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Nuclear receptors: mediators and modifiers of inflammation-induced cholestasis

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1. ABSTRACT

Inflammation-induced cholestasis (IIC) is a frequently occurring phenomenon. A central role in its pathogenesis is played by nuclear receptors (NRs). These ligand-activated transcription factors not only regulate basal expression of hepatobiliary transport systems, but also mediate adaptive responses to inflammation and possess anti-inflammatory characteristics. The latter two functions may be exploited in the search for new treatments for IIC as well as for cholestasis in general. Current knowledge of the pathogenesis of IIC and the dual role NRs in this process are reviewed. Special interest is given to the use of NRs as potential targets for intervention.

2. INTRODUCTION

Inflammation-induced cholestasis (IIC) is a frequently occurring, well-recognized clinical entity. Molecular mechanisms underlying IIC have been partially unravelled over the past decades, facilitated by the increasing knowledge of mechanisms of bile formation and regulation of the transport systems involved, identification of nuclear factors controlling transporter gene expression and a broader understanding of molecular aspects of the inflammatory response. It is now evident that several nuclear receptors (NRs), i.e., ligand-activated transcription factors, play key roles in the regulation of bile formation and the pathogenesis of IIC. These receptors are not only important under physiological conditions but their involvement expands to pathophysiological situations, both as mediators, i.e., in a disease-promoting role, and as important modulators of adaptive responses. Recently, NRs have also been proposed as targets for intervention in IIC. This review focuses on the various roles of NRs in processes that lead to cholestasis during inflammation and on the ways in which NRs can be exploited for design of treatment options.

2.1. Clinical aspects of inflammation-induced cholestasis (IIC)

The link between inflammation and cholestasis has been recognized for centuries, with jaundice as the primary symptom of impaired bile formation (1-4). The underlying mechanisms of this association, however, have gone unexplained for a long time (5).

Cholestasis associated with sepsis is generally regarded as the prototypical example of IIC, but bile formation is also affected in other conditions associated with an inflammatory state and cholestasis may thus be considered as a consequence of the so-called acute phase response (APR). The APR consists of a set of rapid, well-coordinated responses initiated by infection or tissue damage leading to the production of various soluble mediators (e.g., proteases, clotting factors, cytokines, etc.) aimed at restoration of homeostasis (6,7). The APR also includes a broad suppression of many core intermediary metabolic functions within the liver – notably albumin synthesis and the metabolism of carbohydrates, fats, and bile acids are affected, the latter of which directly contributes to cholestasis. Conditions besides sepsis that are associated with cholestasis include extrahepatic infections such as bacterial pneumonia and urinary tract infections (1-3,8-10), but this group can likely be expanded with conditions involving a systemic inflammatory response syndrome following burn injury, severe trauma and major surgery (11). The importance of circulating pro-inflammatory mediators in the pathogenesis of IIC was illustrated by the side-effects of therapeutical administration of these mediators to humans. Treatment of cancer patients with recombinant cytokines (TNF-alpha or IL-2) in phase I/II clinical trials was shown to lead to hyperbilirubinemia and cholestasis (12,13). Cholestasis seen in certain non-metastatic paraneoplastic syndromes, such as Stauffer's syndrome, appears to be caused by secreted cytokines too (14,15). Considering the plethora of conditions associated with IIC, it is not surprising that jaundice is frequently observed in intensive care units for children, most notably neonates, and adults. The importance of sepsis as an underlying cause of clinical cholestasis has often been overlooked (16).

The presence and severity of cholestasis appears to be associated with poor prognosis of septicaemia (17). This obviously does not imply that cholestasis itself is the causative factor of poor outcome: cholestasis is more likely an indicator of the severity of sepsis. Therefore, current treatment modalities are mainly aimed at treating sepsis with antibiotics and further supportive care and not at restoration of hepatic secretory function. Yet, it is easily appreciated that cholestasis *per se* will have immediate repercussions for the metabolism and elimination of drugs and toxins. Moreover, intestinal function will be impaired with reduced bile flow, with subsequent complications of malabsorption as well as bacterial overgrowth and translocation, further worsening the cholestatic state. The long-term effects of sepsis-associated cholestasis are largely unknown.

2.2. Experimental models of IIC

The pathogenesis of IIC has been studied using a variety of *in vivo*, *ex vivo* and *in vitro* models. These models generally involve the induction of a hepatic APR. A frequently used *in vivo* model involves administration of endotoxin, i.e., lipopolysaccharide (LPS), to rodents (18). LPS, a component of the outer membrane of Gram negative bacteria, is a ligand for two different pattern-recognition receptors, i.e., Toll-like receptor (TLR[1])-4 (19) and CD14 (20). LPS signaling is dependent on a complex arrangement that includes binding to soluble proteins (LPS-binding protein) and both CD14 and TLR4. Mice deficient in either Tlr4-signaling (mutant strain C3H/HeJ or null-mice C57BL/1-ScCr) or Cd14 are resistant to LPS (19,21). These receptors are present at the surface of several cell types within the liver, including Kupffer cells (KC), and LPS binding elicits an immune response in these cells (22). KCs are the resident liver macrophages and central mediators of the inflammatory cascade leading to IIC. Mice of the C3H/HeJ strain are often used as LPS-resistant control mice (23-26).

IIC has also been studied using different activators of innate immunity, such as zymosan (27) or lipoteichoic acid (28), or individual pro-inflammatory cytokines (29-31). Other models include administration of chemical agents to rodents, e.g., turpentine (32-34) or surgical procedures to induce polymicrobial sepsis (e.g., cecal-ligation and puncture (CLP) (35-39)).

Isolated perfused rodent livers (40-43) allow for well-controlled experiments with regard to perfusate composition, use of tracers, etc. Precision-cut slices from both human and rodent liver have also been used to study the effects of LPS on cytokine expression and transporter expression (44-46).

In addition to using intact animals/organs, IIC has been examined at the (sub)cellular level using primary

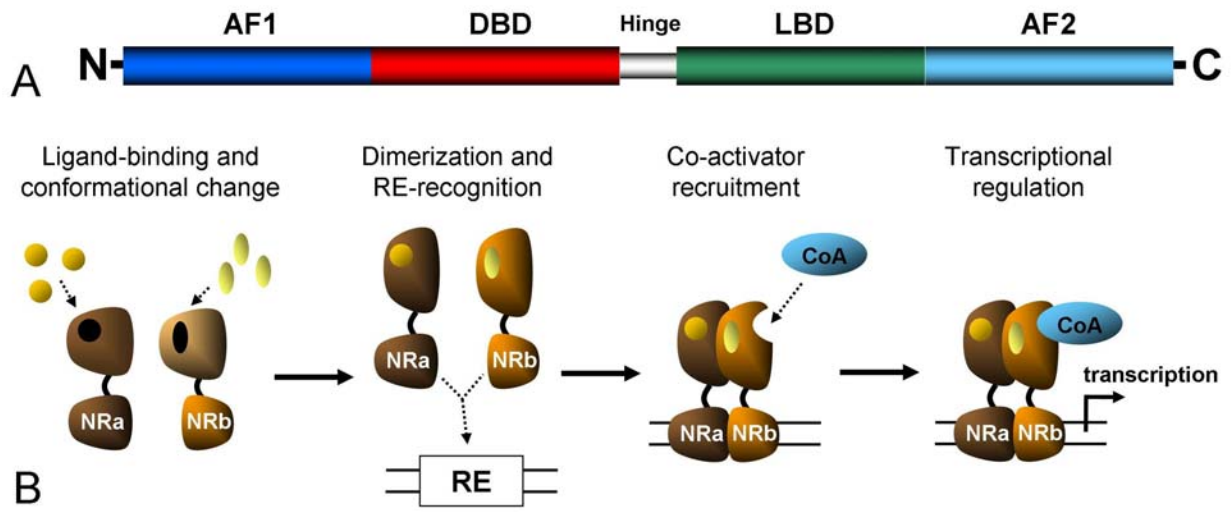

Figure 1. A.) Schematic of NR structure (N = N-terminus, AF = activation function domain, DBD = DNA-binding domain, LBD = ligand-binding domain, C = C-terminus); A.) Schematic of general mechanism of transactivation by ligand-activated NRs (NRa/b, generic NR heterodimer partners, RE = response element, CoA = co-activator). Of note, ligand-binding and conformational change do not necessarily occur prior to DNA-binding, as they may also induce release of co-repressors from RE-bound NRs).

hepatocytes or hepatoma cell-lines (47,48). Treatment with (individual) cytokines or medium obtained from activated Kupffer cells or macrophages mimics the *in vivo* response at the hepatocellular level down-stream of KC activation (49).

3. NUCLEAR RECEPTOR (NR) BIOLOGY

NRs are ligand-activated transcription factors that play important roles in many aspects of metazoan life, including embryonic development, cell differentiation and maintenance of cellular homeostasis (50). NRs are expressed differentially amongst tissue and throughout day-night cycles (51,52). NRs are assumed to have arisen from constitutively active transcription factors (53) having acquired the ability to be activated by hormones (e.g., glucocorticoid receptor (GR), estrogen receptor (ER)) or to sense local environmental and nutritional cues (e.g., liver X receptor (LXR), farnesoid X receptor (FXR)). This allows not only for concerted gene responses throughout the organism, initiating hormone-appropriate responses, such as the stress-response after glucocorticoid release, but also for cell-specific responses to altered local environmental conditions, as exemplified by induction of cholesterol efflux transporters during cellular sterol overload by LXR stimulation.

3.1. Classes and structures of NRs

The various NRs share several structural similarities: a DNA-binding domain (DBD), a ligand-binding domain (LBD) and activation function domains (AF)-1 and AF-2 (Figure 1A). A DBD contains two well-conserved zinc-finger domains, which determine the affinity for specific DNA sequences known as response elements (*cis*-acting elements) (50). The LBD determines the ligand-specificity of NRs and will thus differ most significantly between NR family members (54). Ligand binding will lead to conformational changes in the NR molecule, resulting in altered transcriptional activities through re-organization of the transcription complex at the promoter, generally involving removal of co-repressors and recruitment of co-activators. The AF-1 (situated at the N-terminus) and AF-2 (actually contained within the LBD at the C-terminus) mediate interactions with co-regulators.

The NR superfamily has been subdivided according to different classifications. Traditionally, NRs have been divided into three functional groups (50): 1) classic NRs such as GR, ER, etc. These NRs generally form homodimers and are activated with high affinity by steroid hormones in an endocrine fashion. 2) heterodimers with retinoid X receptor (RXR) and partners such as LXR, FXR, retinoic acid receptor (RAR), peroxisome proliferator activated receptors (PPARs), that are activated at lower affinity by metabolites or nutrients such as fatty acids and oxysterols, and 3) orphan NRs, referring to transcription factors expected to be NRs based on gene/protein structure, for which no specific ligands have been identified yet, or appear to lack a functional LBD based on structural analysis. Once members of this class of NRs have been assigned (specific) ligands, they become adopted, as occurred recently with the identification of heme as ligand of REV-ERB-alpha and REV-ERB-beta (55,56).

More recently, NRs nomenclature has been revised in a way analogous to that for cytochrome p450 (CYP) enzyme systems using a coded numbering system. In this system, NRs are classified into 6 distinct groups based on molecular phylogeny (57). This system allows for classification of NRs from different species as well as NRs identified by genetic analyses without clarified functional and/or biological characteristics (53).

Inherent to their function as transcriptional regulators, NRs selectively recognize and bind to short DNA sequences located in gene-regulatory elements, either in close proximity to the transcriptional start sites (promoters) or in more distant elements (enhancers). These "*cis*-acting" response elements (REs) share several characteristics. REs that mediate transcriptional activity of RXR-heterodimers generally consist of two hexamers that are separated by one to eight nucleotides and are direct, everted or inverted repeats. However, some of the NRs that form heterodimers with RXR may also regulate transcriptional activity by binding as a monomer (e.g., FXR (58,59)).

