

Nuclear receptors in immune function (MS number 11012)

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Glossary

Coactivator: A class of proteins that promote the initiation of transcription via diverse mechanisms. They can serve as a bridge between DNA-bound transcription factors and RNA polymerase II. Some coactivators also have the capacity to modify chromatin structure (e.g. through histone-acetylation) allowing increased access to DNA. Coactivators function as part of large protein complexes comprising additional regulatory proteins.

Corepressor: A class of proteins that inhibit initiation of transcription via several distinct mechanisms, including competition with activator proteins for transcription factor binding, interaction with the basal transcriptional machinery, DNA methylation and recruitment of proteins with histone deacetylase activity.

Hormone response element (HRE): A DNA motif specific for each nuclear receptor usually, but not exclusively found in the vicinity of transcriptional start site of target genes (mostly in the promoter region). HRE allows interaction of the receptor with the DNA leading to transcriptional activation or repression of target gene.

Hypothalamic-pituitary-adrenal axis (HPA axis): Regulatory axis of the neuroendocrine system formed by interactions between the hypothalamus, pituitary gland and adrenal glands that controls various physiological processes including reactions to stress, digestion, immune responses, energy storage and expenditure. The hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates release of adrenocorticotrophic hormone (ACTH) from the pituitary gland. Circulating ACTH acts on the adrenal gland to stimulate biosynthesis of corticosteroids from cholesterol.

Transrepression: A process whereby a transcription factor inhibits transcriptional responses that are mediated by other classes of transcription factors without directly binding to DNA. Transrepression involves protein-protein interactions and its molecular mechanisms depend on the participating transcription factors.

Nomenclature

ABC: ATP-binding cassette

COX-2: cyclooxygenase 2

GALT: Gut-associated lymphoid tissue

GR: glucocorticoid receptor

HRE: hormone response element

iNKT: Invariant natural killer T

iNOS: inducible nitric oxide synthase

LDL: low density lipoprotein

LXR: Liver X receptor

MCP-1: Monocyte chemoattractant protein-1

MHC II: major histocompatibility complex class II

NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells

oxLDL: Oxidized low-density lipoprotein

PI3K: phosphatidylinositol 3-kinases

PPAR: Peroxisome proliferator-associated receptor RA: Rheumatoid arthritis

RAR: Retinoic acid receptor
ROR: retinoic acid receptor-related orphan receptor
RXR: Retinoid X receptor
SMRT: silencing mediator for retinoid and thyroid hormone receptors
SREBP: sterol response element binding protein
STAT: signal transducer and activator of transcription
TLR: Toll-like receptor
Treg: Regulatory T cell
VDR: Vitamin D receptor

Keywords

Glucocorticoid receptor
Immunity
Lipid
Liver x receptor
NR4A receptors
Nuclear receptor
Nur77
Peroxisome proliferator-associated receptor
Retinoic acid receptor
Retinoic acid receptor-related orphan receptor
Retinoid X receptor
Transcription
Vitamin D receptor

Abstract

Immune cells have the capacity to respond to the changing extra- and intracellular lipid environment by altered gene expression and consequential changes in their phenotypes. The effects of many lipid molecules are mediated by a large group of ligand-activated transcription factors, termed nuclear receptors. These molecules are able to bind various lipids and modify gene expression that ultimately impacts cellular functions. In this chapter we summarize our current understanding of the activity of a group of nuclear receptors with established roles in regulating the functions of immune cells including macrophages, dendritic cells and lymphocytes. It appears that lipids have a major impact on immune cell activity and this way provide a link between metabolic state and immune function. Also, some of these receptors are established or emerging drug targets in acute and chronic inflammatory diseases.

General aspects of nuclear receptor signaling

Nuclear receptors are a class of ligand-activated transcription factors, which appeared early during metazoan evolution endowing individual cells with the ability to efficiently sense and respond to their extra- and intracellular lipid milieu. Nuclear receptors are localized intracellularly and bind lipids either diffused through the plasma membrane from the extracellular space or generated by metabolic pathways inside the cell. These lipids include steroids (e.g. glucocorticoids, mineralocorticoids, estrogens, progesterone, androgens, vitamin D, oxysterols, bile acids), thyroid hormones, retinoids and a wide array of fatty acids. Activated nuclear receptors can bind specific regions of the DNA and positively or negatively regulate transcription of a certain number of genes. Thus, unlike membrane-bound receptors, nuclear receptors integrate lipid signal recognition and gene expression regulation within one molecule.

This remarkable combination of different functions is enabled by a modular domain structure, in which each domain possesses a specific function. <Figure 1> The variable N-terminal region includes the AF-1 domain implicated in constitutive ligand-independent transcriptional activation by the receptor. The DNA-binding domain is responsible for binding of specific DNA sequences characteristic for the receptor, called hormone response elements (HRE). The C-terminal region contains a ligand-binding domain, which selectively recognizes lipid molecules determined by the size and polarity of the binding pocket. This domain also serves as a primary surface for homo- or heterodimerization of the receptor. Upon ligand binding, the ligand-binding domain undergoes a conformational change revealing the ligand-dependent transcriptional activation function (AF-2) domain capable of interacting with transcriptional coregulator proteins. The DNA- and ligand-binding domain is connected by a hinge region that permits structural flexibility of the receptor. This structural scheme can be applied to all members of the nuclear receptor superfamily.

