induction of the actin-bundling protein fascin**. *Blood* 2007, **109**:4288–4295.

34. Töröcsik D, Baráth M, Benko S, Széles L, Dezso B, Póliska S, Hegyi Z, Homolya L, Szatmári I, Lányi A, et al.: **Activation of liver X receptor sensitizes human dendritic cells to inflammatory stimuli.** *J Immunol* 2010, **184**:5456–5465.

35. Cui G, Qin X, Wu L, Zhang Y, Sheng X, Yu Q, Sheng H, Xi B, Zhang JZ, Zang YQ: **Liver X receptor (LXR) mediates negative regulation of mouse and human Th17 differentiation**. *J Clin Invest* 2011, **121**:658–670.

36. Yasuda K, Takeuchi Y, Hirota K: **The pathogenicity of Th17 cells in autoimmune diseases**. *Semin Immunopathol* 2019, **41**:283–297.

37. Solt LA, Kamenecka TM, Burris TP: **LXR-Mediated Inhibition of CD4+ T Helper Cells**. *PLoS One* 2012, **7**:e46615.

38. Herold M, Breuer J, Hucke S, Knolle P, Schwab N, Wiendl H, Klotz L: **Liver X receptor activation promotes differentiation of regulatory T cells.** *PLoS One* 2017, **12**:e0184985.

39. Ito A, Hong C, Oka K, Salazar J V, Diehl C, Witztum JL, Diaz M, Castrillo A, Bensinger SJ, Chan L, et al.: **Cholesterol Accumulation in CD11c+ Immune Cells Is a Causal and Targetable Factor in Autoimmune Disease.** *Immunity* 2016, **45**:1311–1326.

40. Huang Y, Fu X, Lyu X, Xu Z, He Z, Zhang Y, Zeng Y, He F, Huang G: **Activation of LXR attenuates collagen-induced arthritis via suppressing BLyS production**. *Clin Immunol* 2015, **161**:339–347.

41. Rong X, Albert CJ, Hong C, Duerr MA, Chamberlain BT, Tarling EJ, Ito A, Gao J, Wang B, Edwards PA, et al.: **LXRs Regulate ER Stress and Inflammation through Dynamic Modulation of Membrane Phospholipid Composition**. *Cell Metab* 2013, **18**:685–697.

42. Eisenreich W, Rudel T, Heesemann J, Goebel W: **How viral and intracellular bacterial pathogens reprogram the metabolism of host cells to allow their intracellular replication**. *Front Cell Infect Microbiol* 2019, **9**:42.

43. Ahsan F, Maertzdorf J, Guhlich-Bornhof U, Kaufmann SHE, Moura-Alves P: **IL-36/LXR axis modulates cholesterol metabolism and immune defense to Mycobacterium tuberculosis**. *Sci Rep* 2018, **8**:1520. *This work describes a novel mechanism of crosstalk by which coordinated IL-36 and LXR signaling limit mycobacterial growth in human macrophages. The conclusions from this study may also have implications in other infection settings.

44. Bouttier M, Laperriere D, Memari B, Mangiapane J, Fiore A, Mitchell E, Verway

M, Behr MA, Sladek R, Barreiro LB, et al.: **Alu repeats as transcriptional regulatory platforms in macrophage responses to M.Tuberculosis infection**. *Nucleic Acids Res* 2016, **44**:10571–10587.

45. Korf H, Vander Beken S, Romano M, Steffensen KR, Stijlemans B, Gustafsson J-Å, Grooten J, Huygen K: **Liver X receptors contribute to the protective immune response against Mycobacterium tuberculosis in mice**. *J Clin Invest* 2009, **119**:1626–1637.

46. Lange PT, Schorl C, Sahoo D, Tarakanova VL: **Liver X receptors suppress activity of cholesterol and fatty acid synthesis pathways to oppose gammaherpesvirus replication**. *MBio* 2018, **9**:e01115-18.

47. Dang E V., McDonald JG, Russell DW, Cyster JG: **Oxysterol Restraint of Cholesterol Synthesis Prevents AIM2 Inflammasome Activation**. *Cell* 2017, **171**:1057-1071.e11.

48. Park K, Scott AL: **Cholesterol 25-hydroxylase production by dendritic cells and macrophages is regulated by type I interferons**. *J Leukoc Biol* 2010, **88**:1081–1087.