3.2. Mechanisms of genomic actions

A key characteristic of NRs that sets them apart from other transcription factors is altered activity upon ligand binding. Although in reality much more intricate due to the involvement of many proteins (53), the general mechanism of NR-regulated gene expression is depicted in Figure 1B. It involves a conformational change of the NR upon binding of its ligand. Some NRs will then relocate to the nucleus, bind to their corresponding response elements and recruit co-activator molecules. Other NRs are already bound to their response elements while being unliganded and associated with co-repressor proteins. Upon ligand binding, these NRs will release co-repressors and start to recruit co-activators. The recruitment of co-activators is a process that involves a multitude of proteins and has specific spatial and temporal characteristics. The conformational changes that occur upon ligand-binding are thought to invoke a closure of the ligand-binding pocket by helix 12 rendering the surface of the NR more available for binding of co-activators, while co-repressors become less able to bind and are released. Since NR-binding to promoters is a cyclic process, i.e., a continuous binding and removal of NRs from the response elements, increased stability of the co-activator complexes will shift the balance from inhibition to stimulation of transcription. One of the important molecular actions of these multi-functional co-activators is chromatin relaxation through histone acetyltransferase activity or mediating the recruitment of other proteins with such function. This will render target genes more accessible for the transcriptional machinery. Co-repressors such as the nuclear receptor co-repressor (NCoR) or silencing mediator of retinoid and thyroid hormone receptor (SMRT) either have histone deacetylating properties or stimulate the recruitment of other co-repressors with such enzymatic activity, the so-called histone deacetylases (HDACs), and thus reverse chromatin relaxation and inhibit gene transcription. In addition to (de)acetylating modification of histones, co-regulators can also modify histones via (de)methylation and (de)phosphorylation mechanisms (60). There are also non-histone-mediated actions by which co-regulators affect gene transcription, including ATP-dependent remodelling of chromatin and the recruitment of both basal transcription factors and co-regulators (60,61). Co-regulators are regarded as the actual determinants of NR-mediated transcriptional regulation and their tissue-specific expression patterns are responsible for the specific effects of NRs and their ligands in different tissues (62).

3.3. Non-genomic actions of NRs

Besides the "classical" NR mode of action, some of the NRs exert effects on gene transcription without DNA-binding, i.e., "non-genomically" by protein-protein interactions (53). The small heterodimer partner (SHP) is one example of a NR that acts non-genomically as it lacks a DBD. SHP is often seen as a transcriptional repressor that acts by binding and interfering with the action of some NRs and transcriptional activators. SHP, however, is not the only NR that has non-genomic actions. There is substantial evidence that many NRs that possess a DBD regulate gene expression through other domains in the protein and without DNA-binding. This group includes GR, ER, PPAR-gamma and LXR, which are known to regulate gene expression via traditional REs, but have also been shown to suppress inflammatory signaling via non-genomic interactions.

3.4. Clinical relevance of NR ligands

The aspect of ligand-induced modification of NR activity and their generally well-matched sets of transcriptional targets has led to the concept that NRs represent attractive targets for pharmacological intervention in a wide range of pathophysiological processes. An estimated 20% of all prescriptions in the United States exert their effects via NRs (63). Interestingly, some of these were already used clinically without knowledge of their primary target or molecular mode of action, such as hypolipidemic fibrates (e.g., clofibrate) and antidiabetic thiazolidinediones (e.g., rosiglitazone) which were later shown to be PPARalpha and PPAR-gamma ligands, respectively (64-66). For several drugs, their identification as ligands of NRs, more specifically of the pregnane X receptor (PXR) and constitutive androstane receptor (CAR), provided an explanation for their known interference with metabolism of other drugs. Examples of the latter group include phenobarbital, a CAR ligand (67-69), and the PXR ligands rifampicin and nifedipine (70). These compounds regulate drug metabolism through activation of CAR and PXR and subsequent changes in the expression of *CYP* and other genes involved in drug metabolism.

3.5. NRs of specific relevance to IIC

A subset of NRs is of particular interest in relation to IIC. This subset includes RXR-alpha for its central role as obligate heterodimerization partner for other class II NRs, RAR-alpha for its role in control of basal hepatocellular gene expression, FXR as bile acid sensor, PXR and CAR as xenobiotic sensors involved in detoxification pathways, LXR and PPAR-gamma for their recently identified anti-inflammatory activities and the orphan receptors liver receptor homologue (LRH)-1, hepatocyte nuclear factor (HNF)-4-alpha and SHP, involved in the transcriptional regulation of various genes involved in bile formation and hepatobiliary transport. Some of the characteristics of these NRs are summarized in Table 1. In the paragraphs reviewing anti-inflammatory properties of NRs involved in IIC, GR will also be discussed as the prototypical example of NRs with anti-

Table 1. NRs involved in bile-formation and IIC, examples of their natural and synthetic ligands and examples of their direct, IIC-related, target genes.

NR	Official name	Ligands		Examples of direct target genes
		Natural	Synthetic	
RXR-alpha	NR2B1	9*cis* retinoic acid	LG268, LG1069	(Obligate heterodimer partner)
RAR-alpha	NR1B1	All-trans retinoic acid	TTNPB	*NTCP, MRP2, CYP7A1*
FXR	NR1H4	Chenodeoxycholic acid, cholic acid	GW4064	*BSEP, SHP, OST-alpha/beta*
LXR-alpha/beta	NR1H3/NR1H2	Oxysterols	T0901317, GW3965	*ABCG5/8*, (rodent) *Cyp7a1*
PPAR-gamma	NR1C3	15d-PGJ2, fatty acids	Thiazolidinediones	
PXR	NR1I2	5beta-pregnane-3,20-dione	PCN, rifampicin, nifedipine	*MRP2, MDR1, CYP*-families
CAR	NR1I3	Androstenol	TCPOBOP	*MRP2, CYP*-families
Orphans				
HNF4-alpha	NR2A1	-	-	*ABCG5/8, CYP8b1*
LRH-1	NR5A2	-	-	*CYP7A1*, (human) *ABCG5/8*
SHP	NR0B2	-	-	(No DBD)

15d-PGJ2 = 15-deoxy-delta-12,14-prostaglandin J2; TCPOBOP = 1,4-bis-2- (3,5-dichloropyridyloxy)-benzene; PCN = pregnenolone-16alpha-carbonitrile. (Adapted from Karpen (54).)

inflammatory properties. Some other NRs, e.g., ER or progesterone receptor (PR), are described to further illustrate general principles regarding NR biology.

4. MECHANISMS UNDERLYING IIC

It is generally accepted that IIC results from impairment of normal hepatobiliary transport functions through the effects of pro-inflammatory mediators (4,18). These mediators can either reach the liver via the systemic circulation or be produced locally in response to a variety of stimulants, including endotoxin (11,18). Pro-inflammatory mediators are able to affect hepatobiliary transport functions via multiple signal transduction pathways, targeting events at the membrane, cytosol and the nucleus.

4.1. Physiology of bile formation

Bile formation represents an osmotic process, driven by active secretion of cholephils by hepatocytes into the minute bile canaliculi (71). These canaliculi are separated from the circulation by tight junctions connecting adjacent hepatocytes. The active secretion of osmotically active solutes, most notably bile salts, leads to the passive transport of water and electrolytes into the canaliculi, thus generating bile flow (71). Total hepatic bile flow is considered to be the sum of bile salt-dependent flow (BSDF) and bile salt-independent flow (BSIF). The latter is mainly driven by the secretion of substances such as glutathione (72,73) and bicarbonate (74). The canaliculi join to form bile ductules that are lined with cholangiocytes and eventually converge into the major bile ducts that drain into the duodenum. Although bile ducts were earlier primarily regarded as a drainage system, it is now clear that the bile duct epithelium plays an active role in the generation of bile flow and regulation of bile composition (75). Approximately 10-13% and 40% of total bile flow in rats and humans, respectively, is driven by secretin-stimulated secretion of chloride and bicarbonate by cholangiocytes (76,77). Not surprisingly, substantial compositional differences exist between canalicular and ductular bile. In humans and mice, but not in rats, bile is stored and further modified by concentration in the gallbladder.

Over the past decades, knowledge of the molecular basis of bile formation has greatly expanded by the identification of several essential transporters that contribute to the process (78). The most important hepatocellular transporters involved in bile formation as well as in protection of hepatocytes against bile salt overload are shown in Figure 2. At the basolateral side of hepatocytes, the Na^+-taurocholate co-transporting polypeptide (NTCP), organic anion transporting proteins (OATPs) and organic cation transporter (OCT)1 are responsible for sodium-dependent and sodium-independent uptake of bile salts, organic anions and cations. At this side of the cell, members of the multidrug resistance-associated proteins (MRPs), i.e., MRP3 and MRP4, are present too. These transporters exert hepatocyte-protecting effects during (extrahepatic) cholestasis most likely by facilitating the basolateral efflux of retained and potentially harmful substances including bile salts (79,80). Recently, an additional bile salt transporter complex has been identified, i.e., the organic solute transporter (OST)-alpha/beta heterodimer (81,82), which appeared to be the elusive bile salt efflux transporter at the basolateral side of enterocytes (83,84). OST-alpha/beta heterodimers are expressed in the liver, both in cholangiocytes and hepatocytes (85), differentially between species. In cholangiocytes, it may very well play a role in "cholehepatic shunting"of bile salts, while in hepatocytes it appears to function as an overflow efflux transporter, as its expression is markedly induced under cholestatic conditions in an FXR-dependent manner (86,87).

At the canalicular membrane of hepatocytes, several transporters are localized that are responsible for the biliary secretion of various biliary components. Many of these transporters belong to the ATP-binding cassette (ABC) transporter family and actively transport their substrates against concentration gradients into the canalicular lumen. The bile salt export pump (BSEP or ABCB11) mediates biliary secretion of monovalent bile salts. MRP2 (ABCC2) is responsible for efflux of divalent anions including conjugated bilirubin and sulfated bile salts. Multidrug resistance (MDR)-3 P-glycoprotein (ABCB4, rodent orthologue Mdr2/Abcb4) is involved in phospholipid secretion into the canalicular lumen by

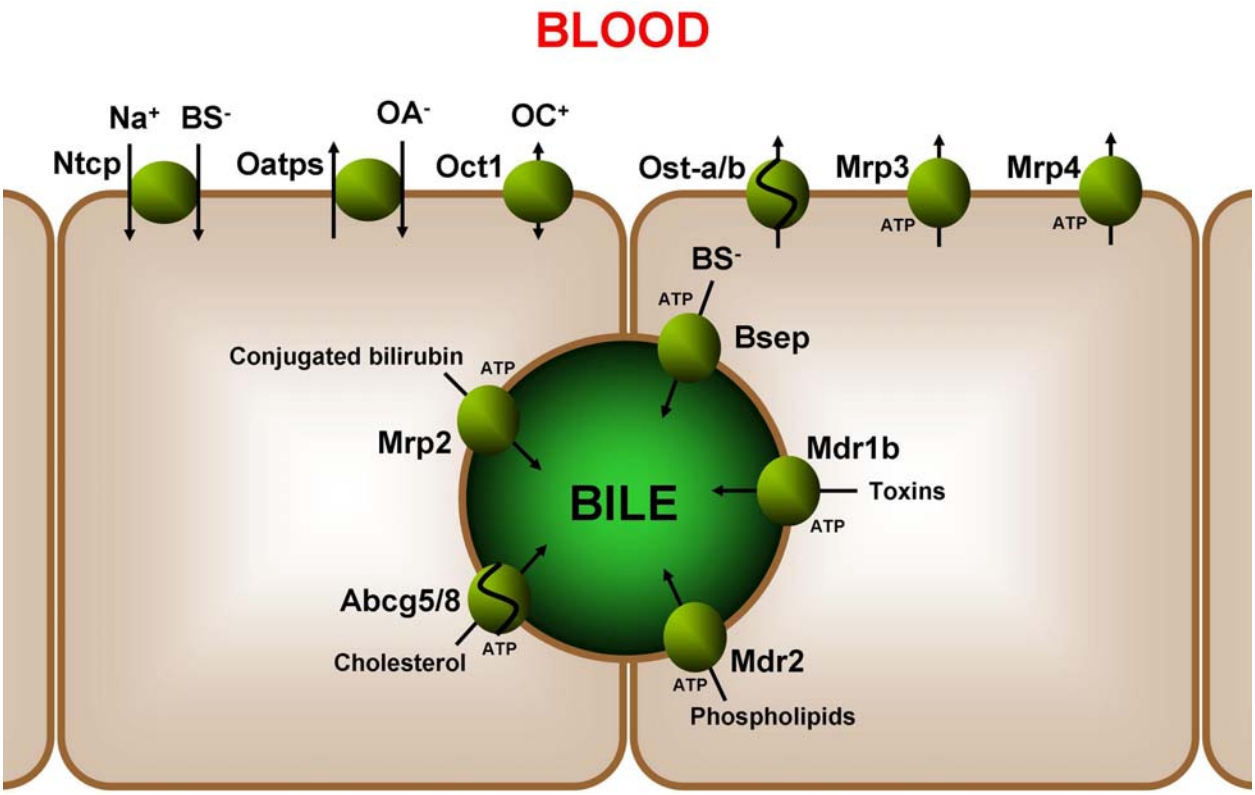

Figure 2. Schematic of hepatobiliary transporters (rodent) involved in bile formation and adaptive responses to cholestasis (see text for details). Adjacent hepatocytes and a bile canaliculus are shown. (BS = bile salt, OA = organic anion, OC = organic cation, Ost-a/b = Ost-alpha/beta)

functioning as a floppase (88). ABCG5 and -8 are two halftransporters that facilitate sterol export and MDR1 (ABCB1, rodent orthologue Mdr1b/Abcb1) is involved in the excretion of many organic cations (endobiotics, xenobiotics). The importance of the various individual transporters in the process of bile formation is demonstrated in various human syndromes as well as various animal knock-out / mutant models (88).