The members of this group of structurally related receptors can be subdivided into four main classes based on their ligand-binding and functional properties <Table 1>. The first class consists of classic endocrine receptors including glucocorticoid receptor (GR), mineralocorticoid receptor (MR), androgen receptor (AR), estrogen receptor (ER), and progesterone receptor (PR). The ligands of this class of receptors are high affinity hormonal lipids that are mostly produced in endocrine tissues and their levels are tightly regulated by the hypothalamic-pituitary axis. The second class consists of so-called orphan nuclear receptors (e.g. Nur77, COUP-TF α , NGF1-B) whose endogenous ligands have not yet been identified. Some of them do not need to bind a ligand at all in order to exert their function. The third class includes adopted orphan nuclear receptors whose physiological ligands have been already identified. Members of this group include retinoid X receptor (RXR) and its heterodimeric partners: peroxisome proliferator-activated receptors (PPAR), liver X receptors (LXR), farnesoid X receptor (FXR), pregnane X receptor (PXR) and constitutive androstane receptor (CAR). Their ligands mainly include low affinity dietary lipid metabolites (fatty acids, oxysterols, bile acids) or xenobiotics that are not regulated by the endocrine system. Adopted orphan receptors form so-called permissive heterodimers with RXR meaning that they can be activated both by ligands of RXR and its partner. Vitamin D receptor (VDR), retinoic acid receptor (RAR), and thyroid receptor (TR) comprise a transitional group of endocrine receptors spanning the gap between classic endocrine receptors and adopted orphan receptors. Similar to the class

of adopted orphans, these receptors function as heterodimers with RXR. However, their essential role in morphogenesis and development together with the tight endocrine regulation of their ligands make them similar to classic endocrine receptors. In contrast to adopted orphan receptors these RXR heterodimer endocrine receptors are non-permissive, meaning that they can only be activated by the ligand of the endocrine receptor and include RXR as a silent component. While dual ligand regulation enables integration of different dietary lipid signals by permissive heterodimers, the non-permissive nature of endocrine RXR heterodimers permits transcriptional regulation that accurately reflects available hormone or vitamin levels.

Different classes of nuclear receptors show distinct modes of transcriptional activation. <Figure 2> Classic endocrine receptors, like GR form functionally inactive complexes with heat-shock proteins in the cytoplasm and/or nucleus. Hormone binding causes dissociation of heat-shock protein complex and subsequent binding of the receptor to hormone response elements in form of a homodimer. DNA binding is followed by coactivator recruitment and initiation of transcription. In contrast, RXR heterodimers formed by adopted orphans or endocrine receptors generally bind their response elements even in the absence of an activating ligand. Unactivated receptors bind corepressor complexes (including nuclear-receptor corepressor (NCOR) or silencing mediator of retinoic acid and thyroid-hormone receptor (SMRT)), which inhibit transcription primarily via modification of chromatin structure. The presence of ligand leads to exchange of corepressors for coactivators leading to activation of target gene transcription. Orphan receptors mostly bind DNA as monomers or homodimers and activate transcription in the absence of ligand. Nevertheless, several orphan receptors have been shown to constitutively bind lipids, which are required for transcriptional activity of the receptor.

Nuclear receptors can negatively regulate gene expression as well. <Figure 3> As we mentioned above, RXR heterodimers repress genes in the absence of activating ligands. However, some corepressor complexes favor binding nuclear receptors in a ligand-bound form. In addition, binding of certain hormone response elements dictate corepressor instead of coactivator recruitment by the liganded nuclear receptor, leading to inhibition of target gene transcription. Several nuclear receptors including GR, PPAR γ and LXR have the capacity to negatively regulate the activity of other signal-dependent transcription factors (e.g. ones involved in inflammatory response, such as NF- κ B or AP-1) by a mechanism termed transrepression. (Glass & Saijo 2010) This process, unlike transcriptional activation, does not require direct binding of response elements of target genes, but instead involves protein-protein interactions. However, the exact molecular details accounting for this effect still remain to be determined.

From the 48 nuclear receptors encoded by the human genome, we will discuss here those, which have a well-established function in immune regulation. Due to space constraints, we cannot include nuclear receptors whose emerging importance in immunity is just being recognized (e.g. sex hormone receptors, REV-ERB). For these topics we refer the reader to the recommended literature in the end of the chapter. Since the function of many members of the receptor family has still not been described extensively, we cannot exclude that others will be linked to immune functions as well.

Glucocorticoid Receptor (GR)

Glucocorticoids are released from the adrenal glands in response to activation of hypothalamic-pituitary-adrenal axis by various stress stimuli including pain, starvation, trauma, and infection. Synthetic derivatives of glucocorticoid hormones serve as highly potent treatments for a wide range of autoimmune diseases, such as rheumatoid arthritis, inflammatory bowel disease, psoriasis and multiple sclerosis. The therapeutic efficiency of these compounds relies on the pleiotropic effect of glucocorticoids on the immune system mediated by GR. Due to the ubiquitous expression of GR, glucocorticoids shape the function of nearly every cell type of the immune system. (Rhen & Cidlowski 2005; Glass & Ogawa 2005; Baschant & Tuckermann 2010; Busillo & Cidlowski 2013). The receptor exerts its function in immune cells through three major mechanisms. First, GR homodimers activate or repress gene expression through binding to HRE (direct activation and repression). Second, GR inhibits the activity of pro-inflammatory transcription factors, such as AP1, NF- κ B, IRF3, NFAT, T-bet and GATA-3, without physical binding to DNA (transrepression). Last, GR can alter the activity of extracellular signal-activated kinases (e.g. PI3K) without directly affecting gene expression (non-genomic effects). The systemic consequence of these mechanisms is a wide-scale inhibition of divergent inflammatory pathways in various immune cells that accounts for the strong immunosuppressive capacity of endogenous and exogenous glucocorticoids (as exemplified by the unfavorable effect of chronic stress or long-term steroid medication on immune defense). Specifically, glucocorticoids inhibit production of a diverse range of pro-inflammatory cytokines, including IL-1 β , IL-2, IL-6, IL-12, TNF α , IFN γ , and chemoattractants, like IL-8, MCP-1, and eotaxin. Activation of GR also suppresses production of eicosanoids derived from arachidonic acid (prostaglandins, thromboxanes, prostacyclins) by multiple mechanisms. The activated receptor induces annexin 1 (also known as lipocortin 1) and MAPK phosphatase, which, by inhibiting cytosolic phospholipase A2 α , block the release of arachidonic acid from membrane phospholipids. Conversion of arachidonic acid to bioactive mediators is also prevented through the repression of cyclooxygenase 2 transcription. Besides suppressing production of protein and lipid mediators GR activation promotes the termination of inflammation by exerting cell-specific effects on the activation state of various immune cells. <Figure 4> Extravasation of neutrophil granulocytes to sites of inflammation is inhibited via reducing the expression of L-selectin and integrins (VLA-4, LFA-1) responsible for adhesion to endothelial cells. Macrophages exposed to glucocorticoid stimulus show enhanced phagocytotic activity thereby facilitating the clearance of cell debris. GR activation also suppresses the maturation process of dendritic cells by preventing the appearance of MHC II and costimulatory molecules (e.g. CD86) on the cell surface. By inducing IL-10 production of macrophages and dendritic cells, glucocorticoids generate an environment favoring T cell anergy and differentiation of regulatory T cells, thus enhance immune tolerance. Acting on lymphocytes, glucocorticoids can cause a shift from Th1 to Th2 immunity also supported by augmented IgE production in B cells.

