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Nuclear Receptors, Cholesterol Homeostasis and the Immune System

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Abstract

Cholesterol is essential for maintaining membrane fluidity in eukaryotes. Additionally, the synthetic cascade of cholesterol results in precursor molecules important for cellular function such as lipid raft formation and protein prenylation. As such, cholesterol homeostasis is tightly regulated. Interestingly, it is now known that some cholesterol precursors and many metabolites serve as active signaling molecules, binding to different classes of receptors including the nuclear receptors. Furthermore, many cholesterol metabolites or their nuclear receptors have been implicated in the regulation of the immune system in normal physiology and disease. Therefore, in this focused review, cholesterol homeostasis and nuclear receptors involved in this regulation will be discussed, with particular emphasis on how these cascades influence the immune system.

Keywords

cholesterol; bile acids; oxysterols; nuclear receptors; immune system; homeostasis

1 Introduction - Cholesterol metabolism; more than just the liver and membrane

Cholesterol is an integral part of the plasma membrane in eukaryotic cells. Demonstrating the functional importance of cholesterol in the membrane is the observation that hopanoids, which are a structurally similar set of compounds, appear to play a similar roles in non-

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eukaryotic cells [1]. The presence of cholesterol in the plasma membrane moderates the fluidity in order to ensure continued dynamic movement even in the face of temperature shifts [2]. Even though extreme temperature fluctuations are not a major stressor among endotherms, the fluidity-buffering of cholesterol is essential for exotherm vertebrates and invertebrates, especially those dwelling in deep seas and migrating long distances at depth [3]. Interestingly, among non-eukaryotes, similar compounds seem to be found and regulated only in members that are exposed to extreme temperature changes in their life cycle [3]. Furthermore, cholesterol is an essential substrate for the synthesis of several classes of biologically important signaling compounds such as steroid hormones and bile acids, each of which mediate processes essential to homeostasis now described in the majority of metazoan Phyla [4–8]. Nuclear receptors, a family of ligand-inducible transcription factors, are a prime target for these cholesterol derivatives.

Cholesterol and several of its metabolites have been implicated in modulating aspects of the immune response. This review will summarize the actions of those nuclear receptors involved in cholesterol homeostasis, their cholesterol metabolite ligands, and the immune system (Fig. 1).

2 Nuclear Receptors

Nuclear receptors are ligand modulated transcription factors that regulate gene expression. This superfamily of proteins is comprised of 48 members in humans, which is further categorized into 7 groups, based on sequence homology and functional characteristics [9]. The canonical receptor consists of a DNA-binding domain with 2 zinc-finger motifs that interact with the receptor's cognate response element. The DNA-binding domain is connected to the ligand-binding domain through a hinge region. Ligands including specific hormones regulate the conformation of the ligand-binding domain, leading to a variety of events associated with the receptor function. At the N terminus as well as within the ligand-binding domain are the so called transactivation domains AF-2 and AF-1 respectively. These domains allow for interactions with other molecules of the same receptor or certain other receptors, essential for their function [8].

In general, depending on the class of nuclear receptor, in the absence of ligands, nuclear receptors are either bound to chaperone proteins away from the DNA, or associated at specific sites on the DNA along with transcriptionally repressive complexes. In the first case, such as for the estrogen receptors, binding of ligand triggers the release of sequestering chaperones, allowing for the dimerization of receptors and their association with specific regions in the DNA, termed hormone response elements [8]. For the second class, such as for the liver X receptors (LXRs), they are typically found as heterodimers with the retinoid X receptors (RXRs), already associated with chromatin. In their unbound state, these heterodimer complexes associate with corepressor proteins ultimately leading to transrepression of target genes. When a ligand binds, the repressive complex is exchanged for coactivator proteins and translational machinery. Intriguingly, nuclear receptor target loci in the genome (termed hormone response elements) are based on a stretch of 6 base pairs, typically forming their response elements in the promoter region. For both classes of receptor, ligand binding triggers the nucleation of a complex that robustly drives or

suppresses translation at the downstream locus [8, 10]. Apart from the thyroid hormones, many of the known ligands for nuclear receptors are derived from cholesterol, making the homeostatic control of its metabolism critical for downstream physiologic actions.