Several other (non-ABC) transporter proteins are also present on the canalicular membrane including FIC1 and Niemann-Pick-1-like-1 protein (NPC1L1). The importance of FIC1 (ATP8B1) is evident since mutations in the *ATP8B1* gene have been recognized as the genetic defects underlying progressive familial intrahepatic cholestasis type 1 (PFIC1, hence its name FIC1), also known as Byler's disease. Different, milder mutations in *ATP8B1* lead to benign recurrent intrahepatic cholestasis type 1 (BRIC1). Although it is known to be a member of sub-family of ATP-transporters that act as aminophospholipid flippases, its exact modes of action under physiological conditions and in the development of PFIC1 and BRIC1 remain to be established (88). NPC1L1 has been identified as the transporter responsible for cholesterol uptake in enterocytes and as the target of the cholesterol-lowering drug ezetimibe (89). NPC1L1 is highly expressed in human (but not mouse) liver (89), where it might mediate re-uptake of cholesterol from the canalicular lumen (90). The physiological relevance of this process is, however, unknown.

4.2. Impaired bile formation during inflammation

LPS-treatment of perfused rat livers reduces bile flow as well as bilirubin and dye transport (29,40,41,43,91-93). Although LPS-treatment was initially thought to primarily affect BSIF (41,92,94), BSDF was also shown to be affected (29,43,93,95), indicating that both components of bile flow are impaired upon inflammation. Similar results were obtained in another sepsis model, i.e., CLP (36).

LPS interferes with normal bile flow generation via several mechanisms, which all ultimately lead to reduced activity of the transporters and enzymes involved in the process. With regard to BSIF, LPS reduces Na^+-K^+-ATPase activity (30,92) and also impairs glutathione secretion (94). The latter has been linked to reduced mRNA and protein expression of Mrp2 (Table 2), but this transporter has also been shown to undergo intracellular re-localization upon LPS-treatment (42,96-98).

It is generally accepted that canalicular transport is rate-controlling in overall hepatobiliary transport (93,99). Hence, impaired biliary secretion is likely to lead to accumulation of potentially toxic substances in hepatocytes. Simultaneous effects on basolateral

Table 2. *In vivo* effects of LPS on hepatobiliary transporters in rodent (mouse/rat) models.

Transporter	Official name	mRNA	References	Protein	References
Basolateral					
Ntcp	Slc10a1	↓	(30,34,108,110,111,126,133,141,152,195,281-285)	↓	(30,108,281,282,284,285)
				↔	(195)
Oatp1	Slco1a1	↓	(34,109-111,126,284,285)	↓	(284-286)
		↔	(286)		
Oatp2	Slco1a4	↓	(109,110,126,152,284,285)	↓	(284,285)
		↔	(111)		
Oatp4	Slco1b2	↓	(24,111,126,284,285,287)	↓	(285)
Oct1	Slc22a1	↓	(126)		
Mrp3	Abcc3	↓	(34,109,152)		
		↔	(111,288)		
		↑	(126)	↑	(288)
Mrp4	Abcc4	↔	(111,285,288)		
Canalicular					
Bsep	Abcb11	↓	(33,34,109,111,125,126,133,152,283-285,289)	↓	(43,283)
				↔	(285)
Mrp2	Abcc2	↓	(34,42,43,96,106,109,110,126,133,152,283,284,288,290)	↓	(43,96,152,283,288,288,290)
		↔	(111)		
Mdr1b	Abcb1b	↓	(33,125)		
		↔	(111,152,284)	↔	(284)
		↑	(96,126)		
Mdr2	Abcb4	↓	(33,125,152)		
		↔	(96)		
Abcg5/8		↓	(133,161)		

Transporters, whether there be a reduction of influx (NTCP, OATPs) or an increase in efflux (MRP3, MRP4, OST-alpha/beta), will therefore strongly influence the degree of bile salt retention and potential cellular damage. Due to their important contribution to bile formation, effects of inflammatory signaling on the cholangiocytes also contribute significantly to the pathogenesis of IIC. Spirli *et al.* (100) analyzed the effects of inflammatory mediators on biliary fluid secretion using isolated bile duct units. These authors found that a mixture of cytokines, but not individual cytokines, reduced cAMP-dependent fluid secretion in isolated bile duct units (100). At the same time, they observed an impaired biliary epithelial barrier function, which most probably contributes to the emergence of cholestasis (100). These data suggest that effects on biliary epithelium indeed have a role in IIC, but further studies are necessary to gain insight into the quantitative and qualitative importance of the effects of inflammation on the biliary epithelium.

Important for bile flow generation, and bile salt metabolism in general, is the synthesis of bile salts by the hepatocytes. This process is tightly regulated and involves the actions of several NRs, e.g., FXR, SHP, LRH-1, HNF4-alpha and LXR (101). Due to the highly efficient reabsorption of bile salts by the epithelia of biliary system and in the distal ileum, leading to cycling of bile salts via the cholehepatic shunt and in the enterohepatic circulation, respectively, the loss of bile salts per cycle is small. Therefore, the quantitative contribution of newly synthesized bile salts to the bile formation process is limited. Inflammation-induced suppression of the expression and activity of Cyp7a1 and Cyp27 (102,103), which catalyze the first step of respectively the classic/neutral and the acidic pathway of bile salt synthesis from cholesterol (101), will only become relevant after prolonged sepsis/inflammation. On the other hand, effects of inflammation on intestinal and gallbladder motility, leading to impaired enterohepatic cycling, may contribute to impaired bile formation.

4.3. Inflammatory cascade and Kupffer cells

IIC can be elicited by various inflammatory mediators, which are either reaching the liver from the circulation or are produced locally (Figure 3). The Kupffer cells (KC), resident liver macrophages, play a central role in local production (104). KCs form the primary line of defense against intestine-derived toxins that enter the liver via the portal circulation, as is illustrated by the nearly complete clearance of endotoxin from portal blood by KCs (105). Activation of KC by LPS occurs via several different signal transduction pathways (22). The importance of KCs in the pathogenesis of IIC has been demonstrated in various in vivo studies in which suppression of transporter expression by LPS-administration was found to be reduced when KC had been inactivated by gadolinium chloride (106,107) or selectively removed using liposomal clodronate (108).

Mediators affecting bile formation in hepatocytes include KC-secreted pro-inflammatory cytokines such as IL-1beta, TNF-alpha and IL-6. The importance of these cytokines was supported by experimental findings showing that individually administered cytokines elicited similar responses *in vivo* as LPS did (30,31,34,109). In addition, cytokine-inactivation through the administration of anti-TNF-alpha and anti-IL-1beta antibodies blunted the cholestatic response to endotoxin-treatment both *in vitro* and *in vivo* (29,49,110), despite the fact that there appeared to be some degree of redundancy in the cytokine-signaling (111).

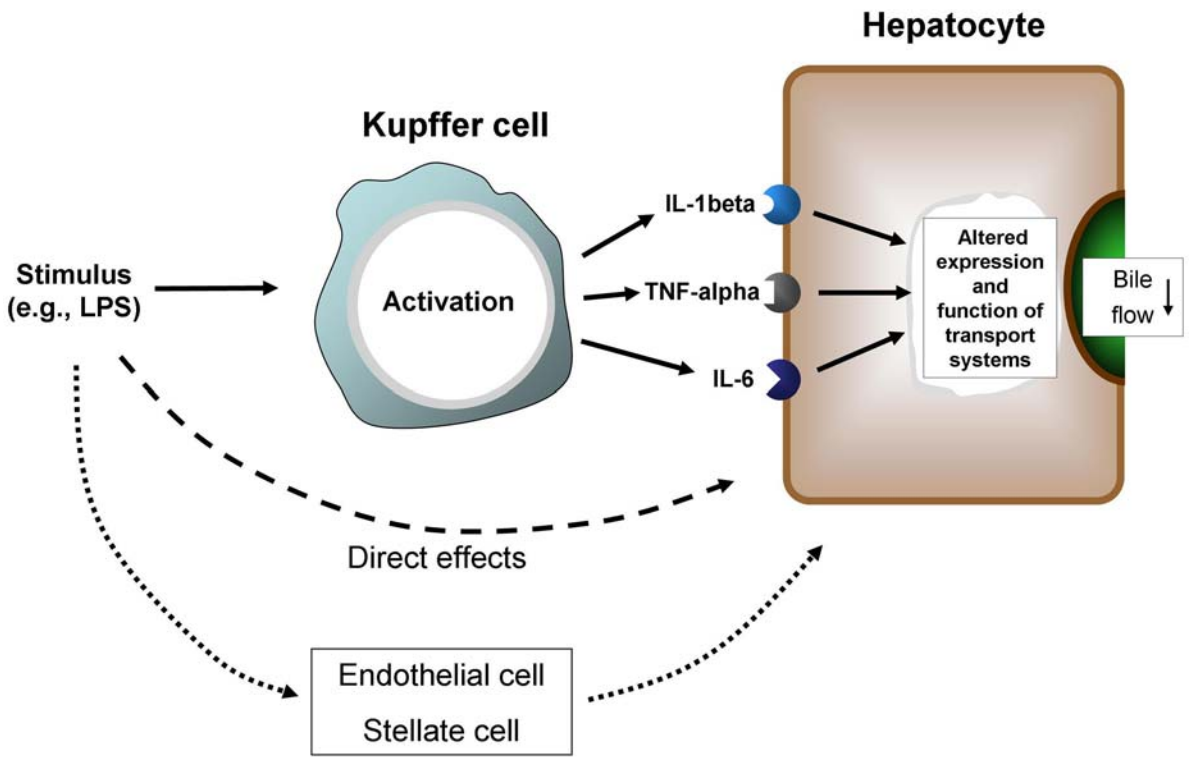

Figure 3. Linking inflammatory signals to hepatocellular effects. Inflammatory stimuli can interfere with normal hepatocellular function either via activation of Kupffer cells and subsequent release of pro-inflammatory mediators (top), through direct effects on hepatocytes (middle) or through effects on other non-parenchymal cells (bottom).

Although the involvement of KCs in the pathogenesis of IIC is evident, it has also become clear that KC-independent mechanisms contribute. Suppression of Ntcp expression after LPS administration persisted in KC-depleted livers, albeit that the degree of suppression was lower than in control livers (108). It is plausible that either direct effects of LPS on hepatocytes or LPS activation of other non-parenchymal liver cells can be involved in the pathogenesis of IIC, e.g., endothelial cells and stellate cells. Endothelial cells, for instance, are known to express TLR4 and to respond to inflammatory stimuli (112,113). The importance of endothelial cells is further illustrated by the findings of Laschke *et al.* (114) using a combination of LPS and D-galactosamine (LPS/Gal) in mice. Pre-treatment of mice with antibodies against the adhesion molecule P-selectin, which is present on endothelial cells upon LPS-treatment (115), reduced the recruitment of leukocytes and concomitantly diminished the effects of LPS/Gal on bile flow, transporter gene expression profile and liver morphology, while levels of inflammatory cytokines were not affected (114). These results indicate that processes such as leukocyte recruitment are also of critical importance in the pathophysiology of sepsis-associated cholestasis. The mechanistic link is between reduced leukocyte recruitment and inhibition of cytokine activity remains to be determined.