Peroxisome Proliferator-Activated Receptors (PPARs)

The PPAR subfamily of nuclear receptors consists of PPAR α , PPAR δ , and PPAR γ , each possessing distinct tissue expression pattern and different but partly overlapping

functions. Due to their relatively large ligand-binding pockets, PPARs can be activated by a wide array of lipids, including polyunsaturated fatty acids, eicosanoids as well as components of VLDL and oxidized LDL. By acting as cellular lipid sensors, PPARs participate in maintaining whole body lipid and glucose homeostasis, which effect is utilized in medicine: PPAR α -activating fibrates and PPAR γ -activating thiazolidinediones are in clinical use to treat dyslipidaemia and diabetes, respectively. In addition to their metabolic functions, all three PPAR isotypes participate in controlling inflammatory responses primarily through ligand-dependent transrepression of inflammatory pathways (similar to GR). This prevents the induction of pro-inflammatory cytokines (e.g. IL-1 β , IL-6, IL-12, TNF α) and enzymes generating bioactive molecules (e.g. iNOS, COX-2). Although there is a growing body of recent studies that suggest the role of PPAR α and δ in regulating T cell and macrophage phenotypes (Dunn et al. 2010; Kang et al. 2008; Mukundan et al. 2009; Dunn et al. 2007), PPAR γ is the most extensively characterized isotype in terms of immunoregulatory functions (Szeles et al. 2007). PPAR γ was originally identified as an indispensable orchestrator of adipocyte differentiation, but it also has a complex regulatory role principally in cells of the myeloid lineage. In macrophages, the predominant consequence of PPAR γ activation is the suppression of inflammatory response by negatively regulating NF- κ B and AP-1 activity (Glass & Ogawa 2005). Another important link between PPAR γ and cytokine signaling is its cooperation with IL-4--STAT6 pathway. The Th2 polarizing cytokine IL-4, via the STAT6 transcription factor, increases the expression of PPAR γ together with enzymes generating endogenous PPAR γ ligands, and also facilitates transcriptional activity of the receptor (Huang et al. 1999; Szanto et al. 2010). The proposed functional relevance of this effect is that IL-4 engages PPAR γ to participate in promoting the differentiation of monocytes towards the so-called M2 macrophage phenotype characterized by regenerative and anti-inflammatory functions. PPAR γ is also a crucial member of a regulatory circuit organizing cholesterol homeostasis in macrophages also comprising LXR, RAR and RXR nuclear receptors <Figure 5> Certain components of oxidized low-density lipoprotein (oxLDL) have the capacity to induce expression of PPAR γ and serve as an activating ligand at the same time. Activation of the receptor increases expression of CD36 scavenger receptor leading to further lipid uptake and subsequent activation of PPAR γ (Nagy et al. 1998). This positive feedback loop is counterbalanced through simultaneous initiation of cholesterol efflux processes orchestrated by LXR. PPAR γ induces the expression of LXR and also CYP27A1, an enzyme capable of generating 27- hydroxycholesterol serving as an endogenous LXR activator. Along with PPAR γ , RAR and RXR can also induce CYP27A1. The resulting augmented LXR activity leads to increased expression of ABC transporters ABCA1 and ABCG1, which mediate efflux of cholesterol from the cell. Acquired disturbances in the integrity of this circuit are crucial during formation of cholesterol-laden macrophages, known as foam cells, that is considered one of the earliest steps in the pathogenesis of atherosclerosis (Moore & Tabas 2011). PPAR γ is involved in shaping the function of dendritic cells as well (Nagy et al. 2012). Activation of the receptor inhibits the secretion of IL-12 (a pivotal cytokine in T cell activation) along with impeding surface expression of costimulatory molecules (e.g. CD40, CD80) and by inducing coinhibitory molecules, like PD-L1. Thus, PPAR γ perpetuates a state of dendritic cell immaturity characterized by diminished T cell-stimulatory capacity. It seems that this is achieved, at least partly, by interfering with other extracellular signal-activated pathways including NF- κ B signaling, similar to macrophages. In addition to restricting activation, PPAR γ alters