3 Cholesterol Homeostasis

3.1 Cholesterol Synthesis and Uptake

De novo synthesis of cholesterol occurs through the mevalonate pathway, in which acetyl-CoA is first converted to mevalonate by the rate limiting enzyme, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR). Mevalonate is phosphorylated and subsequently converted to isopentenyl pyrophosphate. This is then converted to farnesyl-pyrophosphate (farnesyl-PP) [11]. Farnesyl-PP is the substrate for the synthesis of essential compounds such as heme, geranylgeranyl-PP and squalene, squalene being the building block for cholesterol, by way of either desmosterol or 7-dehydrocholesterol [12].

Once synthesized, in addition to intracellular trafficking, cholesterol can be complexed with lipoproteins for export and transport via the bloodstream. There are five different categories of lipoprotein: high density (HDL), intermediate density (IDL), low density (LDL), very low density (VLDL) and ultra-low density (ULDL, also known as Chylomicrons) [13, 14]. In addition to synthesizing their own cholesterol, peripheral tissues can also take up LDL-cholesterol via LDL receptor (LDLR)-mediated endocytosis. After lysosomal degradation of LDL, released cholesterol can be utilized directly, metabolized or repackaged for secretion.

3.2 Cholesterol Metabolism

There are two major pathways by which cholesterol is catabolized. The classic bile acid synthesis pathway is the predominant pathway, at least with respect to the liver, and is initiated by the conversion of cholesterol into 7- α -hydroxycholesterol by CYP7A1. The alternative or acidic bile acid synthesis pathway is characterized by the hydroxylation of cholesterol on carbon 26 by CYP27A1, forming 27-hydroxycholesterol ((25R)-26-Hydroxycholesterol, or 27HC) [15]. Although both bile acid synthesis pathways have been primarily described in the liver, and it is thought that the liver is the predominant organ with regards to cholesterol metabolism, the expression of enzymes involved in both bile acid synthesis pathways are found across other tissues, albeit at lower levels compared to the liver [16]. This is particularly the case for the acidic pathway as the enzymes involved are more ubiquitously active across tissues, especially CYP27A1, which is highly expressed in myeloid immune cells [17]. Although both pathways form bile acids at their completion, several intermediate oxysterol metabolites serve important roles both within liver and the periphery.

Among peripheral tissues, the acidic pathway is particularly active in certain myeloid immune populations, including lung macrophages and marrow osteoclast progenitors [18–20]. This is evidenced by the high expression of CYP27A1 among these cell types; CYP27A1 being the enzyme responsible for the hydroxylation of cholesterol, the first step in the acidic bile acid synthesis pathway. The precise physiological reasons for why this pathway is active in myeloid immune cells remains uncertain, although it is thought that it

may be due to the role of macrophages in clearance of cholesterol deposits such as in atherosclerotic plaques [21, 22]. Furthermore, there is accumulating evidence that metabolites within the pathway have specific signaling activities, thereby exerting distinct biological activities in relevant corresponding tissues [23].

3.3 Regulation of Cholesterol Homeostasis

Intracellular cholesterol levels are tightly regulated by two different regulatory loops. In the first, responsible for short-term regulation of cholesterol, sterol levels are monitored by the sterol regulatory element-binding proteins (SREBPs) [24]. There are three isoforms of SREBP, all of which being transmembrane proteins that span the endoplasmic reticulum membrane. In the presence of certain sterols such as cholesterol, SREBPs associate with SREBP cleavage-activating protein (SCAP), while SCAP associates with insulin-induced gene (INSIG); both SCAP and INSIG being able to bind sterols [25]. Absence of cholesterol within the lumen of the endoplasmic reticulum allows the dissociation of INSIG from SCAP and the exposure of a domain that targets it (along with SREBP) to the Golgi complex. Once there, the S1P protease cleaves SREBP to free it from SCAP, and S2P protease cleaves the cytoplasmic domain of SREBP, which in turn traffics to the nucleus where it serves as a transcription factor. SREBPs promote the transcription of cholesterol-related molecules, downregulating genes that catabolize or excrete cholesterol, upregulating cholesterol synthesis genes, and upregulating genes associated with cholesterol uptake such as LDLR. Upon the return of intracellular cholesterol levels to baseline, SREBP cleavage arrests as does its associated changes in gene expression, thereby completing the loop.