Finally, activated KCs not only secrete cytokines but also inflammatory mediators such as nitric oxide (NO) and prostaglandins (116). Although NO had been shown to stimulate bile flow (117), LPS-induced NO-production did not have a significant effect on LPS-induced suppression of bile flow (94). Prostaglandins have also been shown to reduce bile flow and bile salt secretion (118,119).

4.4. Inflammatory signaling in hepatocytes

Pro-inflammatory cytokines and other inflammatory mediators affect hepatocellular function via various, sometimes parallel, pathways (7,120). These include, but are definitely not limited to, NF-kappaB and mitogen-activated protein kinase routes. Examples of how these pathways can contribute to the development of IIC include NF-kappaB-mediated induction of *Mdr1b* expression (121), c-jun N-terminal kinase (JNK)-mediated nuclear export of RXR-alpha *in vitro* (47,122) and extracellular regulated kinase-mediated inhibition of interferon-regulated factor-3 nuclear import (48). Of note, the specific roles of these pathways have often been examined *in vitro* using hepatocytes or hepatoma cells treated with (individual) cytokines. Although this is an elegant approach to elucidate underlying mechanisms, one has to bear in mind that in the *in vivo* situation multiple, parallel pathways may be active with a certain degree of redundancy, while LPS affects hepatocytes directly to some extent as well (123,124).

4.5. Hepatobiliary transporters

Inflammatory signaling has been shown to differentially affect hepatocellular transport function at both transcriptional and post-transcriptional level. The

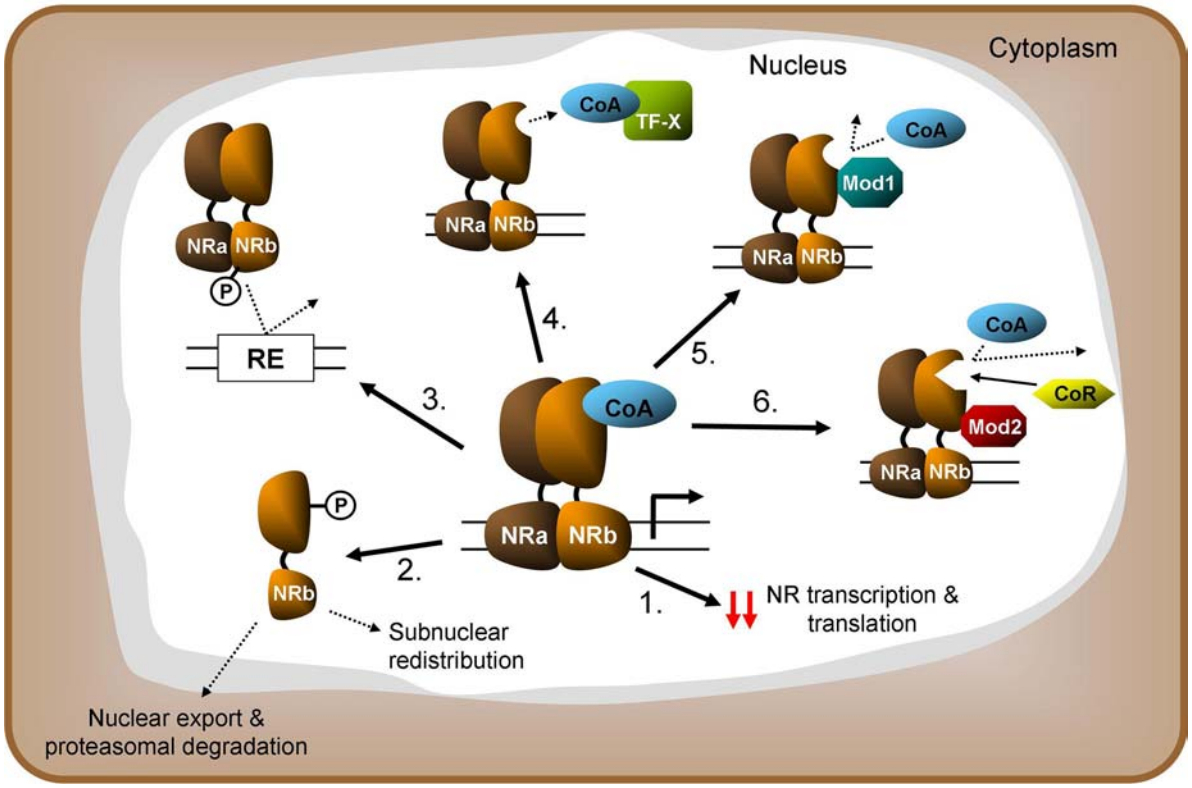

Figure 4. General effects of inflammatory signaling on NR expression and function: 1.) suppression of NR transcription or translation; 2.) signal dependent relocalization and degradation; 3.) reduced DNA binding by post-transcriptional modification of NR (e.g., phosphorylation (P)); 4.) competition for co-activator (CoA) by inflammation-induced transcription factor (TF-X); 5) inhibition of CoA recruitment by NR-modification (Mod1) leading to steric hindrance and/or conformational change; 6) increased affinity for co-repressor (CoR) binding by NR-modification (Mod2). (Other abbreviations: NRa/b, generic NR heterodimer partners, RE = response element, CoA = co-activator.)

effects of LPS on the expression of several physiologically relevant transporters in rats and mice are summarized in Table 2. This list, however, is not complete and ought to be seen as an indicator of general effects. The localization of the various transporters is shown in Figure 2. Most extensively studied transporters in this regard are Ntcp, Bsep and Mrp2. These three transporters are generally suppressed, both at mRNA and protein level. To our knowledge, no information is available on the effects of LPS on the expression of the canalicular transporters Fic1 and Npc1l1 or on that of the basolateral Ost-alpha/beta transporting complex.

Most of the transporters are suppressed under inflammatory conditions with rodent Mdr1b (Abcb1b) being the most consistent exception. Although some demonstrated suppressive effects of LPS on *Mdr1b* expression (33,125), other groups have observed an induction of *Mdr1b in vivo* (96,126) (Mulder *et al.* (unpublished data)). Mdr1b, as a member of the multidrug resistance protein family serves as an inducible efflux transporter for organic cations, xenobiotics and toxins (78) and is directly regulated by NF-kappaB signaling (121,127). This role of inducible efflux transporter appears to be in contrast to that of its closely related family member Mdr1a, which is more consistently expressed (96).

Water channels, the so-called aquaporins (AQP), have so far received relatively little attention in IIC research, despite the expression of at least 7 family members in the hepatobiliary system (128). A recent report by Lehmann *et al.* showed that LPS-treatment of rats led to down-regulation of Aqp8 protein expression, while Aqp9 expression was not affected (129). The suppression of Aqp8 expression was TNF-alpha-dependent and post-transcriptionally mediated through both lysosomal and proteasomal degradation (129). This led to a reduced osmotic water permeability of the canalicular membrane, which was suggested to contribute to the pathogenesis of inflammation-induced cholestasis (129).

4.6. NR expression and function during inflammation

The observed reduction in hormone sensitivity during inflammatory conditions, for instance growth hormone (GH) resistance during chronic inflammation (130), illustrates that there is a direct link between inflammation and altered NR function. This link has been addressed experimentally in many studies. Although some NRs are differentially regulated, e.g., induction of Nur77 in LPS-stimulated macrophages (131), inflammation suppresses expression and function of most NRs studied at multiple levels (Figure 4). This general response is considered to be a crucial step in the negative hepatic APR (132-134).

4.6.1. RXR-alpha

In view of its importance as obligate heterodimer partner of class II NRs, the effects of inflammatory signaling on RXR-alpha expression and function have been studied extensively. Beigneux *et al.* (132) showed that both mRNA and protein expression of Rxr-alpha in livers of Syrian hamsters were strongly reduced upon treatment with either LPS or cytokines, which led to reduced binding activity of Rxr-homodimers. This effect was shown to be mediated, at least partially, by reduced *Rxr-alpha* transcription (132). Similar effects of LPS on hepatic *Rxr-alpha* gene expression were obtained in mice (135) and rats (136) as well as in non-hepatic tissues, i.e., adipose tissue (137) and heart (138).

At least two other mechanisms have been identified by which inflammatory signaling alters RXR-alpha transcriptional activity. Firstly, as part of the protein complex originally known as Footprint B binding protein, Rxr-alpha was shown to be a key regulator of the rat *Ntcp* gene expression (139,140). Follow-up studies revealed that the nuclear abundance of this protein is rapidly reduced upon LPS-treatment of mice with a simultaneous, transient appearance in the cytosolic compartment, suggesting active nuclear export followed by degradation (133). At the same time, Jnk activity, important in the regulation of Ntcp promoter activity by Rxr-alpha during inflammation (47), was increased (133). This led to a reduced mRNA expression of NR-target genes, despite preserved expression of most heterodimer partners (133). Subsequent *in vitro* studies provided a potential mechanism for the nuclear export of RXR-alpha (122). JNK-dependent phosphorylation of a serine residue at position 260 of RXR-alpha was shown to induce nuclear export and subsequent proteasomal degradation (122). Reduced nuclear protein levels of Rxr-alpha have also been reported by others (141). Secondly, Gu *et al.* (142) presented another mechanism involved in altered RXR function during inflammation. Activated NF-kappaB suppressed DNA-binding of the PXR:RXR heterodimer to the promoter of the *CYP3A4* gene and this effect was in part mediated by direct interaction between the p65 subunit of NF-kappaB and RXR (142). Although this has not been shown to occur with other heterodimers, it may represent a more generally occurring mechanism and thus not only pertain to the PXR:RXR heterodimer.

These results combined suggest that inflammatory signaling suppresses RXR-alpha expression and function in multiple ways. Considering its important role in the function of class II NRs, the regulation of RXR-alpha alone could already be a central mediator of the negative hepatic APR.

4.6.2. RAR-alpha

In contrast to RXR-alpha, limited information is available on the effects of inflammatory signaling specifically on RAR-alpha expression. TNF-alpha treatment of mice led to a transient reduction in nuclear protein levels of Rar-alpha in liver, which corresponded well with transiently reduced mRNA expression (31). Interestingly, treatment with IL-1beta also led to reduced nuclear protein levels of Rar-alpha, but at a later timepoint, while *Rar-alpha* mRNA expression was not altered (31).

This indicates that the reduction in nuclear Rar-alpha by inflammatory cytokines is not only regulated at the transcriptional level.

Results obtained with individual cytokines were different from those with LPS-treatment. Ghose *et al.* (133) showed that Rar-alpha mRNA expression and nuclear protein levels remained unaffected in mice after LPS-treatment, while nuclear Rxr-alpha protein levels were rapidly reduced. The cause of these differential effects of individual cytokines and LPS remains unclear, but may be related to the dose of inflammatory mediators used, as Rar-alpha nuclear protein levels were strongly reduced after very high dose of LPS in mice (141).

4.6.3. FXR

Since its identification as an intracellular bile salt sensor (143,144), FXR has been shown to play a central role in control of expression of transporter genes such as *BSEP* as well as in the adaptive response to a bile salt challenge (145). Studies with Fxr-null mice revealed an impaired hepatocellular protection against bile salt overload (145). The expression of FXR at both mRNA and protein level is reduced upon LPS- or cytokine treatment of rodents or cytokine-treatment of hepatoma cells (31,136,146). In the CLP-model, expression and binding activity of Fxr:Rxr were also reduced (39). These results not only illustrate that FXR is similarly affected by inflammatory signaling as other NRs and thus may contribute to the pathogenesis of IIC, but also indicate that impaired FXR function will most likely prevent a proper adaptive response during IIC as well.