49. Cyster JG, Dang E V., Reboldi A, Yi T: **25-Hydroxycholesterols in innate and adaptive immunity**. *Nat Rev Immunol* 2014, **14**:731–743.

50. Choi JY, Seo JY, Yoon YS, Lee YJ, Kim HS, Kang JL: **Mer signaling increases the abundance of the transcription factor LXR to promote the resolution of acute sterile inflammation**. *Sci Signal* 2015, **8**:ra21.

51. Han D, Li X, Li S, Su T, Fan L, Fan W-S, Qiao H-Y, Chen J-W, Fan M-M, Li X-J, et al.: **Reduced silent information regulator 1 signaling exacerbates sepsis-induced myocardial injury and mitigates the protective effect of a liver X**

receptor agonist. *Free Radic Biol Med* 2017, **113**:291–303.

52. Wang YY, Ryg U, Dahle MK, Steffensen KR, Thiemermann C, Chaudry IH, Reinholt FP, Collins JL, Nebb HI, Aasen AO, et al.: **Liver X receptor protects against liver injury in sepsis caused by rodent cecal ligation and puncture**. *Surg Infect (Larchmt)* 2011, **12**:283–289.

53. Castrillo A, Joseph SB, Vaidya S a, Haberland M, Fogelman AM, Cheng G, Tontonoz P: **Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism.** *Mol Cell* 2003, **12**:805–816.

54. Sanjurjo L, Amézaga N, Vilaplana C, Cáceres N, Marzo E, Valeri M, Cardona PJ, Sarrias MR: **The scavenger protein apoptosis inhibitor of macrophages (AIM) potentiates the antimicrobial response against Mycobacterium tuberculosis by enhancing autophagy**. *PLoS One* 2013, **8**:e79670.

55. Long J, Roy RB, Zhang YJ, Antrobus R, Du Y, Smith DL, Weekes MP, Javid B: **Plasma membrane profiling reveals upregulation of ABCA1 by infected macrophages leading to restriction of mycobacterial growth**. *Front Microbiol* 2016, **7**:1086.

56. Huang L, Nazarova E V., Russell DG: **Mycobacterium tuberculosis: Bacterial Fitness within the Host Macrophage**. *Microbiol Spectr* 2019, **7**.

57. Chen S, Sorrentino R, Shimada K, Bulut Y, Doherty TM, Crother TR, Arditi M: **Chlamydia pneumoniae -Induced Foam Cell Formation Requires MyD88-Dependent and -Independent Signaling and Is Reciprocally Modulated by Liver X Receptor Activation** . *J Immunol* 2008, **181**:7186–7193.

58. Bukrinsky MI, Mukhamedova N, Sviridov D: **Lipid Rafts and Pathogens: The**

Art of Deception and Exploitation. *J Lipid Res* 2019, **61**:601–610.

59. Samanta D, Mulye M, Clemente TM, Justis A V., Gilk SD: **Manipulation of host cholesterol by obligate intracellular bacteria**. *Front Cell Infect Microbiol* 2017, **7**:165.

60. Mujawar Z, Rose H, Morrow MP, Pushkarsky T, Dubrovsky L, Mukhamedova N, Fu Y, Dart A, Orenstein JM, Bobryshev Y V, et al.: **Human immunodeficiency virus impairs reverse cholesterol transport from macrophages.** *PLoS Biol* 2006, **4**:e365.

61. Jiang H, Badralmaa Y, Yang J, Lempicki R, Hazen A, Natarajan V: **Retinoic acid and liver X receptor agonist synergistically inhibit HIV infection in CD4+ T cells by up-regulating ABCA1-mediated cholesterol efflux**. *Lipids Health Dis* 2012, **11**:69.

62. Morrow MP, Grant A, Mujawar Z, Dubrovsky L, Pushkarsky T, Kiselyeva Y, Jennelle L, Mukhamedova N, Remaley AT, Kashanchi F, et al.: **Stimulation of the liver X receptor pathway inhibits HIV-1 replication via induction of ATP-binding cassette transporter A1**. *Mol Pharmacol* 2010, **78**:215–225.

63. Hanley TM, Blay Puryear W, Gummuluru S, Viglianti G a: **PPARgamma and LXR signaling inhibit dendritic cell-mediated HIV-1 capture and trans-infection.** *PLoS Pathog* 2010, **6**:e1000981.