The cholesterol-lowering effect of oxysterol production is extremely important in the context of metabolic stress, such as foam cells of an atherosclerotic plaque or steatohepatitis [21, 26]. Furthermore, when cholesterol is metabolized into oxysterols, these can serve as ligands for the liver X receptors (LXRs). LXRs, being part of the nuclear receptor superfamily, are ligand inducible transcription factors. They heterodimerize with the retinoid X receptors (RXRs) and localize to specific response elements in the genome, where they typically repress transcription in the absence of ligand. Upon binding of ligands such as various oxysterols, LXRs are activated and enact a transcriptional program resulting in increased expression of several rate-limiting enzymes in the classic bile acid synthesis pathway (CYP7A1, CYP8B1, etc.) and transporter proteins such as ATP-binding cassette A1 (ABCA1) and ABCG1, leading to increased cholesterol catabolism and efflux, respectively, thereby restoring cholesterol levels [27]. The liver receptor homologue 1 (LRH-1) is another nuclear receptor that partakes in the regulation of key cholesterol metabolism genes such as CYP7A1 and CYP8B1 [28].

3.4 Regulation of Bile Acid Synthesis

As described above, the synthesis of bile acids is primarily regulated by the catabolism of cholesterol. Bile acids themselves are ligands for the nuclear farnesoid X receptors (FXRs) [29–32]. When bile acid levels rise they activate the FXRs, which in turn induce a transcriptional program resulting in complex physiological changes. One gene upregulated by FXR is small heterodimer partner (SHP). SHP is an orphan nuclear receptor that lacks a DNA-binding domain. As such, it does not regulate transcription directly, but does associate

with other nuclear receptors to repress their activity. Thus, by binding to the LXRs and LRH-1, SHP represses their activity, ultimately resulting in decreased bile acid synthesis and cholesterol efflux. Upon the return of bile acid levels to normal, FXR activation and SHP expression arrests, thus completing the feedback loop.

4 Cholesterol, nuclear receptors and the immune system.

Interestingly, in addition to many of the metabolic enzymes, many of the nuclear receptors involved in cholesterol homeostasis are also expressed in immune cells, and in particular those of the myeloid cell lineage [33]. This is perhaps due to the activity of the acidic bile acid synthesis pathway in these cells or simply because of the imperative biological need for the transcriptional regulation by these receptors. Therefore, the activities of nuclear receptors under both normal and metabolically stressed conditions have immunological outcomes that range from generalized local inflammation to altered cross-talk between to secondary immune populations.

4.1 Non-nuclear-receptor-mediated Effects of Cholesterol Metabolites.

Intermediates of the mevalonate pathway, cholesterol and its oxysterol metabolites have been described to impact the biology of peripheral immune cells in several ways. In terms of non-nuclear-receptor-mediated effects, isoprenoid modification (prenylation) is necessary for the function of several proteins in these cells. Indeed, it is thought that a major mechanism by which bisphosphonates spare bone resorption is due to inhibition of normal prenylation in osteoclasts, a bone cell type derived from the myeloid immune lineage [34]. Furthermore, cholesterol participates in the formation of membrane lipid rafts, a hot spot for cell surface receptor-mediated signaling [35, 36]. In this regard, it has been shown that altered cholesterol levels affect membrane receptor signaling in T lymphocytes [35]. These activities, as well as other intracrine aspects of cholesterol within immune cells are nicely reviewed elsewhere [37, 38]. This includes (1) the ability of SREBP2 to activate the NLRP3 inflammasome by trans-activating NADPH oxidase 2 and NLRP3, (2) intracellular cholesterol crystals themselves can behave as Damage-Associated Molecular Patterns, (3) influence of the mevalonate synthesis pathway on the IFN response pathway, (4) cholesterol as a metabolic checkpoint for T cell proliferation, and (5) the ability of membrane cholesterol to regulate TCR signaling in an allosteric manner [reviewed in 37]. Synthesis of steroids such as glucocorticoids may also occur within immune cells, with intracrine or paracrine effects. It appears that the rate-limiting step of steroidogenesis, mediated by steroidogenic acute regulatory protein (StAR), functions in myeloid cells, suggesting the capacity of these cells to synthesize steroids *de novo* [39, 40]. However, in the absence of this step in other immune cells, steroidogenesis is carried out upon the availability of downstream intermediates such as androstenedione or dehydroepiandrosterone (DHEA) [41].