lipid antigen presentation by coordinately regulating surface expression of CD1 proteins, MHC I-like molecules required for presenting lipid antigens for T cells. PPAR γ -instructed dendritic cells reduce their surface expression of CD1a, CD1b, CD1c, and CD1e, while increasing expression of CD1d. Enhanced expression of CD1d confers the ability to selectively activate a particular class of T cells carrying invariant T cell receptors, termed invariant natural killer T (iNKT) cells (Szatmari et al. 2004). These CD1d-restricted T cells recognize self as well as microbial lipid antigens and possess complex regulatory functions both during innate and adaptive immune responses. Finally, PPAR γ regulates a number of genes in dendritic cells that are implicated in various aspects of lipid metabolism, involving fatty acid oxidation or phospholipid and triacylglycerol synthesis. However, the exact metabolic changes brought about by the receptor are still not clear. T cell-expressed PPAR γ has been found to inhibit naïve T cell differentiation towards IL-17 producing Th17 cells by interfering with the expression of ROR γ , master regulator of Th17 differentiation (see below) (Klotz et al. 2007). Specifically, PPAR γ prevents removal of SMRT corepressor from the promoter of ROR γ in response to Th17 polarizing stimuli, thereby blocking ROR γ transcription and Th17 expansion, which is thought to play a major role in tissue destruction observed in a number of autoimmune conditions.

Liver X Receptors (LXRs)

LXR α and LXR β act as sensors of cholesterol precursors and oxysterols like desmosterol, 24S-hydroxycholesterol or 27-hydroxycholesterol, and turn these signals into a transcriptional program that regulates both cellular and systemic cholesterol homeostasis. As described above, LXR activation protects macrophages from cholesterol overload by inducing the expression of lipid-transport proteins, like ABCA1, ABCG1, or ApoE that promote elimination of excess cholesterol. Simultaneously, LXRs decrease further cholesterol uptake by inducing degradation of low-density lipoprotein receptors. Nevertheless, cholesterol metabolites via LXRs can influence diverse pathways in immune cells that are distinct from lipid homeostasis. LXRs have been also shown to counterregulate inflammatory responses through transrepression analogous to GR and PPARs, affecting a similar set of crucial inflammatory genes (e.g. iNOS, COX-2, IL-6, IL-1 β and MCP-1). It is important to note, however, that the target genes of inflammatory stimuli (e.g. bacterial lipopolysaccharide) inhibited by GR, PPAR and LXR show only partial overlap (Ogawa et al. 2005). Therefore, each receptor skews immune response to pathogens and tissue damage in a unique way that is attributable to differences in molecular determinants of the transrepression process depending on the signal, the target gene and the nuclear receptor. Interestingly, only LXR α , but not LXR β , has been shown to be indispensable for the development of macrophages in the marginal zone of the spleen (A-González et al. 2013). Generally, LXR α and β signaling is cooperatively activated in macrophages following phagocytosis of bacteria and apoptotic cells, impacting multiple aspects of immune homeostasis (A-González & Castrillo 2011). Intracellular bacteria (e.g. *Listeria monocytogenes*) taken up via phagocytosis activate LXR, which, in turn, promotes bacterial clearance and macrophage survival during the infection. Lipid components of engulfed apoptotic cells also turn on LXR signaling leading to further enhancement in phagocytotic activity by inducing a receptor tyrosine kinase crucial for apoptotic cell clearance, called Mer. At the same time, the anti-inflammatory actions of LXR are utilized as activation of the receptor negatively modulates inflammatory gene expression to avoid unwanted immune

responses against apoptotic cell debris. The importance of LXR-induced immune tolerance is highlighted by the observation that mice lacking LXR α and β develop autoimmune kidney disease resembling certain aspects of human systemic lupus erythematosus (A-González et al. 2009). (Interestingly, recent studies have shown that mice lacking PPAR δ or PPAR γ in macrophages exhibit similar deficiencies, suggesting the overlapping activities of these receptors in phagocytosis (Mukundan et al. 2009; Röszer et al. 2011)). LXR expressed by macrophages participates in limiting the steady-state levels of neutrophil granulocytes, too. Phagocytosis of senescent neutrophils activates the receptor leading to the suppression of IL-23/IL-17/G-CSF cytokine cascade, which stimulates granulopoiesis and granulocyte egress from the bone marrow (Hong et al. 2012). LXR of T lymphocytes serves as an intriguing example of the close link between metabolic state and activation status of the cell. Endogenous cholesterol content regulates lymphocyte proliferation through coordinate regulation of SREBP and LXR pathways. Lymphocyte activation and proliferation is associated with inhibition of LXR activation and resulting cholesterol efflux due to conversion of oxysterols to metabolites that are unable to activate the receptor. At the same time, SREBP is activated leading to enhanced cholesterol synthesis that seems to be required for efficient T cell activation. Conversely, activation of LXR limits lymphocyte expansion by triggering cholesterol transport through ABCG1 (Bensinger et al. 2008). Similar to PPAR γ , LXR has also been shown to restrict Th17 differentiation of naïve T cells. This effect, as in the case of PPAR γ , is an indirect one that is mediated through SREBP-1. Activated LXR promotes SREBP-1 expression, which in turn decreases aryl hydrocarbon receptor-mediated initiation of IL-17 transcription (Cui et al. 2011).