64. Ramezani A, Dubrovsky L, Pushkarsky T, Sviridov D, Karandish S, Raj DS, Fitzgerald ML, Bukrinsky M: **Stimulation of Liver X Receptor Has Potent Anti-HIV Effects in a Humanized Mouse Model of HIV Infection**. *J Pharmacol Exp Ther* 2015, **354**:376–383.

65. Bocchetta S, Maillard P, Yamamoto M, Gondeau C, Douam F, Lebreton S,

Lagaye S, Pol S, Helle F, Plengpanich W, et al.: **Up-regulation of the ATP-binding cassette transporter A1 inhibits hepatitis C virus infection.** *PLoS One* 2014, **9**:e92140.

66. Sheng X xiang, Sun Y jie, Zhan Y, Qu Y rong, Wang H xia, Luo M, Liao Y, Qiu X sheng, Ding C, Fan H jie, et al.: **The LXR ligand GW3965 inhibits Newcastle disease virus infection by affecting cholesterol homeostasis**. *Arch Virol* 2016, **161**:2491–2501.

67. Zelcer N, Hong C, Boyadjian R, Tontonoz P: **LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor**. *Science* 2009, **325**:100–104.

68. Grassi G, Di Caprio G, Fimia GM, Ippolito G, Tripodi M, Alonzi T: **Hepatitis C virus relies on lipoproteins for its life cycle**. *World J Gastroenterol* 2016, **22**:1953–1965.

69. Zeng J, Wu Y, Liao Q, Li L, Chen X, Chen X: **Liver X receptors agonists impede hepatitis C virus infection in an Idol-dependent manner**. *Antiviral Res* 2012, **95**:245–256.

70. Lange PT, Jondle CN, Darrah EJ, Johnson KE, Tarakanova VL: **LXR Alpha Restricts Gammaherpesvirus Reactivation from Latently Infected Peritoneal Cells**. *J Virol* 2019, **93**:e02071-18.

71. Matalonga J, Glaria E, Bresque M, Escande C, Carbó JM, Kiefer K, Vicente R, León TE, Beceiro S, Pascual-García M, et al.: **The Nuclear Receptor LXR Limits Bacterial Infection of Host Macrophages through a Mechanism that Impacts Cellular NAD Metabolism**. *Cell Rep* 2017, **18**:1241–1255. *This report described for the first time that the multifunctional enzyme CD38 is a

transcriptional target of LXR. Through upregulation of this enzyme, LXR agonists modulated the levels of intracellular NAD+, which might have physiological implications beyond the management of infection.

72. Glaría E, Valledor AF: **Roles of CD38 in the Immune Response to Infection**. *Cells* 2020, **9**:228.

73. Chini EN, Chini CCS, Espindola Netto JM, de Oliveira GC, van Schooten W: **The Pharmacology of CD38/NADase: An Emerging Target in Cancer and Diseases of Aging**. *Trends Pharmacol Sci* 2018, **39**:424–436.

74. Hanley TM, Viglianti GA: **Nuclear receptor signaling inhibits HIV-1 replication in macrophages through multiple trans-repression mechanisms.** *J Virol* 2011, **85**:10834–10850.

75. Wang YY, Dahle MK, Steffensen KR, Reinholt FP, Collins JL, Thiemermann C, Aasen AO, Gustafsson JÅ, Wang JE: **Liver x receptor agonist GW3965 dose-dependently regulates lps-mediated liver injury and modulates posttranscriptional TNF-α production and p38 mitogen-activated protein kinase activation in liver macrophages**. *Shock* 2009, **32**:548–553.

76. Bruhn KW, Marathe C, Maretti-Mira AC, Nguyen H, Haskell J, Tran TA, Vanchinathan V, Gaur U, Wilson ME, Tontonoz P, et al.: **LXR deficiency confers increased protection against visceral Leishmania infection in mice.** *PLoS Negl Trop Dis* 2010, **4**:e886.

77. Kumar GA, Jafurulla M, Chattopadhyay A: **The membrane as the gatekeeper of infection: Cholesterol in host–pathogen interaction**. *Chem Phys Lipids* 2016, **199**:179–185.