4.6.4. CAR and PXR

CAR and PXR play critical roles in xenobiotic metabolism and detoxification (147). These NRs are distinct NR family members and show important differences in their LBD, with PXR being more promiscuous (147). Nevertheless, they are often grouped together due to several shared characteristics, including expression profile, with highest expression in liver and intestine, shared target genes as well as a subset of ligands. CAR and PXR have been studied extensively in relation to drug metabolism, which is altered by inflammation (148-150). *Car* and *Pxr* mRNA expression levels were found to be suppressed in LPS-treated rodents (107,133,136,151,152). Suppressed Pxr-function was shown by Kim *et al.* (153), who demonstrated that induction of dehydroepiandrosterone-sulfotransferase (*Sult2a1*) expression by pregnenolone 16alpha-carbonitrile was suppressed after LPS administration in mice. This effect was most likely mediated by cytokines (TNF, IL-1, but not IL-6) as these induced similar effects in hepatoma cells (153). Interestingly, *Sult2a1* expression was suppressed at much lower LPS doses than expression of Pxr and Car (153). It is unclear whether the transactivation capacity of PXR and CAR *per se* is affected, since both NRs were shown to retain their transactivational activity,

albeit when overexpressed, in IL-6-treated human hepatocytes (154).

Mechanisms underlying suppression of CAR expression were analyzed by Assenat *et al.* (155), who showed that inflammatory signaling interfered with GR-regulated CAR-expression via NF-kappaB signaling. Activation of the latter led to decreased histone acetylation of the proximal CAR promoter (155). Of note, NF-kappaB signaling has also been shown to affect PXR function (142,156).

Apart from their roles in xenobiotic/drug metabolism, CAR and PXR are also involved in the protection against cholestatic liver injury in general (157) and, more specifically, against various "cholestatic compounds" such as hepatotoxic bile salts, notably lithocholate (158), bilirubin (159) and cholesterol metabolites (160). It can therefore be anticipated that inflammation will not only affect drug metabolism, but will also render the liver less capable to deal with the ensuing cholestatic insult.

4.6.5. LXR

LPS-treatment of both hamsters and mice led to reduced Lxr DNA-binding in the liver (132,133). This occurred simultaneously with reduced *Lxr-alpha* mRNA expression in hamster (132), while hepatic *Lxr-alpha* mRNA expression was actually slightly increased in LPS-treated mice (133). Reduced DNA-binding in mouse liver, associated with suppressed expression of the Lxr target gene *Abcg5*, was ascribed to reduced nuclear Rxr-alpha levels (133). Treatment of mice with either TNF-alpha or IL-1 was found to slightly reduce liver *Lxr-alpha* mRNA level (135). *In vitro* experiments with Hep3B cells confirmed suppression of *LXR-alpha* mRNA expression, reduced transactivating activity and suppressed expression of its target gene sterol regulatory element binding protein-1c (135). In contrast to LPS-treated livers tissue and cytokine-treated hepatoma cells, LPS treatment of macrophages did not affect Lxr DNA-binding (161), indicating cell-specific effects. Expression of the *Lxr-beta* isoform was not significantly reduced upon LPS-treatment in mouse liver (132).

4.6.6. PPAR-gamma

Hepatic *Ppar-gamma* mRNA expression was suppressed by LPS treatment of Syrian hamsters (132). Similar results were obtained in mice, where LPS-induced suppression of Ppar-gamma mRNA and protein expression was shown to be dependent on TNF-alpha release (162). *In vitro*, TNF-alpha and IL-1beta were also shown to suppress *PPAR-gamma* expression in Hep3B cells (135).

The mechanism underlying the reduced gene expression of *PPAR-gamma* by inflammatory signaling may involve NF-kappaB as was shown for LPS-induced suppression of *Ppar-gamma* in macrophages (163).

4.6.7. HNF4-alpha

HNF4-alpha is a transcriptional regulator of many genes involved in hepatic lipid and bile salt metabolism (164). HNF4-alpha appears to be constitutively active, supposedly due to a permanent association of lipids with its LBD (165). However, HNF4-alpha is still considered an orphan NR, since no specific ligand has been identified. Considering the broad impact of HNF4-alpha on hepatocellular gene expression profiles (166), it has been postulated that effects of inflammatory signaling on HNF4-alpha function might provide a central mechanism for initiation of the hepatic APR (134).

Inflammatory signaling affects HNF4-alpha function via multiple, often simultaneously acting, mechanisms. These mechanisms include reduction of mRNA expression, acceleration of proteasomal degradation, reduction of DNA-binding through post-transcriptional modifications and inhibition of co-activator recruitment (134,141,167-174). Since these data suggest that inflammatory signaling indiscriminately leads to reduced HNF4-alpha activity, it is important to emphasize that HNF4-alpha activity can also be regulated in an opposite manner by other inflammation-related cues. Kuo and colleagues showed that in a different *in vitro* model of sepsis/shock, i.e., combined treatment of hepatocytes with cytokines and hydrogen peroxide, alteration of the specific phosphorylation pattern of HNF4-alpha was critical for interaction with a specific co-activator and thus for enhancement of cytokine-induced iNOS expression by oxidative stress (175-177). This indicates that post-transcriptional modification of HNF4-alpha in the context of inflammatory processes has promoter-specific effects.

Finally, HNF4-alpha was also shown to undergo tyrosine-phosphorylation signal-dependent intranuclear redistribution (178). Whether this also occurs in the setting of inflammation is unclear. It may, however, provide a new level of complexity to the regulation of NR function.

4.6.8. LRH-1

Gerbod-Giannone *et al.* (179) identified another mechanism by which NR function can be inhibited during inflammation. Inflammation-induced production of alpha1-antitrypsin leads to increased production of the alpha1-antitrypsin-derived peptide C-36. This peptide was shown to specifically reduce DNA-binding of Lrh-1 and to inhibit Lrh-1 regulated gene transcription (including *Cyp7a1*, *alpha-fetoprotein*). C-36 physically interacted with Lrh-1, but did not bind to its DBD suggesting that C-36 induced conformational changes in Lrh-1 (179). This interaction between bio-active peptides and a NR suggests that there may be more of these unanticipated interactions.

4.6.9. SHP

The expression of the atypical NR SHP is regulated by several other NR superfamily members, including FXR, ER and HNF4-alpha, but also via an AP-1 binding site (145,180-182). The latter is most likely regulated by inflammatory signaling. LPS-treatment of mice leads to strongly reduced hepatic *Shp* mRNA expression (133,141). However, the exact mechanism underlying this effect and whether reduced mRNA levels are translated into reduced protein expression and activity, remains to be elucidated. Considering the general

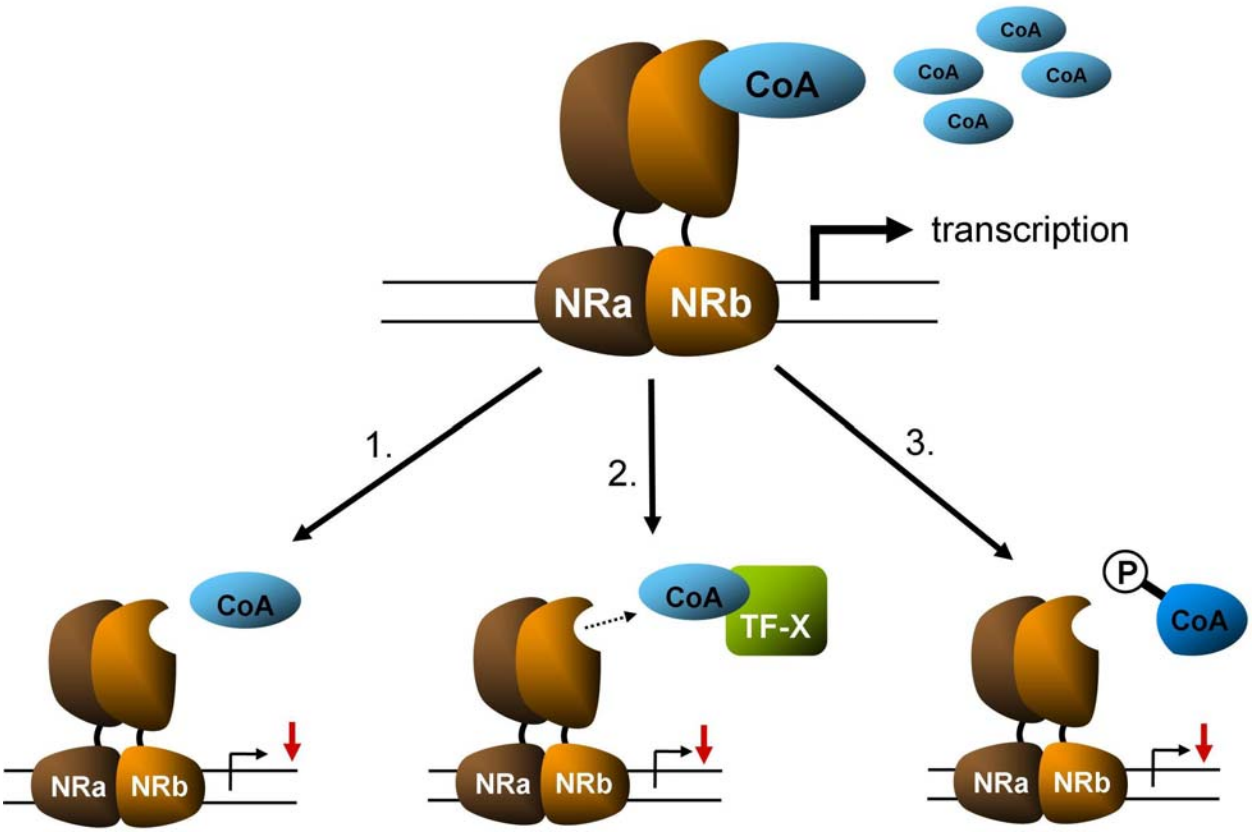

Figure 5. General effects of inflammatory signaling on co-activators (CoA): 1.) reduction of CoA abundance; 2.) competition for CoA-recruitment by inflammation-induced transcription factors (TF-X); 3.) post-transcriptional modification of CoA (e.g., phosphorylation (P)) leading to reduced NR-binding. (Other abbreviations: NRa/b, generic NR heterodimer partners.)

repressive effect of SHP on other NRs, it is interesting to speculate on the functional consequences of reduced SHP expression. The net outcome will depend on whether reduced repression by SHP or reduced expression of the other NRs prevails.

4.7. Effects of inflammation on co-factor expression and function

The importance of co-regulators in NR action on gene transcription implies that interactions between inflammatory signaling and these co-regulators will directly affect NR-regulated gene transcription. Effects of inflammation on co-factor expression and function can be categorized into three different general categories, i. reduction of co-regulator expression/abundances, ii. competition between transcription factors for critical co-factors and iii. post-transcriptional modification of co-factors (Figure 5).

Kim *et al.* showed that inflammation leads to a reduced expression of a subset of co-factors *in vitro* and *in vivo* (135). Cytokine treatment (IL-1 and TNF, but not IL-6) of hepatoma cells and cytokine- or LPS-treatment of mice led to a specific reduction in mRNA expression of the co-activators PPAR-gamma co-activator (PGC)-1alpha, PGC-1beta and steroid receptor co-activator (SRC)-1, while the expression of other co-activators, including CRE-binding protein (CBP), as well as co-repressors NCoR and SMRT was not affected (135). This selective effect in combination with reduced NR expression led to reduced NR-mediated transcription using reporter constructs (541}. This supports the concept that a specific reduction in co-activating complexes shifts the balance between transactivating and transrepressing complexes towards the latter, ultimately suppressing gene transcription. Reduced expression of specific subsets of co-activators upon inflammatory stimuli was not only seen in liver, but also in other organs and tissues, including heart (138), adipose tissue (137) and uterine smooth muscle cells (183).

Leite *et al.* (183) provided evidence that there is a critical window of co-activator abundance, as the activity of the progesterone receptor (PR) is reduced during inflammation despite the fact that protein levels are not altered. This suggests that the concomitantly reduced levels of co-activators SRC-1 and SRC-2 are responsible for reduced PR function (183). This is further supported by the fact that exogenous SRC-1 or SRC-2 can at least partially reverse the effects of TNF-alpha on PR function (183).