Retinoid Receptors (RAR, RXR)

Vitamin A-deficiency causes immune dysfunction and increased susceptibility to infections, an observation, which underlines the fundamental importance of vitamin A in immune regulation (Mora et al. 2008). The diverse biological effects of vitamin A (retinol), including its essential function in embryonic and postnatal development, epithelial differentiation, reproduction and vision, are all exerted through its metabolites, mainly retinoic acid (Ross et al. 2000). All-trans and 9-cis stereoisomers of retinoic acid bind two types of retinoid receptors, namely retinoic acid receptor (RAR) and retinoid X receptor (RXR). Both receptors have α , β , and γ isotypes, as well as different tissue expression, but partially overlapping actions. Retinoid receptors show different ligand specificities: RAR binds both all-trans and 9-cis stereoisomers of retinoic acid, whereas RXR only binds 9-cis retinoic acid. RXR serves as a heterodimeric partner of one third of human nuclear receptors by enabling them to bind DNA and regulate transcription as part of a heterodimer. Hence, ligand binding of RXR leads to activation of parallel nuclear receptor pathways mediated by permissive RXR heterodimers (Szanto et al. 2004). It is still an open question though, whether 9-cis retinoic acid is a physiologic activator of RXR, or whether the actual ligands are other lipids, such as certain fatty acids (e.g. phytanic acid and docosahexaenoic acid) that were also demonstrated to bind and activate the receptor under nonphysiological settings. Due to the fact that vitamin A is provided exclusively through diet, its serum levels largely depend on dietary uptake. Therefore, the magnitude of its biological effects must be regulated by the activity of its converting enzymes, retinaldehyde dehydrogenases, which modify the availability of biologically active retinoic acid. Synthesis of retinoic acid by these enzymes in

dendritic cells, macrophages, epithelial cells and stromal cells located along environmental interfaces of the body plays a central role in orchestrating mucosal immune homeostasis (Hall et al. 2011). Retinoic acid produced by dendritic cells enhances expression of gut-homing molecules (integrin heterodimer $\alpha 4\beta 7$ and chemokine receptor CCR9) on $CD4^+$ T cells, thus promoting their migration into intestinal *lamina propria* and GALT (gut-associated lymphoid tissue) (Iwata et al. 2004). Moreover, local retinoic acid in the intestine and GALT cooperates with $TGF\beta$ to promote differentiation of $CD4^+$ T cells into so-called induced regulatory T (iTreg) cells, characterized by FoxP3 expression. Remarkably, retinoic acid sustains iTreg generation even in a cytokine environment that would favor an inflammatory response. Differentiation of naïve T cells into Th17 cells also requires $TGF\beta$ (along with IL-6 and IL-1). However, retinoic acid regulates Th17 differentiation adversely by suppressing the expression of $ROR\gamma$ and IL-6 receptor, thus shifting T cell responses away from inflammation and autoimmunity towards tolerance. Accordingly, the constant level of homing and expansion of iTregs in the intestines maintained by retinoic acid is an essential determinant of oral tolerance, the suppression of immune responses against dietary antigens. An additional layer of retinoic acid effects on mucosal immunity is that retinoic acid synthesis by GALT dendritic cells is also required for generation of immunoglobulin A (IgA)-secreting B cells. Hence it seems that a sufficient amount of vitamin A-derived retinoic acid in the intestines is indispensable for maintaining the delicate balance between efficient immune protection and tolerance against the vast amount of dietary antigens. Recent results pointed out that retinoic acid derived from pulmonary macrophages and dendritic cells sustains airway tolerance through an analogous mechanism. Retinoic acid, however, also acts in an autocrine manner, shaping the function of dendritic cells. Activation of RAR endows dendritic cells with lipid antigen presentation capacity to iNKT cells via CD1d, but also suppresses secretion of inflammatory mediators and promotes a tolerogenic dendritic cell phenotype. In macrophages, retinoid receptors participate in the regulation of cholesterol homeostasis via two distinct mechanisms. First, the RAR/RXR heterodimer induces CYP27A1 enzyme producing LXR ligands, thereby turning on LXR-mediated cholesterol efflux (see above). Second, RAR/RXR itself directly induces expression of ABCA1 cholesterol transporter. As can be expected, presence of RXR is essential for macrophage functions regulated by RXR heterodimers. This is best illustrated by the observation that mice lacking RXR in their macrophages show similar defects in apoptotic cell clearance, compared to those observed in the absence of LXR, $PPAR\gamma$ and $PPAR\delta$ (Röszer et al. 2011).

Retinoic Acid Receptor-Related Orphan Receptors (RORs)

RAR-related orphan receptor α , β , and γ were initially identified in a screen for RAR- and RXR-related genes that might have additional functions in vitamin A physiology. Ensuing research revealed a broad range of ROR-dependent physiological functions, which turned out to be separate from those of retinoid receptors (Solt & Burris 2012; Jetten 2009). Unlike the dimeric receptors described above, RORs bind their response elements as monomers, and appear to function constitutively. Although all-trans retinoic acid and cholesterol derivatives have been shown to modulate ROR activity *in vitro*, a genuine physiological ligand for the receptor has not been established yet. Beside the crucial importance of $ROR\alpha$ and β in brain and retinal development, $ROR\gamma$ expressed by so-called lymphoid tissue inducer cells is essential for the formation of

lymph nodes. Moreover, ROR γ promotes survival and differentiation of developing lymphocytes in the thymus. Most importantly, ROR α and ROR γ act in concert to direct the lineage specification of naïve T cells towards Th17 cells (Yang et al. 2008; Ivanov et al. 2006). Th17 cells are characterized by the production of IL-17, IL-21 and IL-22, and are abundant in inflamed tissues often associated with autoimmune conditions. These cells are generated in a cytokine environment that is dominated by TGF β in combination with IL-1 β , IL-6 or IL-21. ROR α and ROR γ are highly induced in response to these factors via the STAT3 transcription factor, and are key requirements for functional Th17 cell development. As mentioned above, several nuclear receptors are involved in the regulation of Th17 expansion, including PPAR γ , LXR, and RAR, further supporting the idea that intracellular lipid milieu sensed by nuclear receptors holds (at least partial) control over T cell fate. <Figure 6>

Vitamin D Receptor (VDR)