On the other hand, several studies have shown that inhibitors of HMGCoA Reductase, statins, have direct immune-modulatory roles. Many of these attributes can be ascribed to the direct depletion of cholesterol itself, or mevalonate pathway intermediates, although some effects may be independent of this. This is a very interesting avenue of research, especially

given the recent approval of PCSK9 inhibitors and the rapid development of bempedoic acid [42]. However, this is outside the scope of this paper, and discussed nicely elsewhere [43–45].

Oxysterols have also been identified in lipid rafts, although their functional relevance in this capacity has not been fully elucidated [46]. In addition, oxysterols have been shown to function as putative chemokines; upon activating the membrane G-protein-coupled receptor CXCR2, tumor oxysterols recruit neutrophils to promote an immunosuppressive milieu within the tumor microenvironment [47], while 27HC can stimulate osteoblasts to recruit monocytes in an estrogen receptor and CXCL12 dependent fashion [20]. Oxysterols have also been implicated in several physiological or pathological processes, in a non-nuclear receptor dependent manner, or where the precise mechanisms remain unknown [48].

4.2 Nuclear Receptor-mediated Mechanisms

It is now recognized that in addition to their roles in cholesterol clearance and detergents for lipid emulsification in the gut, oxidized cholesterol products such as oxysterols and bile acids are also signaling molecules with significant physiological roles. In fact, it has been shown that the solubilization of intracellular cholesterol crystals in macrophages results in accessibility of enzymes within the acidic pathway to cholesterol [21]. The resulting oxysterols not only serve as a route to eliminate highly insoluble cholesterol crystals, but also serve as signaling molecules to initiate a genetic program, opposite to that of SREBPs, to eliminate cholesterol. Nuclear receptor involvement has been implicated for many of these effects. Here, we highlight some of these mechanisms.

4.2.1 Retinoic Acid Receptor-related Orphan Receptor (ROR)—The ROR has three isoforms with extremely distinct expression patterns across the body. Pertinent to the goal of this review, ROR- γ is expressed at high levels in the thymus, hence the name ROR- γ t [49]. It has been shown that ROR- γ is a master regulator of T cell fate, driving a transcriptional program that differentiates T cells into anti-inflammatory subtypes, such as T helper 17 (Th17) and regulatory T cells (Tregs) [50–53]. The initiation and stability of the ROR- γ t-driven phenotype in non-conventional T cells is of immense physiological importance. It seems that ROR- γ t is in an active transcriptional state in intact cells, which supports the idea that it is already engaged with endogenous ligands [54]. In fact, cholesterol and a variety of oxysterols, such as 24HC and 25HC, have been shown to act as inverse or full agonists of ROR- γ t at low nanomolar concentrations, which are often found in tissues [55, 56]. However, the precise physiological relevance of regulation of ROR- γ t in terms of T cell differentiation remains to be determined. Elucidation of this would shed light on the therapeutic potential of targeting the cholesterol biosynthetic, classic and the acidic pathways for treating autoimmune and inflammatory diseases [57, 58].