The concept of critical co-activator levels within the nucleus for proper NR function also provides the basis for another mechanism by which inflammation may interfere with NR function, namely competition between

transcription factors. Despite large functional and structural differences, the individual NRs often recruit the same co-factors as other types of transcription factors (60). Therefore, initiation of inflammatory signaling within cells and the subsequent activation of transcription factors such as NF-kappaB and AP-1 will lead to the recruitment of co-activators. If co-activators are not redundantly present within cells, competition for co-activators can lead to insufficient co-activator-NR interaction and thus to reduced NR function. This principle of competition has been shown to occur between GR and NF-kappaB, as these transcription factors were shown to both be dependent on co-activators SRC-1 and CBP for maximal activity (184). Again, exogenous supplementation of either co-activator was able to reverse this competition (184). Similar findings were reported for other NRs, including RXR (185).

A third mechanism by which inflammation can affect co-factor function is by post-transcriptional modification. It is known that, similarly to NRs, co-factors can be subjected to (de)phosphorylation, (de)acetylation and (de)methylation and their activity can further be controlled by proteolytic processes and shuttling between nucleus and cytoplasm in response to various signals (186). For example, TNF-alpha-induced, inhibitor of kappaB kinase-mediated phosphorylation of SRC-3 leads to preferential nuclear localization of this co-activator and enhanced NF-kappaB mediated gene transcription (187). Although this is an example of increased co-activator activity, it is plausible that inflammatory signaling may lead to reduced activity of co-activators in other settings. The multiple sites in co-regulators for post-transcriptional/translational modification provide a means to rapidly regulate co-regulator function with a need to alter gene expression (188).

Thus, inflammatory stimuli can have multiple effects on co-factors that may directly translate into disturbed NR function. The overall effect, however, is most likely context-dependent, i.e., specific for type of stimulus and tissue involved, and not easily predicted. Moreover, a recent report by Lu *et al.* (189) adds another level of complexity of NR-co-factor function. These authors showed that the co-activator Src-3 is specifically involved in suppressing the innate immune response. Src-3 knock-out mice were shown to be highly sensitive to LPS-treatment. Surprisingly, Src-3 was shown to act as a suppressor of mRNA translation. This unexpected interaction between inflammation and co-factor function warrants caution in predicting how co-factors will affect inflammatory processes.

5. PHARMACOLOGICAL/EXPERIMENTAL INTERVENTIONS IN MODELS OF IIC

Better insight into the pathogenesis of specifically IIC and the negative APR in general has provided the opportunity to explore different, intriguing approaches to intervene in these processes. These approaches have been aimed at different levels of the cascade leading to IIC (Figure 3).

Administration of high-density lipoprotein particles (190,191), recombinant LPS-binding protein (192) or TLR4 antagonists, e.g., M62812 (113) have been used to attenuate the initial step of KC activation. Liposomal siRNA against TNF-alpha was used to specifically suppress production of this cytokine in KC, which rapidly take up liposomes (193). Inhibition of signaling downstream of KCs has been achieved by using cytokine-inactivating antibodies, e.g. anti-TNF-alpha, anti-IL-1beta (29,49,110), or inhibitors of hepatocellular signaling, e.g., JNK-inhibitors as SP600125 (122).

6. DIFFERENT ROLES OF NRs IN IIC: MEDIATORS AND MODIFIERS

Thus far, this review has mainly dealt with effects of inflammatory signaling on NR function that contribute to the pathogenesis of IIC. Inflammatory signaling has multiple potential effects on NR function through reduction of NR mRNA and protein expression, subcellular localization, post-transcriptional modifications with subsequent reduced DNA-binding and/or co-regulator recruitment and altered co-regulator expression or activity (Figures 4 and 5). In this regard, NRs can be seen as mediators in the development of IIC. On the other hand, NRs can also play modifying roles, which can be divided into two modes of action. NRs can exert adaptive responses aimed at restoration of normal hepatocellular homeostasis and NRs have been shown to have anti-inflammatory properties.

6.1. Modifier function 1: adaptive responses

During cholestasis, in particular of extrahepatic origin, hepatocellular accumulation of bile salts and other potentially toxic compounds will lead to an adaptive cellular response (194). This means that, in theory, accumulation of bile salts could lead to an adapative response during IIC too. High intracellular bile salt concentrations will activate FXR and thus induce their export from the liver while reducing import and production. However, this adaptive response requires “sound” NR signaling. It is, however, unlikely that such an appropriate adaptive response can be fully engaged, since inflammation affects NR signaling in many different ways. Although the quantitative contribution of adaptive responses remains to be determined, it probably is limited. The latter idea is best illustrated by the reported findings of Zollner *et al.*: bile-duct ligation and bile salt treatment of mice leads to increased *Shp* mRNA expression, while LPS-treatment strongly suppresses *Shp* mRNA expression (141,195). The latter suggests that in the context of LPS, direct suppressive effects of inflammatory signaling on SHP expression overrule the anticipated stimulating effect of bile salt accumulation.

In this regard, it is important to mention the concept of pharmacologic enhancement of normal adaptive responses (196) as a means to intervene in the pathogenesis of IIC. Although inflammation generally impairs NR function, boosting the remaining activity using pharmacological agents may provide an avenue to at least

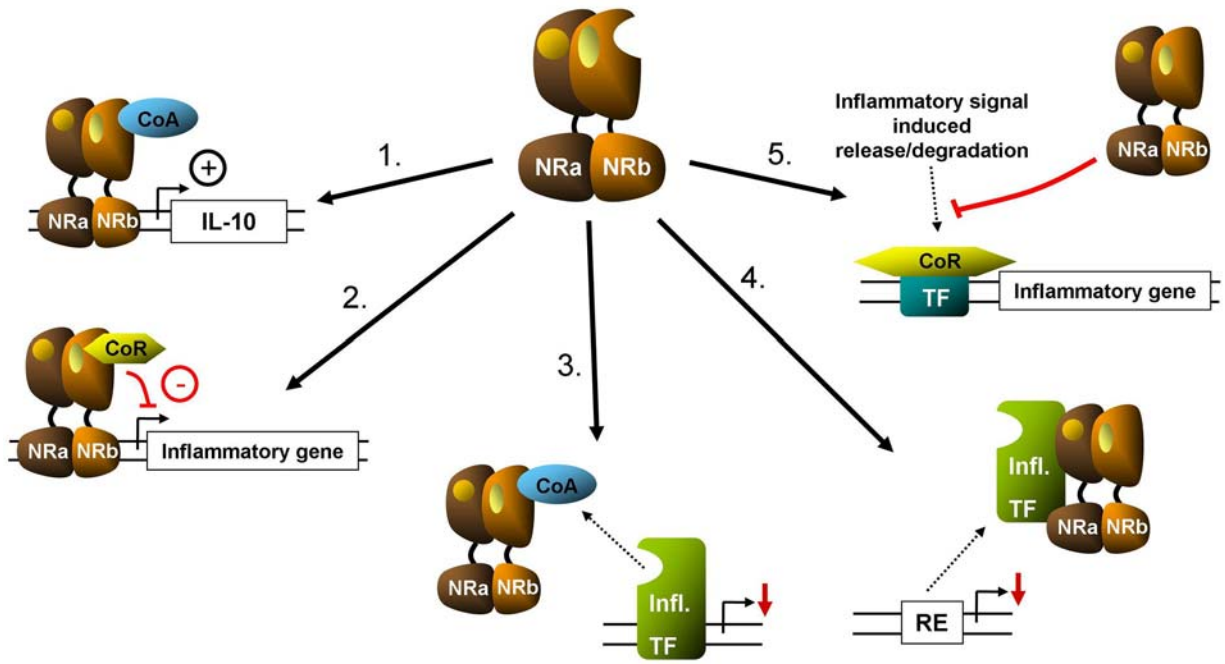

Figure 6. Mechanisms behind anti-inflammatory actions of NRs: 1.) induction of anti-inflammatory mediators through transactivation; 2.) transrepression of (pro-)inflammatory genes; 3.) competition for co-activators (CoA) with inflammation-induced transcription factors (Infl. TF); 4.) direct interaction with transcription factors induced by inflammatory signaling; 5.) interference with release of co-repressors (CoR) from transcription factors associated with inflammatory genes. (Other abbreviations: NRa/b, generic NR heterodimer partners, RE = response element, CoA = co-activator.)

attenuate the effects of inflammation on hepatobiliary transporter expression.

6.2. Modifier function 2: anti-inflammatory actions

Several NRs have been shown to possess anti-inflammatory properties. Although most of the studies exploring these NR properties have dealt with other models of inflammatory diseases, most notably atherosclerosis, it implies that use of NR ligands to suppress inflammatory signaling may be a useful means to intervene in the pathogenesis of IIC as well. This approach has been explored already by several groups (23,197,198). The following sections will further focus on the mechanisms of anti-inflammatory effects of NRs (Figure 6).

6.2.1 GR

GR has been studied extensively for its anti-inflammatory properties (199). Glucocorticoids remain the mainstay of therapeutic options for a wide variety of conditions involving a detrimental inflammatory response, including sepsis, asthma, and autoimmune diseases (200). GR, more in particular the GR-alpha isoform, appears to have multiple effects on inflammatory signaling, both through activation of gene expression (transactivation) and through inhibition of gene expression (transrepression) (201). Examples of transactivation include the induction of the anti-inflammatory cytokine IL-10, the IL-1 receptor antagonist and the inhibitor of NF-kappaB. The transrepressive mechanisms appear to be more diverse and include, but are not limited to, inhibition of gene expression, competition for co-factors, the physical interaction between GR and other transcription factors, and effects on chromatin-remodelling (201). Glucocorticoids have been found to be effective in various experimental settings of inflammation-induced cholestasis, both *in vitro* and *in vivo* (39,42,126,202). However, despite these effects in experimental settings, this has not led to the widespread clinical use of glucocorticoids for IIC.

6.2.2 PPAR-gamma

Although it had initially been recognized as an important regulator of lipid and glucose metabolism as well as cellular differentiation, PPAR-gamma was among the first class II NRs to be attributed with anti-inflammatory properties (203,204). Much of this early work dealt with the development of atherosclerosis, in which peripheral macrophages play an important role. It is, therefore, not surprising that the anti-inflammatory effects of PPAR-gamma and its ligands have been studied particularly well in this cell-type (205). Since the discovery of their anti-inflammatory potential, PPAR-gamma ligands have been applied experimentally in various models of inflammation-related disorders, e.g., rheumatoid arthritis (206), inflammatory bowel disease (207) and lung disease (208).

Considering the role of KCs, as resident liver macrophages. in the pathogenesis of endotoxemic shock, Uchimura and colleagues (209) investigated the potential effect of the PPAR-gamma agonist pioglitazone and of the RXR agonist Ro47-5944, on LPS-stimulated rat Kupffer cells and found that both were able to suppress the production of TNF-alpha and NO and the transactivation

activity of NF-kappaB. This effect, however, was not mediated by reduced DNA-binding of the transcription factors AP-1/NF-kappaB (209). Since no Ppar-gamma/Rxr response element was found in either the *Tnf-alpha* or *iNos* promoter, these authors suggested activated Ppar-gamma/Rxr to interfere non-genomically with the transcriptional activity of the pro-inflammatory transcription factors, perhaps through competition for important co-activators. This concept had been already been addressed by Li *et al.* (210), who showed that in macrophages Ppar-gamma transrepressed *iNos* gene expression through interaction with co-activator Cbp.

A different mechanism for PPAR-gamma induced transrepression was elucidated by Pascual *et al.* (211) who showed that the transrepression of NF-kappaB signaling by ligand-activated Ppar-gamma involved prevention of the clearance of NCoR-Hdac3 from the basally repressed promoter of the *iNos* gene. This clearance is normally initiated upon a pro-inflammatory stimulus, but ligand-dependent SUMOylation of Ppar-gamma prevented this process. In retrospect, this mode of action appears to be in agreement with the anti-inflammatory effects of Ppar-gamma/Rxr in rat Kupffer cells (209), which might also involve prevention of co-repressor clearance.