Vitamin D₃ (cholecalciferol) is central in the strict control of calcium homeostasis, but it has also been long known that a sufficient amount of the vitamin is a prerequisite for healthy immune function (Mora et al. 2008; Nagy et al. 2012). The biological effects of vitamin D₃ are mediated by its derivative 1,25-dihydroxyvitamin D₃ that binds and activates VDR forming a heterodimer with RXR. Analogous to vitamin A, the amount of available active vitamin is limited by its conversion that is completed through a two-step process. Vitamin D₃, either derived from dietary uptake or from endogenous synthesis in the skin requiring sunlight exposure, is converted by the CYP27A1 enzyme in the liver to 25-hydroxyvitamin D₃, which is further metabolized in the kidneys to 1,25-dihydroxyvitamin D₃ by the CYP27B1 enzyme. Certain cells of the immune system, including macrophages, dendritic cells and lymphocytes are also equipped with the enzymatic machinery required for synthesis of active vitamin D₃ endowing them with the capacity to produce 1,25-dihydroxyvitamin D₃ acting in both autocrine and paracrine manner to modulate local cellular responses. Activation of Toll-like receptors by microbial signals in macrophages triggers the generation of 1,25-dihydroxyvitamin D₃ by CYP27B1 and expression of VDR as well. Activated VDR, in turn, confers enhanced antibacterial activity by increasing the production of bactericidal proteins defensin B2/B4 and cathelicidin (Liu et al. 2006). In parallel, 1,25-dihydroxyvitamin D₃ also induces autophagy, an additional cellular response to infection. Together, these actions are critical in the defense against various pathogens, in particular *Mycobacterium tuberculosis*. Intriguingly, the antimicrobial effect of enhanced vitamin D synthesis was already utilized by Niels Ryberg Finsen in the late 19th century, who successfully treated cutaneous tuberculosis with concentrated light irradiation, and was later awarded the Nobel Prize for his achievements. Dendritic cells exposed to 1,25-dihydroxyvitamin D₃ fail to initiate their maturation program and acquire a tolerogenic phenotype. Tolerogenic dendritic cells are characterized by low surface expression of MHC class II and costimulatory molecules (eg, CD40, CD80, and CD86), decreased secretion of IL-12, high expression of T cell inhibitory molecules (e.g. ILT-3, PD-L1), and increased IL-10 secretion. These cells therefore inefficiently stimulate Th1 and Th2 cell activation, but foster generation of Treg cells, promote T cell anergy and effector T cell death, thus play an important role in maintaining self-tolerance. VDR is also expressed in T cells, but only in response to TCR activation. Initiation of vitamin D signaling, in turn, curbs T cell activation by reducing IFN γ and IL-2 production that is associated with a diminished capacity to proliferate. Moreover, active vitamin D₃ inhibits IL-17 secretion further broadening

the spectrum of nuclear receptors controlling Th17 activity. While acting only as a fine-tuner of T helper cell responses, VDR expression proved to be essential for the development of iNKT cells reacting to lipids presented by CD1d (Yu & Cantorna 2008). Ultimately, the fundamental importance of vitamin D in healthy immune responses is underpinned by numerous studies showing that vitamin D-deficiency, often associated with insufficient sunlight exposure, can be correlated with an increased risk of several autoimmune diseases.

NR4A Receptors (NURR1, NUR77, NOR1)

NR4A receptors are a class of orphan receptors, whose essential roles in immune system are just being recognized. Although their target genes and regulated pathways remain to be elucidated, they have been proven to be indispensable for the development of several immune cell types (Hamers et al. 2013). Mice lacking all three receptors fail to develop Treg cells and die early due to the consequential systemic autoimmunity. In addition, Nur77 receptor is required for generation of a leukocyte population, called patrolling monocytes, which are primarily responsible for removing cellular debris from the vasculature. Nur77 is also involved in the negative regulation of inflammatory pathways, like the one mediated by the NF- κ B transcription factor. Nevertheless, the exact molecular mechanisms behind these actions are still not known necessitating further research.

List of relevant web pages

<http://www.nursa.org> - Nuclear Receptor Signaling Atlas (NURSA), the most comprehensive database of nuclear receptors including coregulator proteins, ligands, tissue expression, interactions, and phenotype of mice with targeted deletion (knock-out) of each receptor.

Reference list

- A-González, N. & Castrillo, A., 2011. Biochimica et Biophysica Acta. *BBA - Molecular Basis of Disease*, 1812(8), pp.982–994.
- A-González, N. et al., 2009. Apoptotic Cells Promote Their Own Clearance and Immune Tolerance through Activation of the Nuclear Receptor LXR. *Immunity*, 31(2), pp.245–258.
- A-González, N. et al., 2013. The nuclear receptor LXR α controls the functional specialization of splenic macrophages. *Nature Immunology*, 14(8), pp.831–839.
- Baschant, U. & Tuckermann, J., 2010. Journal of Steroid Biochemistry and Molecular Biology. *Journal of Steroid Biochemistry and Molecular Biology*, 120(2-3), pp.69–75.
- Bensinger, S.J. et al., 2008. LXR Signaling Couples Sterol Metabolism to Proliferation in the Acquired Immune Response. *Cell*, 134(1), pp.97–111.
- Busillo, J.M. & Cidlowski, J.A., 2013. The five Rs of glucocorticoid action during inflammation: ready, reinforce, repress, resolve, and restore. *Trends in Endocrinology & Metabolism*, 24(3), pp.109–119.
- Cui, G. et al., 2011. Liver X receptor (LXR) mediates negative regulation of mouse and human Th17 differentiation. *Journal of Clinical Investigation*, 121(2), pp.658–670.
- Dunn, S.E. et al., 2007. Peroxisome proliferator-activated receptor (PPAR) expression in T cells mediates gender differences in development of T cell-mediated autoimmunity. *Journal of Experimental Medicine*, 204(2), pp.321–330.
- Dunn, S.E. et al., 2010. Peroxisome proliferator-activated receptor limits the expansion of pathogenic Th cells during central nervous system autoimmunity. *Journal of Experimental Medicine*, 207(8), pp.1599–1608.
- Evans, R.M. & Mangelsdorf, D.J., 2014. Nuclear Receptors, RXR, and the Big Bang. *Cell*, 157(1), pp.255–266.
- Glass, C.K. & Ogawa, S., 2005. Combinatorial roles of nuclear receptors in inflammation and immunity. *Nature Reviews Immunology*, 6(1), pp.44–55.
- Glass, C.K. & Saijo, K., 2010. Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells. *Nature Reviews Immunology*, 10(5), pp.365–376.