4.2.2 Oxysterols and the LXRs—LXR has an α isoform that is expressed highly in tissues such as liver and adipose and lower expression across other tissues, whereas the β isoform is more universally expressed, including myeloid cells and lymphocytes [59]. It has been shown that LXRs dampen the inflammatory response in macrophages [60]. This is perhaps most evident in the context of atherosclerosis and obesity-induced insulin resistance,

where ablation of LXRs sensitizes preclinical models to the development of these pathologies, while the symptoms are partially ameliorated upon pharmacological activation of LXR [61, 62]. LXRs reverse these pathologies by various downstream effects. LXRs modulate macrophage reception of the inflammatory trigger, potentially by modulating the lipid raft composition thus affecting Toll-like receptor signaling [63]. Furthermore, LXRs modulate the cellular fatty acid composition to reduce saturated fatty acid-induced inflammation [64]. Interestingly, LXRs have also been shown to be necessary for the regulation of T lymphocyte responses and subtype-specific differentiation [65–67]. Overall, it appears that dampening of LXR activity by lowering oxysterol ligands and the subsequent cholesterol availability is required for a pro-inflammatory phenotype in T cells. When the T cell receptor is activated, SULT2B1 is induced, resulting in the sulfation and subsequent inactivation of oxysterols, thereby removing LXR stimulus [67]. This requirement is due in part to the reliance of pro-inflammatory T cells on processes such as plasma membrane signaling, organelle turnover and cell division, all of which require cholesterol [35, 67].

Although not specifically addressed in many of the reports describing the role of LXRs in inflammation, it is likely that their activities and subsequent impact on inflammation are regulated by cholesterol metabolites such as the oxysterols, or the cholesterol precursor desmosterol, which is also an LXR ligand [68]. In the case of desmosterol, it has been shown the buildup of cholesterol by experimentally ablating the main enzymes that catabolize it to oxysterols blocks 24-dehydrocholesterol reductase. The blockade of this enzyme lowers desmosterol conversion to cholesterol, resulting in the accumulation of desmosterol [68]. When cholesterol levels are elevated, it is also oxidized into oxysterols such as 27HC by enzymes in the acidic pathway. Together, these two ligands activate LXR to initiate a genetic program to reduce inflammatory cues, such as upregulation of cholesterol efflux pumps and enzymes involved in unsaturated fatty acid biosynthesis. This transcriptional reprogramming has the capacity to resolve atherosclerotic plaques and adipose tissue insulin resistance in mice [21, 62].

4.2.3 Farnesoid X Receptor (FXR)—FXR was originally discovered as the protein that bound farnesoid compounds and bile acids [69]. It was shown that, within hepatocytes, FXR is the sensor for bile acids. Buildup of bile acids activates FXR, which in turn initiates a transcriptional program associated with shutdown of bile acid synthesis [31, 32]. As described in section 3.4, a major part of FXR's biological actions, in terms of bile acid homeostasis, is mediated by SHP. In addition, it is now known that FXR contributes to systemic bile acid homeostasis by sensing bile acid levels in the gut epithelium [70]. There exists an intricate endocrine loop between the small intestine epithelium and the liver, mediated by fibroblast growth factor 19 (FGF19 in human or FGF15 in mouse), that shuts down hepatic bile acid synthesis upon increased concentrations in the gut lumen [71]. Due to its central roles in liver and gut, the immune functions of FXR have largely been described in regards to these tissues in spite of its expression across the body [72].

FXR has been shown to modulate the inflammatory response in myeloid cells, mainly of the monocytic lineage, within the gut. Since the gut is an immune-rich, inflammation-prone tissue it is perhaps not surprising that bile acids exert immunomodulatory roles within this tissue. Using models of induced colitis, it has been shown that the absence of FXR results in

uncontrolled inflammation and subsequent tissue damage, whereas small-molecule FXR agonists dampen them [73, 74]. On the other hand, it has been shown that, ex vivo, small-molecule FXR agonists dampen the inflammatory response within gut myeloid cells of mice with colitis [73]. Overall, it appears that in myeloid cells FXR is upregulated by the activity of certain inflammatory cascades to modulate the severity of the inflammatory response [74]. Although it is likely that FXR protects against colitis progression mainly through myeloid cell modulation, cell-depletion and conditional-knockout approaches are required to establish the precise cell type involved. The mechanism underlying these outcomes is most likely a direct transcriptional regulation over inflammatory genes by FXR. It will be of interest as to whether FXR plays immunomodulatory roles outside the gastrointestinal tract.