The effect of PPAR-gamma agonist treatment in the setting of IIC has been addressed by Ghose *et al.* (197), who showed that pre-treatment of mice with rosiglitazone partially preserved gene expression of critical hepatobiliary transporters involved in bile formation. This, however, did not seem to be mediated by KCs, as cytokine expression and production were not significantly altered by rosiglitazone-pretreatment, but most likely involves the preservation of nuclear Rxr-alpha levels in the hepatocytes (197). Miyake *et al.* (23) used rosiglitazone in a mouse model of hepatic inflammation induced by a bile-acid containing pro-atherogenic diet. Rosiglitazone was able to suppress the effect of diet-induced suppression of Cyp7a1, which was indirectly shown to be dependent on macrophage/KC inflammatory signaling (23).

Lastly, PPAR-gamma ligands also exert anti-inflammatory actions through gene transactivation. Similarly to the induction of IL-10 and the IL-1 receptor antagonist by glucocorticoids, PPAR-gamma ligands induce the soluble IL-1 receptor antagonist in the THP-1 macrophage cell-line (212).

6.2.3 LXR

LXR is critical for maintenance of cellular cholesterol homeostasis (213,214). In peripheral macrophages, Lxr activation leads to an increased expression of transporters involved in reverse cholesterol transport, including Abca1 (215) and Abcg1 (216,217) and functionally to a reduction of atherosclerosis in relevant mouse models (218-220). LXR activation in hepatocytes induces expression of the canalicular half-transporters Abcg5 and Abcg8 and stimulates biliary cholesterol excretion (221,222). Joseph *et al.* (223) reported that Lxr activation inhibited the macrophage inflammatory gene response both *in vitro* and *in vivo*. This effect could be exerted by either isoform of Lxr and appeared to involve inhibition of NF-kappaB signaling (223). Follow-up studies by Castrillo *et al.* (224) demonstrated that ligand-activated Lxr inhibited inflammatory signaling, leading to macrophage matrix metalloproteinase (Mmp)-9 expression at least in part through interference with NF-kappaB signaling downstream of its binding to DNA. No evidence was found for interference with AP-1 signaling or for direct Lxr:Rxr binding to the 5'-flanking region (224). Since then, multiple groups have reported on this anti-inflammatory effect of LXR (-agonists) in macrophages (225-228) and other cell-types, including lymphocytes (229), keratinocytes (230), Kupffer cells (198), hepatocytes (231), microglia and astrocytes (232) and polymorphonuclear neutrophils (233). Interestingly, Ogawa *et al.* (226) found LXR-agonists to inhibit inflammation-induced expression of osteopontin through interference with AP-1 signaling. It remains unclear what causes the discrepancy between this finding and the earlier observed lack of effect on AP-1 signaling (224,225). There may also be a difference between the anti-inflammatory actions in mice and humans as Fontaine *et al.* (228) showed that in human macrophages LXR (-agonists) increased the expression of TLR4 and the response to LPS-challenge via an LXR-RE in the TLR4 promoter, which is not conserved in mice.

Inspired by studies on anti-inflammatory actions of PPAR-gamma, Ghisletti *et al.* (234) sought to determine whether similar mechanisms might be involved in the anti-inflammatory actions of LXRs. Interestingly, similar mechanisms involving SUMOylation (albeit by SUMO2/3 rather than SUMO1) of ligand-bound Lxr were found, which prevented the clearance of NCoR from the *iNos* (or other inflammatory gene) promoter (234). Reduced clearance of NCoR by ligand activated Lxr has also been shown to be the mechanism underlying the inhibition of hepatocellular C-reactive protein expression (231).

Considering the anti-inflammatory effects of LXRs and their expression in KCs, we recently examined the potential of LXR-agonists to interrupt the inflammatory cascade leading to LPS-induced suppression of hepatobiliary transporter expression (Mulder *et al.* (unpublished data)). Although treatment of mice with T0901317 led to partial preservation of transporter gene expression and preserved Mrp2 protein expression, this appeared to be KC-independent, since cytokine expression was not altered. Wang *et al.* employed a different LXR-agonist in a more severe model of hepatic inflammation (LPS/Gal-treatment of rats) and found the LXR agonist to be protective and associated with a reduced KC-response (198).

Similarly to GR, LXR can also modify inflammatory response "genomically" through direct transactivation, e.g., through induction of *arginase II*, which can compete with iNOS for substrate (235). Furthermore, it was recently shown that LXR-alpha can directly repress gene expression through promoter specific recruitment of the co-repressor RIP140 (236). It is tempting to speculate that LXR may repress inflammatory genes via such a mechanism too.

6.2.4 RXR-alpha and RAR-alpha

The anti-inflammatory effects of retinoids have been used clinically for several decades, especially in the treatment of various dermatological conditions (e.g., psoriasis, acne) (237). Initial studies addressed the effects of both types of receptor on regulation of transforming growth factor (TGF)-beta1 (238). Both receptors were shown to inhibit AP-1 signaling through physical interaction (238).

Pertaining to IIC, Rxr-alpha agonists were shown to inhibit TNF-alpha release and NO production by primary Kupffer cell (239). These results were confirmed by Uchimura *et al.* (209), who went on to show that this effect was associated with reduced NF-kappaB transcriptional activity. Rar-alpha-selective ligands, on the other hand, were not effective *in vitro* (239). The latter was surprising as all-trans retinoic acid was shown to blunt LPS and Propionibacterium acnes induced liver injury *in vivo* (240), indicating that RAR-alpha agonists can initiate anti-inflammatory mechanisms at least *in vivo*.

Ligand-bound Rxr-alpha was able to inhibit NF-kappaB transcriptional activity (209) and appeared to do so similarly to Ppar-gamma:Rxr-alpha heterodimer, suggesting that similar mechanisms as described for PPAR-gamma and LXR might be active (i.e., inhibition of co-repressor clearance). Na *et al.* showed that RXR ligands reduced LPS-induced cytokine expression in mouse macrophages via at least two mechanisms, i.e., either through physical interactions with NF-kappaB subunits p50 and p65, but also through co-activator competition (185). Anti-inflammatory effects of retinoids have also been shown in other cell-types, e.g., microglia and astrocytes (232). Finally, the observation of increased liver cytokine gene expression in hepatocyte-specific Rxr-alpha-deficient mice after alcohol-treatment suggests that Rxr-alpha may very well have anti-inflammatory effects under basal conditions (241).

6.2.5 Anti-inflammatory effects of other NRs

Besides GR, PPAR-gamma and LXR, other NRs involved in bile formation, lipid homeostasis and bile salt metabolism have also been attributed anti-inflammatory properties.

Although FXR has been shown to modify disease processes involving inflammation (including atherosclerosis (242) and cholesterol gallstone disease (243)), limited information is available on direct effects of FXR on inflammatory signaling in liver cells. Hepatic cytokine gene expression after LPS treatment was not different between in wild-type and Fxr-null mice (141). However, one has to bear in mind that, in this study, FXR agonists were not used to analyze the effect of pharmacological activation of FXR on LPS-induced cytokine expression (141). In bile-duct ligated mice, FXR-activation by GW4064 led to a decreased expression of the pro-fibrogenic cytokine TGFbeta (244), although no underlying mechanism was provided. It remains to be determined whether this reduced expression is a concurrent effect of disease process modification rather than a direct effect on TGFbeta expression. Interestingly, it was recently shown in models of diabetic nephropathy, which is associated with increased fibrogenesis/inflammation, that FXR agonists such as cholate and GW4064 reduced *IL-6* and *TGFbeta* mRNA expression (245). This suggests that this effect of FXR is a more general phenomenon, because it is not limited to liver cells and because different disease processes that lead to fibrogenesis in different cell types respond similarly. Li *et al.* (246) showed that FXR ligands were able to inhibit inflammatory response in rat/human vascular smooth muscle cells in a FXR- and SHP-dependent manner, with concurrent reduction in NF-kappaB activation.

In contrast with a potential anti-inflammatory role, Qin *et al.* (247) showed that FXR mediates the bile salt-induced expression of intracellular adhesion molecule-1, which is involved in the recruitment of neutrophils to the site of inflammation. By this induction, FXR will actually enhance the inflammatory process. Furthermore, FXR activation led to a SHP-mediated derepression of MMP-9 in endothelial cells (248). MMP-9, involved in tissue remodelling including atherosclerosis, was shown to be negatively regulated by LXR (224), suggesting opposite effects of these two nuclear receptors. It clearly remains to be further investigated how FXR modulates inflammatory processes overall and whether some the effects described above are concurrent effects of FXR agonists on disease progression or distinct anti-inflammatory effects.

PXR is another NR that is able to interfere with NF-kappaB signaling (156). Different PXR ligands were able to suppress basal or induced expression of NF-kappaB target genes and this suppression was dependent on PXR expression. Furthermore, primary hepatocytes derived from Pxr-null mice showed increased expression of NF-kappaB target genes, indicating a general anti-inflammatory effect. A mechanistic explanation, however, was not provided for these observations. Similar observations of the anti-inflammatory characteristics of Pxr were done in a mouse model of inflammatory-bowel disease (249).

LRH-1 was recently also shown to inhibit the hepatic inflammatory response via at least two different mechanisms (250,251). First, LRH-1 was shown to specifically interfere with C/EBPbeta transcriptional activity induced by different cytokines, while it had no effect on simultaneous induction of NF-kappaB and AP-1 activity (250). Subsequent studies revealed that LRH-1 also induces expression of secreted IL-1RA by hepatocytes under inflammatory conditions (251). The latter indicates that LRH-1 not only directly interferes with inflammatory signaling (C/EBPbeta), but also indirectly through induction of an anti-inflammatory mediator (IL-1 receptor antagonist). These mechanisms appear to act synergistically. Of interest, Mueller *et al.* reported that Lrh-1 mediates the induction of extra-adrenal glucocorticoid synthesis, i.e. by the intestine (252), which has been linked to reduced inflammatory responses in the setting of inflammatory bowel disease (253). It would be relevant to examine whether such mechanisms might also be at work in the pathogenesis of IIC.

Although generally regarded as a transcriptional suppressor, only limited information is available on a potential role of SHP in modulation of inflammatory signaling. Some of the mechanisms by which SHP exerts its repressive effects on gene transcription, e.g., competition for binding with co-activators through physical interaction with NRs and other transcription factors (254,255) are very similar to those by which other NRs interfere with inflammatory signaling. It seems therefore plausible that SHP interferes with inflammatory cascades. This has already been shown for TGFbeta1 signaling (256). TGFbeta1, a cytokine involved liver regeneration and fibrosis, activates, amongst other pathways, SMAD-signaling (257). SHP was shown to inhibit TGFbeta1-induced gene responses through physical interaction with SMAD3, preventing binding of the co-activator p300 (256). As mentioned above, Li (246) showed that FXR-mediated induction of SHP expression was able to inhibit the inflammatory response in rat and human vascular smooth muscle cells by interfering with NF-kappaB activation. The exact molecular mechanism of action, however, remains to be revealed.

6.2.6 General considerations regarding the anti-inflammatory effects of NRs

Although some NRs seem to act clearly in an anti-inflammatory fashion (e.g., GR), the effects of others, i.e., FXR and LXR, appear to be rather unpredictable and are gene-specific. The inflammatory response is an extremely complex process which balances effects aimed at protection of the organism with potential detrimental effects. This balance, in turn, involves multiple delicately organized processes in different cell-types in response to multiple stimuli which change over time with various feedback systems. It might therefore be an oversimplification to expect that one particular NR will either be pro-inflammatory, neutral or anti-inflammatory as this may be cell type-, species- and context-dependent. In its overall complexity, it appears that inflammatory signaling involves several different, often redundant pathways. Considering the different modes of anti-inflammatory actions of the NRs, one could propose that targeting multiple NRs simultaneously may lead to synergistic anti-inflammatory effects. The latter has indeed been shown for combinations of GR- and PPAR-gamma ligands (258) as well as LXR- and PPAR-gamma ligands (259). This concept is especially interesting with regard to clinical application of NR ligands in inflammatory therapeutic regimes as combining ligands may reduce side-effects associated with the use of single ligands (260).