- Hall, J.A. et al., 2011. The Role of Retinoic Acid in Tolerance and Immunity. *Immunity*, 35(1), pp.13–22.
- Hamers, A.A.J. et al., 2013. NR4A nuclear receptors in immunity and atherosclerosis. *Current Opinion in Lipidology*, p.1.
- Hong, C. et al., 2012. Coordinate regulation of neutrophil homeostasis by liver X receptors in mice. *Journal of Clinical Investigation*, 122(1), pp.337–347.
- Huang, J.T.A. et al., 1999. Interleukin-4-dependent production of PPAR- γ ligands in macrophages by 12/15-lipoxygenase. *Nature*, 400(6742), pp.378–382.
- Ivanov, I.I. et al., 2006. The Orphan Nuclear Receptor ROR γ t Directs the Differentiation Program of Proinflammatory IL-17+ T Helper Cells. *Cell*, 126(6), pp.1121–1133.
- Iwata, M. et al., 2004. Retinoic acid imprints gut-homing specificity on T cells. *Immunity*, 21(4), pp.527–538.
- Jetten, A.M., 2009. Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. *Nuclear Receptor Signaling*, 7, p.e003.
- Kang, K. et al., 2008. Adipocyte-Derived Th2 Cytokines and Myeloid PPAR δ Regulate Macrophage Polarization and Insulin Sensitivity. *Cell Metabolism*, 7(6), pp.485–495.
- Klotz, L. et al., 2007. Peroxisome proliferator-activated receptor gamma control of dendritic cell function contributes to development of CD4+ T cell anergy. *Journal of immunology (Baltimore, Md. : 1950)*, 178(4), pp.2122–2131.
- Liu, P.T. et al., 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*, 311(5768), pp.1770–1773.
- Moore, K.J. & Tabas, I., 2011. Macrophages in the Pathogenesis of Atherosclerosis. *Cell*, 145(3), pp.341–355.
- Mora, J.R., Iwata, M. & Andrian, von, U.H., 2008. Vitamin effects on the immune system: vitamins A and D take centre stage. *Nature Reviews Immunology*, 8(9), pp.685–698.
- Mukundan, L. et al., 2009. PPAR- δ senses and orchestrates clearance of apoptotic cells to promote tolerance. *Nat Med*, 15(11), pp.1266–1272.
- Nagy, L. et al., 2012. Nuclear Hormone Receptors Enable Macrophages and Dendritic Cells to Sense Their Lipid Environment and Shape Their Immune Response. *Physiological Reviews*, 92(2), pp.739–789.
- Nagy, L. et al., 1998. Oxidized LDL Regulates Macrophage Gene Expression through Ligand Activation of PPAR γ . *Cell*, 93(2), pp.229–240.
- Ogawa, S. et al., 2005. Molecular Determinants of Crosstalk between Nuclear

- Receptors and Toll-like Receptors. *Cell*, 122(5), pp.707–721.
- Rhen, T. & Cidlowski, J.A., 2005. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. *New England Journal of Medicine*, 353(16), pp.1711–1723.
- Ross, S.A. et al., 2000. Retinoids in embryonal development. *Physiological Reviews*, 80(3), pp.1021–1054.
- Röszer, T. et al., 2011. Autoimmune kidney disease and impaired engulfment of apoptotic cells in mice with macrophage peroxisome proliferator-activated receptor gamma or retinoid X receptor alpha deficiency. *Journal of immunology (Baltimore, Md. : 1950)*, 186(1), pp.621–631.
- Solt, L.A. & Burris, T.P., 2012. Action of RORs and their ligands in(patho)physiology. *Trends in Endocrinology & Metabolism*, 23(12), pp.573–581.
- Szanto, A. et al., 2004. Retinoid X receptors: X-ploring their (patho)physiological functions. *Cell Death and Differentiation*, 11, pp.S126–S143.
- Szanto, A. et al., 2010. STAT6 Transcription Factor Is a Facilitator of the Nuclear Receptor PPAR γ -Regulated Gene Expression in Macrophages and Dendritic Cells. *Immunity*, 33(5), pp.699–712.
- Szatmari, I. et al., 2004. Activation of PPAR γ Specifies a Dendritic Cell Subtype Capable of Enhanced Induction of iNKT Cell Expansion. *Immunity*, 21(1), pp.95–106.
- Szeles, L., Torocsik, D. & Nagy, L., 2007. PPAR γ in immunity and inflammation: cell types and diseases. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 1771(8), pp.1014–1030.
- Yang, X.O. et al., 2008. T Helper 17 Lineage Differentiation Is Programmed by Orphan Nuclear Receptors ROR α and ROR γ . *Immunity*, 28(1), pp.29–39.
- Yu, S. & Cantorna, M.T., 2008. The vitamin D receptor is required for iNKT cell development. *Proc Natl Acad Sci U S A*, 105(13), pp.5207–5212.

Further Reading

- Aranda, A. & Pascual, A., 2001. Nuclear hormone receptors and gene expression. *Physiological Reviews*, 81(3), pp.1269–1304.
- Evans, R.M. & Mangelsdorf, D.J., 2014. Nuclear Receptors, RXR, and the Big Bang. *Cell*, 157(1), pp.255–266.
- Fish, E.N., 2008. The X-files in immunity: sex-based differences predispose immune responses. *Nature Reviews Immunology*, 8(9), pp.737–744.