78. Smoak K, Madenspacher J, Jeyaseelan S, Williams B, Dixon D, Poch KR, Nick J

a, Worthen GS, Fessler MB: **Effects of liver X receptor agonist treatment on pulmonary inflammation and host defense.** *J Immunol* 2008, **180**:3305–3312.

79. Price J V., Vance RE: **The Macrophage Paradox**. *Immunity* 2014, **41**:685–693.

80. Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ, et al.: **Role of LXRs in control of lipogenesis**. *Genes Dev* 2000, **14**:2831–2838.

81. Muse ED, Yu S, Edillor CR, Tao J, Spann NJ, Troutman TD, Seidman JS, Henke A, Roland JT, Ozeki KA, et al.: **Cell-specific discrimination of desmosterol and desmosterol mimetics confers selective regulation of LXR and SREBP in macrophages**. *Proc Natl Acad Sci U S A* 2018, **115**:E4680–E4689. ** This study reveals the capacity of certain endogenous LXR ligands to dissociate LXR functions in a cell-specific manner, which is important for the development of new agonists with diminished adverse effects.

82. Cao E, Lindgren A, Martinsson S, Hu L, Lindfors L, Sigfridsson K, Skantze U, Michaëlsson E, Trevaskis NL, Porter CJH: **Promoting intestinal lymphatic transport targets a liver-X receptor (LXR) agonist (WAY-252,623) to lymphocytes and enhances immunomodulation.** *J Control Release* 2019, **296**:29–39. *This paper reports a new delivery route for an LXR agonist, in combination with a lymph-directing long chain lipid-based formulation, which targets the agonist specifically to lymphocytes. This type of approach may facilitate the modulation of immune responses while avoiding the adverse lipogenic effects of conventional LXR agonist administration.

FIGURE LEGENDS

Figure 1. LXRs inhibit the inflammatory response in macrophages through multiple mechanisms. TLR signaling or IFN-γ stimulation induce inflammatory gene expression. Agonist-bound LXRs mediate mechanisms of transrepression, which interfere with the release of corepressors or with the activity/recruitment of transcription factors (NF-κB, STAT1) required for inflammatory gene expression. In addition, LXRs inhibit inflammatory responses indirectly through the transcriptional activation of LXR targets (in blue) involved in the modulation of metabolic and/or immune responses. The cholesterol efflux mediated by ABCA1 results in changes in the lipid composition of the membrane, which interferes with TLR signaling. SREBP1c induces the expresion of enzymes involved in the generation of lipids with anti-inflammatory properties. MER couples efferocytosis with the suppression of the inflammatory response. AIM/CD5L enhances the expression of molecules involved in the resolution of inflammation and promotes an anti-inflammatory profile. IRF8 induces the expression of IL18BP, which binds to secreted IL-18 and inhibits its biological actions. Some elements in this image have been downloaded from SMART - Servier Medical ART. *Arg1*, arginase 1; *Casp1*, caspase 1; *Fas*, fatty acid synthase; HDL, high-density lipoprotein; IFNγR, IFN-γ receptor; *Il1b*, interleukin 1β; *Il12b*, interleukin 12 subunit b; *Il6*, interleukin 6; MyD88, Myeloid differentiation primary response 88; TRAF6, tumor necrosis factor receptor associated factor 6.

Figure 2. LXR activation induces protective mechanisms that limit viral and bacterial infection. LXR agonists upregulate the expression of LXR targets (in blue) that contribute in reducing the infection by several pathogens (names of pathogens in green).

AIM/CD5L confers resistance to apoptosis and induces the synthesis of antimicrobial peptides. CD38 reduces intracellular NAD+ levels and the infection by *S.* Typhimurium. ABCA1 promotes cholesterol efflux. As a consequence, reduced intracellular cholesterol limits the growth of mycobacteria and, potentially, of other bacterial strains that depend on intracellular cholesterol. In addition, changes in the cholesterol levels within lipid rafts may interfere with the entry of several viruses into host cells. IDOL, by virtue of its role in controling the turnover of the LDLR, inhibits the capability of HCV to infect host cells. LXRs can also affect the intracellular replication of HIV-1 through mechanisms of transrepression, which affect corepressor release or transcription factor recruitment to the proviral DNA. Ub, ubiquitin. Some elements in this image have been downloaded from SMART - Servier Medical ART.