7. DESIGN OF OPTIMAL NR LIGANDS FOR INTERVENTION IN IIC

In the previous sections, the involvement of NRs in the pathophysiology of IIC as well as their potential role as modifiers of this condition have been discussed. The latter concept makes use of the key characteristic of NRs that separates them from other transcription factors, namely their ligand-binding properties. Although natural ligands have been identified for several of the NRs discussed above and synthetic ligands have become available, there remains a clear necessity for improvement. Besides optimizing the general pharmacokinetic characteristics, i.e., absorption, distribution, elimination and metabolism of NR ligands, and perhaps the application of advanced drug targeting strategies, the concept of selective NR modulators (SNuRMs) may provide an alternative approach in the quest for ideal NR ligands.

NRs function as molecular links between genomic information (genes/promoters), environmental/hormonal cues and the effector machinery for active gene transcription. However, NRs should not be regarded as rigid mediators that serve as “on/off switches”, whose interactions with ligands resemble key-lock interactions. The overall outcome of ligand-binding to a NR is determined at multiple levels. First of all, although not all NRs are as promiscuous with regard to their ligand-binding as for instance CAR and PXR, NRs can generally be bound by different, more or less related ligands. Differential ligand binding will lead to different conformational changes, which will affect co-factor recruitment. Secondly, it has also been shown that very subtle differences in *cis*-acting response-elements can have an influence of co-factor recruitment by NRs as well as other transcription factors. The latter was illustrated by Leung *et al.* who showed that a single-nucleotide difference in a kappaB-site affected co-factor recruitment to NF-kappaB dimers. This illustrates the extent to which NR/transcription factor actions are fine-tuned and that DNA can be considered as an allosteric modifier of transcription factor function (261) Thirdly, co-factors can be regarded as the effector molecules of NR function. Since this large group of molecules has its own (spatial and temporal) expression profile, binding of a single ligand to a specific NR may have transcriptional effects that are context-dependent (organ, tissue, cell-type, promoter). These aspects all contribute to the complexity of DNA-NR-co-factor interaction. This feature of NR biology makes it near to impossible to accurately predict the outcome of NR-binding by a single ligand, yet, it simultaneously provides an opportunity to search for compounds that acts as SNuRMs. Kremoser *et al.* reviewed a series of approaches to search for effective SNuRMs (262).

A good example of a clinically used SNuRMs are the selective modulators of the estrogen receptor (SERMs), tamoxifen and raloxifene (62). Tamoxifen is widely used as an adjuvant treatment in breast cancer care, while raloxifene is used as anti-osteoporosis agent. These drugs act as antagonists of the estrogen receptor in some tissues, but as agonists of the same receptor in other tissues. The underlying mechanism of this tissue specificity primarily involves differences in co-regulator expression and activity (62). SERMs are actually mixed agonists/antagonists and their binding induces conformational changes, which lead to changes co-activator and co-regulator recruitment. The exact pattern of recruitment depends on co-regulator expression and post-transcriptional modification. The development/identification of such selective modulators for other NRs would potentially be a great stride forward, especially for those NRs whose general modulation/activation appears to be of benefit in certain

settings, but whose further development is hampered by undesired activation of other subsets of genes.

Tissue- and gene-specific activation of LXR is probably one of the greatest challenges in the NR field. Although the anti-inflammatory (223) and plaque-reducing effects (218-220) are obvious from mouse studies, current LXR agonists have also been shown to induce de novo lipogenesis in hepatocytes with increased plasma triglycerides and hepatic steatotosis as detrimental results. The metabolic consequences of these particular agonists currently preclude their clinical use. In the search for other LXR ligands with great therapeutic potential, one can take different approaches. First of all, ligands which are preferentially taken up by macrophages or specifically targeted to KCs, rather than by hepatocytes, should in theory limit the detrimental hepatocellular LXR-activation. Organ selectivity has been described for one of the current LXR agonists, i.e., GW3965 (263). LXR-beta selective ligands would also be appealing alternatives, since LXR-beta expression is lower than that of LXR-alpha in hepatocytes, while expression levels of both are more comparable in macrophages. Although LXR-beta-selectivity may *a priori* not be easily achieved as the LBDs of the two LXR-isoforms are highly similar, several groups have reported on LXR-beta selective LXR ligands (264,265). In addition to cell-, organ- and isoform-specific LXR ligands, development of selective LXR modulators that are specifically active in non-hepatocyte cells or inactive on promoter of lipogenic genes would be a great therapeutic potential.

7.1. Other approaches

As described previously, co-regulators are very important in regulation of gene transcription by NRs. Initially considered to be mere executors in the regulation of gene expression by NRs, this clearly underestimates the role of co-regulators. This can be illustrated by the transcriptional regulation of phosphoenolpyruvate carboxykinase (PEPCK), an enzyme centrally involved in gluconeogenesis. The gluconeogenic response is dependent on proper HNF4-alpha function, but binding of this NR is not altered during fasting, when *PEPCK* transcription is induced. This induction was regulated by increased expression of co-activator PGC1alpha (266,267), illustrating both that co-regulators are more than mere bystanders in the process of gene transcription and that methods to alter co-regulator abundance/modification may provide another approach to modify NR-function. Although even more far-fetched, the concept of co-activator rescue, e.g., restoring NF-kappaB transactivation capacity by supplying an exogenous co-activator (268) is also intriguing. However, due to the complexity of the transcriptional complex, it is difficult to predict what the implications of alterations of single co-regulators may be *in vivo*. So far, this concept has not been pursued in the context of IIC.

8. SUMMARY AND PERSPECTIVE

IIC is a frequently occurring phenomenon and with substantial morbidity and mortality. NRs play a dual role in the pathogenesis of IIC. On one hand, NRs contribute to disease development, as they are targets of inflammatory signaling themselves. Whether it be through direct effects on their expression levels or functionality or through indirect effects on their co-regulators, altered NR activity affects expression levels of important hepatobiliary transport systems. Hence, NRs can be considered to be important mediators of IIC. On the other hand, NRs also play important roles in adaptive responses, for instance during bile salt accumulation within hepatocytes through FXR activation. Several NRs have also been shown to possess anti-inflammatory effects. Therefore, NRs can also act as modifiers of IIC. The latter concept makes it interesting to determine whether NRs can be exploited as pharmacological targets to intervene in IIC, either through boosting adaptive responses or induce anti-inflammatory responses. Identification of new NR ligands or SNuRMs with characteristics as described in the previous section would be ideal.

Although sepsis-associated cholestasis is the most widely known example of IIC, cholestasis can also occur in the setting of many other inflammation-related conditions associated with a generalized APR, e.g., severe burn injury or trauma. Moreover, inflammatory signaling also appears to be involved in other conditions characterized by cholestasis. An example of the latter is biliary atresia (BA), the most common neonatal cholestatic disorder, which is characterized by progressive inflammation, fibrosis and, subsequently, obliteration of the bile ducts ultimately leading to biliary cirrhosis (269). This multifactorial pathophysiological process appears to include a persistent (auto)immune response after a perinatal, infectious insult or aberrant bile duct formation (269). Several cytokines, e.g., TNF-alpha, IFN-gamma and osteopontin (270-272), have been implicated as potentially important mediators and are in part secreted by recruited macrophages/monocytes (273). This indicates that anti-inflammatory agents aimed at macrophages may have a role in treatment of BA. Perhaps PPAR-gamma or LXR agonists may be suitable agents to explore. Interestingly, glucocorticoids are often used in the treatment of BA, but only after the primary surgical intervention, i.e., Kasai portoenterostomy, with the underlying thoughts that glucocorticoids will enhance bile formation and reduce inflammation, and thus increase the chances of establishing permanent post-Kasai bile drainage (274).

Total parenteral nutrition associated cholestasis (TPNAC) is another, rather frequently occurring condition that shows the close link between inflammation and cholestasis. Although the exact pathogenesis of TPNAC remains unclear, many factors have be implicated to play a causative role, including increased portal LPS-levels and individual components of TPN formulas (275). With regard to the latter, it was recently shown that the phytosterol, stigmasterol, can act as an FXR antagonist (276), which is expected to impair hepatic adaptive responses to bile salt retention. Implicitly, this also suggests that FXR agonists are potentially of benefit under such conditions by presumably enhancing adaptive responses. Furthermore, FXR also appears to have anti-inflammatory characteristics

within the intestine (277). This suggests that NR ligands are of potential use in TPNAC either as anti-inflammatory agents or as adaptation-boosting agents.

These two examples (BA and TPNAC) further illustrate the link between inflammation and cholestasis, which probably ought to be regarded as intertwined phenomena. Not only does inflammation lead to cholestasis (as in IIC), cholestasis *per se* leads to hepatic inflammation as well, as reduced intestinal delivery of bile may lead to bacterial overgrowth, translocation and an inflammatory response (11). Finally, hepatocellular damage, regardless of its cause, will activate inflammatory signaling and thus further impair liver function in part via inducing cholestasis. Studies in bile duct-ligated mice, a well known model of acute, extrahepatic cholestasis, have shown that there is an inflammatory response that further worsens liver injury (278,279). The underlying mechanisms, however, remain unclear as bile-duct ligation also leads to reduced expression of hepatic transporters independently of cytokine expression (280) or degree of inflammatory response (25).

In conclusion, NRs play dual roles in the setting of IIC. Increased understanding of the pathogenesis of IIC has shown us how centrally involved NRs and their co-regulators are in the regulation of hepatobiliary transport systems. Simultaneously, this has provided us with novel therapeutic strategies aimed at maintaining or even boosting NR function during inflammation, enabling proper/necessary adaptive responses. Furthermore, future application of new anti-inflammatory agents (such as selective PPAR-gamma or LXR modulators) would expand the therapeutic arsenal importantly making us less dependent on traditional compounds as glucocorticoids.

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Footnote: [1]According to general convention, names of human proteins and genes are presented in uppercase with the latter in italics, while those for other species are presented in lowercase. Throughout this review, uppercase is used when human and other species are discussed simultaneously and when general topics/mechanisms are discussed. When proteins and encoding genes are discussed simultaneously, only the protein nomenclature is used for reasons of clarity.

Abbreviations: ABC: ATP-binding cassette (transporter), AF: activation function (domain), APR: acute phase response, AQP: aquaporin, BA: biliary atresia, BRIC: benign recurrent intrahepatic cholestasis, BSDF: bile salt dependent flow, BSEP: bile salt export pump, BSIF: bile salt independent flow, CAR: constitutive androstane receptor, CBP: CRE-binding protein, CLP: cecal-ligation and puncture, CYP: cytochrome P450, DBD: DNA-binding domain, ER: estrogen receptor, FXR: farnesoid X receptor, Gal: D-galactosamine, GH: growth hormone, GR: glucocorticoid receptor, HDAC: histone deacetylase, HNF: hepatocyte nuclear factor, IIC: inflammation-induced cholestasis, IL: interleukin, iNOS: inducible nitric oxide synthase, JNK: c-jun N-terminal kinase, KC: Kupffer cell, LBD: ligand-binding domain, LPS: lipopolysaccharide, LRH: liver receptor homolog, LXR: liver X receptor, MDR: multidrug resistance (P-glycoprotein), MMP: matrix metalloproteinase, MRP: multidrug resistance-associated protein, NCoR: nuclear receptor co-repressor, NO: nitric oxide, NPC1L1: Niemann-Pick-1-like-1 (protein), NR: nuclear receptor, NTCP: Na^+-taurocholate co-transporting polypeptide, OATP: organic anion transporting protein, OCT: organic cation transporter, OST: organic solute transporter, PEPCK: phosphoenolpyruvate carboxykinase, (P)FIC: (progressive) familial intrahepatic cholestasis, PGC: PPAR-gamma co-activator, PPAR: peroxisome proliferator activated receptors, PR: progesterone receptor, PXR: pregnane X receptor, RE: response element, RAR: retinoic acid receptor, RXR: retinoid X receptor, SERM: selective estrogen receptor modulator, SHP: small heterodimer partner, SMRT: silencing mediator of retinoid and thyroid hormone receptor, SNuRM: selective nuclear receptor modulator, SRC: steroid receptor co-activator, Sult2a1: dehydroepiandrosterone-sulfotransferase, TGF: transforming growth factor, TLR: Toll-like receptor, TNF: tumor necrosis factor, TPNAC: total parenteral nutrition associated cholestasis

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