4.2.4 Small Heterodimer Partner (SHP)—SHP was originally described as the negative feedback mediator of the classic bile acid synthesis pathway in liver (see section 3.4). However, the focus of SHP's hepatobiliary role has overshadowed its potential roles in other cell types and contexts. About a decade after the original description of SHP, it was shown that whole-body SHP-knockout mice undergo a more severe septic shock compared to wild type mice when challenged with lipopolysaccharide [75]. The more severe septic shock observed in the absence of SHP was attributed to a more robust inflammatory response in macrophages, driven at least in part by NF κ B and NLRP3 (NACHT, LRR and PYD domains-containing protein 3) [76]. The modulatory role of SHP on NF κ B and NLRP3 is intriguing given the many aspects of immune response initiated by NF κ B and NLRP3 signaling, and suggests that it likely plays roles beyond acute pathologies such as sepsis. In this regard, it was recently shown that, in the absence of SHP, liver tissue harbors a more inflammatory milieu due to increased C-X-C motif ligand 2 (CXCL2) release and subsequent neutrophil recruitment [77]. These reports demonstrate that SHP likely plays important, physiologically relevant regulatory roles in the immune response, the precise nature of which remain to be elucidated.

4.2.5 Estrogen Receptor (ER)—Although not traditionally thought of as a receptor mediating either cholesterol homeostasis or aspects of the immune system, several recent reports have identified the estrogen receptors (ERs) as involved in both processes. The two isoforms of the nuclear receptor ER have distinct expression profiles, with ER α being expressed in a variety of tissues and ER β being more restricted to male and female reproductive tissues [8]. In some cases, it appears that ER β can modulate ER α -mediated responses to ligands [78, 79]. Perhaps biased by its clear roles in reproductive biology, the influence of steroid hormones such as 17- β -estradiol on immune cells has gone relatively unexplored. The role of classic ER ligands such as estradiol has now been implicated in several aspects of immune regulation, including promoting a pro-inflammatory phenotype in cells of the myeloid lineage and, therefore suppressing lymphocyte activity [80, 81]. Furthermore, the metabolite A-diol has been found to modulate glial cell biology by promoting ER β tethering and transrepression over inflammation-driving transcriptional complexes [82]. Furthermore, ER signaling has been shown to directly modulate T lymphocyte generation, differentiation and function, affecting osteoporosis and autoimmune diseases [83, 84]. It appears that the findings of these studies indicate opposite outcomes on T lymphocyte responses upon manipulation of ER signaling [85, 86]. The paradoxical

findings, however could be due to differences in (1) methodology, such as genetic approaches that ablate the receptor [87] as opposed to pharmacological approaches that administer exogenous ligands [88], and (2) pathophysiological context, in which mouse models may have differences in baseline T cell homeostasis, such as ovary-intact antigen-challenged compared to ovariectomized antigen-intact female mice. However, given the focus of this review on cholesterol metabolites and immunology, we will highlight the effects of 27HC, a recently described selective estrogen receptor modulator (SERM). 27HC was the first endogenous SERM identified [17]. The characterization of 27HC as a SERM came from two papers which found that (1) 27HC binds to both ER α and β , (2) 27HC binding to ER α resulted in a conformational change different than that observed for either estradiol or tamoxifen, (3) 27HC inhibited the ER-mediated atherosclerosis-protection of estradiol (thus behaving as an “antagonist”) and (4) stimulating proliferation of ER-positive breast cancer cell lines in an ER dependent manner (thus behaving as an “agonist”) [89, 90].