UNIVERSITAT DE BARCELONA

Departament de Biologia Cel·lular, Fisiologia i Immunologia
Secció d'Immunologia
Facultat de Biologia

Dra. Annabel Valledor
Edifici Ramon Margalef (plantes 3-A & 4-A)
Av. Diagonal, 643. E-08028 Barcelona
Tel: +34 934039384/ Fax: +34 934110358
e-mail: afernandezvalledor@ub.edu
https://sites.google.com/site/avalledorf/
http://www.ub.edu/fisiod3

Barcelona, January 21, 2020

Re: Manuscript entitled "Integrating the roles of Liver X Receptors in inflammation and infection: mechanisms and outcomes"

The authors declare no conflicts of interest.

Annabel F. Valledor, PhD

Estibaliz Glaría

Nicole A. Letelier

Figure 1

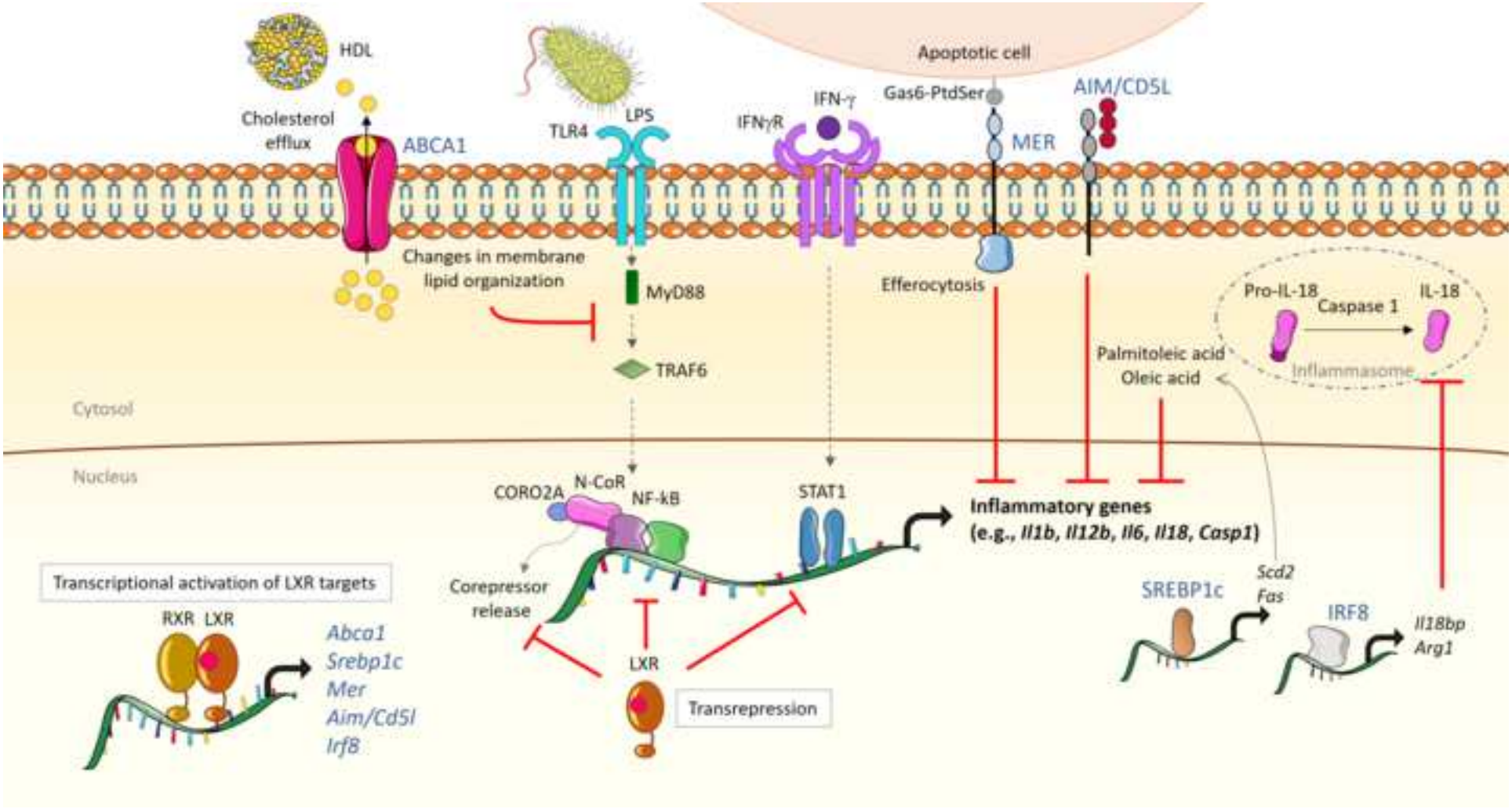

Figure 2

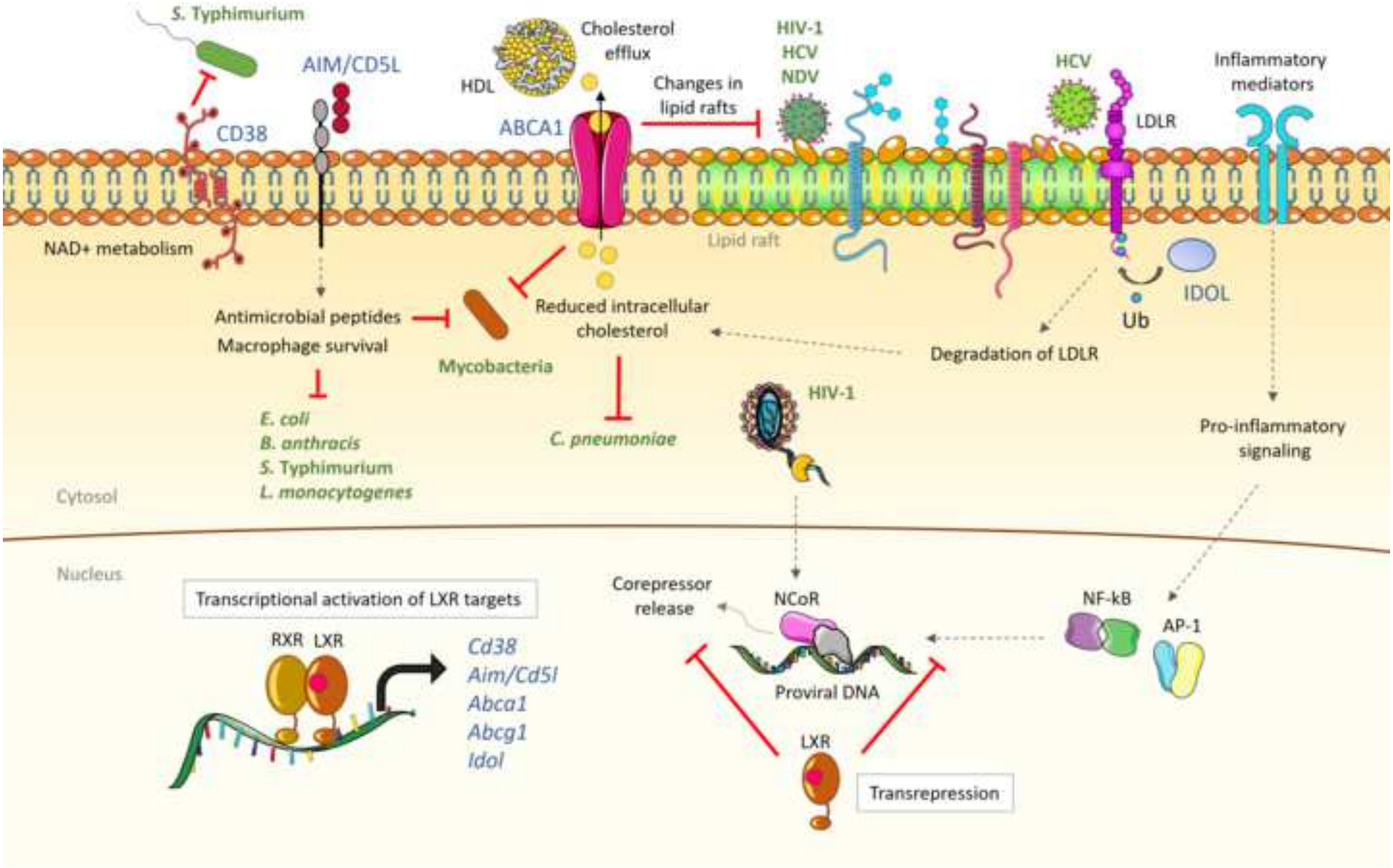

Table I

Table I. Specific contributions of LXR isoforms to the control of inflammation and infection. Ref., reference. TPA, phorbol 12-myristate-13-acetate.

Disease / Cellular model	Trigger	Species	LXR isoform	Effects	Ref.
Macrophages (*in vitro*)	LPS; IFN-γ	Mouse	LXRα / LXRβ	Repression of inflammatory genes	[12,14]
Astrocytes (*in vitro*)	IFN-γ	Mouse	LXRα / LXRβ	Repression of inflammatory genes	[10]
Lupus-like autoimmunity	Aging	Mouse	LXRα / LXRβ	Protection from autoimmunity	[17]
Hepatic acute phase response (*in vivo*)	LPS	Mouse	LXRβ	Repression of acute phase response	[22]
Ear inflammation (*in vivo*)	TPA	Mouse	LXRβ	Inhibition of inflammation	[11]
Mast cells (*in vitro*)	LPS; FcεRI crosslinking	Mouse	LXRβ	Repression of inflammatory cytokine production	[24]
T cells (*in vivo*; *in vitro*)	Aging; mitogens	Mouse	LXRβ	Inhibition of proliferation	[3]
Antigen presenting cells (*in vivo*)	Cholesterol accumulation	Mouse	LXRβ	Limitation of B cell expansion	[39]
B cell lines (*in vitro*)	Basal conditions	Human	LXRα	Repression of BAFF production	[40]

Macrophages (*in vitro*)	*M. tuberculosis*	Human	LXRα / LXRβ	Limitation of mycobacterial infection	[43]
M. tuberculosis infection *in vivo*	*M. tuberculosis*	Mouse	LXRα	Increased resistance to infection	[45]
L. monocytogenes infection *in vivo*	*L. monocytogenes*	Mouse	LXRα	Increased resistance to infection	[30]
GHV infection *in vivo*	GHV	Mouse	LXRα	Restriction of viral reactivation in peritoneal cells	[70]
Cecal ligation and puncture (*in vivo*)	Sepsis	Mouse	LXRα	Protection against liver injury	[52]

ANSWERS TO REVIEWERS

Reviewer 1: In this manuscript, the authors review the roles of LXRs in immunity.

1. Discussion is superficial. Molecular mechanisms of LXR actions in immunity should be reviewed in more detail.

2. Cell type-specific actions and LXR isoform-specific actions should be discussed in detail.

3. The figure is not informative. Please draw figures that show details of the molecular mechanisms.

4. English should be edited carefully.

Answers from the authors:

First of all, we thank the reviewer for constructive comments.

We must apologize for significant delay in our revision, but the situation has been extremely difficult for us because of all the complications derived from the covid-19 pandemia.

We have addressed all the items requested by the reviewer. All our changes in the text are highlighted in yellow.

1-We have provided much more information on detailed mechanisms than in the previous version of the manuscript. However, this implies that we are now over the word limit. We still think it is worthy keeping the level of detail as it is now.

2-Most of the studies exploring the roles of LXRs and infection/inflammatory responses have been performed in macrophages. This is why a large fraction of the information provided in this review is focused on this cell type. However, we have made the effort to discuss studies performed in other cell types, and, in particular, in in vivo models. We have also discussed the relative contribution of LXR isoforms in some immune responses, although in most studies this aspect was not determined (most studies used agonists without complementing with LXR-deficient models or used animals/cells that are knockout for both isoforms). Nevertheless, we have generated a table (Table I) that integrates all the data obtained from reports in which isoform-specific actions have been explored.

3- We have now generated two figures. Fig 1 represents mechanisms involved in the control of inflammatory responses in macrophages and Fig 2 represents mechanisms involved in limiting infection in general. We have also improved the figure legends so that the interpretation is easier. But, please, let us know if these graphics are still not informative.

4. A professional native English speaker has reviewed the manuscript.

CREDIT AUTHOR STATEMENT

Estibaliz Glaría: Conceptualization, Writing - original draft, revision & editing.

Nicole A. Letelier: Conceptualization, Writing - original draft & revision.

Annabel F. Valledor: Conceptualization, Writing - original draft, revision & editing, Supervision, Funding acquisition.