Although it is clear that ER agonists including 27HC directly promote primary breast tumor growth [91, 92], a recent report has found that 27HC can also promote metastasis, but it does so by engaging the immune system [93]. Specifically, it has been found that 27HC recruits granulocytic myeloid cells to distal metastatic sites. The 27HC recruited granulocytic myeloid cells are positively correlated with $\gamma\delta$ T lymphocytes, which likely feedback to further polarize the granulocytic myeloid cells into an alternative anti-inflammatory state [94]. Collectively, the $\gamma\delta$ T lymphocytes and granulocytes result in decreased CD8+ cytotoxic T cells, allowing for the increased metastatic outgrowth of tumors [93]. This is even more intriguing when one considers that myeloid cells have show high expression of CYP27A1, and thus may be able to provide their own source of 27HC [95]. It is yet to be determined what the relative contributions of myeloid cell-synthesized 27HC are compared those from circulating 27HC (largely hepatic synthesized). However, given the robust effects of 27HC in promoting metastasis, the regulation of CYP27A1 within myeloid cells is of considerable interest. The precise contributions of the ERs in mediating this process are not yet clear, and it is important to note that 27HC also signals through the LXRs [20, 96], adding another layer of complexity. Furthermore, there is some evidence that 27HC may also modulate the activity of the C-X-C chemokine receptor 2 (CXCR2), a critical mediator of granulocyte migration, and CXCR2 activity is required for the pro-metastatic effects of 27HC [93, 97]. However, in support of a role for ERs in myeloid cell biology is another recent report indicating that estradiol alters myelopoiesis by both enhancing myeloid cell trafficking as well as their ability to suppress the expansion of activated T cells [80]. By doing so, estradiol contributes to the progression of ovarian tumors in preclinical models.

4.2.6 Nuclear Receptors with Emerging Evidence of Involvement in Cholesterol Homeostasis and Immunology.—The glucocorticoid receptor (GR) has long been known to exert potent anti-inflammatory effects, and as such has been a target for several steroidal anti-inflammatory medications. One mechanism by which the GR dampens the inflammatory response is by associating and inhibiting the transcriptional complex of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) [98]. GR signaling has recently been linked to cholesterol biology as it was found that GR mediates the breast tumor promoting effects of 6-oxo-cholestan-3 β ,5 α -diol [99]. The effects reported were

ascribed to its direct effects on cancer cells [99]. It will be of significant interest to determine whether this cholesterol metabolite or others actively signal in immune cells such as macrophages.

Peroxisome proliferator-activated receptor (PPAR) gamma and to a smaller extent delta are the PPAR isoforms mainly associated with an immunomodulatory role, perhaps due to their expression in macrophages and lymphatic tissues, such as spleen. PPAR γ is mainly associated with an anti-inflammatory outcome [100], while PPAR δ is known to exert pro-inflammatory functions [101]. PPAR γ is highly expressed in adipocytes, which is postulated as the basis for the efficacy of PPAR γ agonists in ameliorating the metabolic syndrome [53, 102, 103]. However, with accumulating evidence on the contribution of macrophage-mediated inflammation within the adipose tissue to the progression of the metabolic syndrome, it has to be explored what portion of PPAR γ agonist efficacy is due to modulating adipose-resident macrophage inflammation. Of interest, it has long been known that PPAR γ and PPAR α lower the progression of atherosclerosis by modulating macrophages [104]. It has been shown that by upregulating LXR signaling, PPARs upregulate cholesterol efflux to lower the inflammatory response in foam cells [105]. Similar beneficial outcomes of PPAR γ activity has been shown in other pathophysiological contexts, caused by imbalanced cholesterol homeostasis in macrophages such as pulmonary alveolar proteinosis [106].

The estrogen related receptor (ERR) has three isoforms, with each isoform being expressed with a unique pattern across tissues and developmental periods. ERRs are well-known for their ubiquitous regulation over metabolic enzymes, especially in tissues with a preferred fatty acid energy source [107]. However, there is accumulating evidence that, particularly ERR α , has roles beyond metabolic regulation, specifically in myeloid immune cells [108, 109]. It was shown that whole-body ERR α -knockout mice undergo a more severe septic shock compared to wild type, when challenged with lipopolysaccharide. This phenotype was attributed to higher NF κ B activity and glycolytic metabolism in the knockout mice, both promoting a severe inflammatory response [109]. In addition, it has been shown that the regulation of metabolism by ERR α is necessary and critical for ubiquitous and subtype-specific T cell differentiation [110].

Importantly, although previously an orphan nuclear receptor, ERR α was recently 'adopted', with a report indicating that cholesterol itself likely binds to and modulates its activity [111]. Specifically, using the ligand-binding domain of ERR α , cholesterol was pulled down from tissue lysates. Based on this finding, it was shown that the muscular cytotoxicity associated with statin-therapy, as well as the bone-depleting effects of cholesterol, depend on ERR α . Although molecular modeling would predict binding, this has not been rigorously tested yet. This, as well as the potential binding of related compounds such as oxysterols is an important area of future investigation. Furthermore, the precise contributions of cholesterol regulated ERR α in terms of immune regulation are exciting avenues to pursue, given that ERR α represents a suitable drug target, with several small molecule agonists and antagonists being described [112, 113].

Conclusions and Perspectives

It appears that the importance of cholesterol surpasses its crucial structural roles in the cells, which spans kingdoms of life. Cholesterol itself serves as a precursor for steroids, bile acids and oxysterols. Bile acids are now known to be essential signaling molecules in addition to their actions as detergents. Delineating the roles and mediators of oxysterol function will further clarify whether mammals have evolved to use this class of molecules as hormonal signals, perhaps due to their better solubility compared to cholesterol. Many oxysterols and other cholesterol metabolites serve as ligands for various nuclear receptors. Importantly, a set of tissues that appear to be affected by cholesterol and oxysterols are those populated by immune cells. Therefore, cholesterol biology seems to have strong implications in the generation, differentiation and function of the major immune lineages: myeloid and lymphoid. Whether this phenomenon is an evolutionary footprint or has evolved with an immune-driven selection is yet to be determined.

Given the broad effects of cholesterol and oxysterols among several types of immune cells, and the central role these immune populations play in the pathology of diseases that range from autoimmune to cancer, from a therapeutic standpoint, cholesterol pathways are promising targets for a variety of diseases that remain a health burden (Fig. 1). The therapeutic approaches could include (1) targeting the nuclear receptors that mediate feedback within these pathways, such as the LRH-1, FXR or SHP, (2) targeting nuclear receptors that mediate the biological function of cholesterol and oxysterols, such as LXR, GR or ERR α , or (3) targeting the enzymes within these pathways, particularly those catalyzing rate-limiting steps, such as HMGCR (statins) and CYP27A1.

In this regard, a number of areas remain to be explored. First and foremost, the specific regulation of nuclear receptors by cholesterol metabolites needs to be further elucidated. There is strong evidence that either cholesterol metabolites or various nuclear receptors regulate the immune system, but which attributes of cholesterol metabolites can be ascribed to nuclear receptor signaling requires elucidation. Furthermore, phenotypic differences observed when different cholesterol metabolites are perturbed should be explored at the receptor level. For example, in terms of breast cancer, 27HC appears to have very different biological responses compared to dendiogenin A, although whether these effects are receptor-mediated is still to be determined [91–93, 114]. These efforts will be essential for the future development of these axes for therapeutic intervention.

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Highlights:

- Cholesterol and Bile Acid homeostasis involve intricate feedback loops
- Many cholesterol metabolites serve as ligands for nuclear receptors
- Cholesterol metabolites their respective nuclear receptors implicated in immunology

Figure 1: Cholesterol Metabolism, Nuclear Receptors, Immune Outcomes and Pathophysiology of Disease.

Cholesterol precursors, cholesterol itself and downstream metabolites affect myeloid and lymphoid immune cells, triggering or resolving a pro-inflammatory state. These effects are mediated by membrane receptors (such as CXCR2, Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2/GPR183), GPR17 or Smoothened), nuclear receptors (such as the LXRs for oxysterols and FXR in the case of bile acids), SREBPs (in the case of cholesterol and oxysterols) and potentially other unknown mechanisms. The immunomodulation of immune populations affects the tissue microenvironment and ultimately organ physiology, resulting in progression or resolution of pathologies such as atherosclerotic plaques.