Gibbs, J.E. et al., 2012. The nuclear receptor REV-ERB α mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines. *Proceedings of the National Academy of Sciences*, 109(2), pp.582–587.

Kiss, M., MSc, Z.C. & PhD, L.N.M., 2013. The role of lipid-activated nuclear receptors in shaping macrophage and dendritic cell function: From physiology to pathology. *The Journal of allergy and clinical immunology*, 132(2), pp.264–286.

Nagy, L. et al., 2012. Nuclear Hormone Receptors Enable Macrophages and Dendritic Cells to Sense Their Lipid Environment and Shape Their Immune Response. *Physiological Reviews*, 92(2), pp.739–789.

Tata, J.R., 2002. Signalling through nuclear receptors. *Nature Reviews Molecular Cell Biology*, 3(9), pp.702–709.

Illustrations, Tables and Multimedia

Figure legends:

1. **Domain structure of nuclear receptors.** Colors on the domain structure above correspond to the identically colored domains on the schematic 3-dimensional structure below. AF-1: ligand-independent activation function domain; AF-2: ligand-dependent activation function domain.
2. **Distinct ways of transcriptional activation by different classes of nuclear receptors.** Hormone receptors form functionally inactive complexes with heat-shock proteins mostly in the cytoplasm. Hormone binding causes dissociation of heat-shock protein complex, formation of a homodimer and subsequent binding of the receptor to response elements. DNA binding is followed by coactivator recruitment and initiation of transcription. RXR heterodimers generally bind their response elements even in the absence of an activating ligand. Unactivated receptors bind corepressor complexes which inhibit transcription of target genes. The presence of ligand leads to exchange of corepressors for coactivators leading to activation of target gene transcription. Orphan receptors mostly bind DNA as monomers or homodimers and activate transcription in the absence of ligand. HRE: hormone response element
3. **Mechanisms of transcriptional repression by nuclear receptors**
4. **Effects of glucocorticoids on major immune cell populations contributing to inflammation.**
5. **Regulation of macrophage cholesterol homeostasis by the cross-talk of nuclear receptors.** Fatty acids from oxidized low-density lipoprotein (oxLDL) have the capacity to induce expression of PPAR γ and serve as an activating ligand at the same time. Activation of the receptor increases expression of CD36 scavenger receptor leading to further lipid uptake and subsequent activation of PPAR γ . PPAR γ also induces the expression of LXR and CYP27A1, an enzyme capable of generating 27-hydroxycholesterol serving as an endogenous LXR activator. Along with PPAR γ , RAR and RXR can also induce CYP27A1. The resulting augmented LXR activity leads to increased expression of ABC transporters ABCA1 and ABCG1, which mediate efflux of cholesterol from the cell. Green arrows indicate positive transcriptional regulation.

6. Positive and negative regulation of Th17 development by nuclear receptors.

Tables:

1. List of mammalian nuclear receptors and their known ligands. Ligands in brackets indicate atypical ligands that appear to be constitutively bound to their receptors based on structural studies. FXR β exists only as a pseudogene in humans. (adapted from (Evans & Mangelsdorf 2014))

Name	Common abbreviation	Unified nomenclature	Ligands
Classic endocrine receptors			
Androgen receptor	AR	NR3C4	androgens
Estrogen receptor α	ER α	NR3A1	estrogens
Estrogen receptor β	ER β	NR3A2	estrogens
Glucocorticoid receptor	GR	NR3C1	glucocorticoids
Mineralocorticoid receptor	MR	NR3C2	mineralocorticoids and glucocorticoids
Progesterone receptor	PR	NR3C3	progesterone
RXR heterodimer endocrine receptors			
Retinoic acid receptor α	RAR α	NR1B1	retinoic acids
Retinoic acid receptor β	RAR β	NR1B2	retinoic acids
Retinoic acid receptor γ	RAR γ	NR1B3	retinoic acids
Thyroid hormone receptor α	TR α	NR1A1	thyroid hormones
Thyroid hormone receptor β	TR β	NR1A2	thyroid hormones
Vitamin D receptor	VDR	NR1H1	1 α ,25-dihydroxyvitamin D ₃ and lithocholic acid
Adopted orphan receptors			
Constitutive androstane receptor	CAR	NR1H3	xenobiotics
Farnesoid X receptor α	FXR α	NR1H4	bile acids
Farnesoid X receptor β	FXR β	NR1H5	bile acids
Liver X receptor α	LXR α	NR1H3	oxysterols
Liver X receptor β	LXR β	NR1H2	oxysterols
Peroxisome proliferator-activated receptor α	PPAR α	NR1C1	fatty acids
Peroxisome proliferator-activated receptor β/δ	PPAR β/δ	NR1C2	fatty acids
Peroxisome proliferator-activated receptor γ	PPAR γ	NR1C3	fatty acids
Pregnane X receptor	PXR	NR1I2	endobiotics and xenobiotics
Retinoid X receptor α	RXR α	NR2B1	9-cis retinoic acid and docosahexanoic acid
Retinoid X receptor β	RXR β	NR2B2	9-cis retinoic acid and docosahexanoic acid

Retinoid X receptor γ	RXR γ	NR2B3	9-cis retinoic acid and docosahexanoic acid
Orphan receptors			
Chicken ovalbumin upstream promoter-transcription factor α	COUP-TF α	NR2F1	
Chicken ovalbumin upstream promoter-transcription factor β	COUP-TF β	NR2F2	
Chicken ovalbumin upstream promoter-transcription factor γ	COUP-TF γ	NR2F6	
Dosage-sensitive sex reversal-adrenal hypoplasia congenital critical region on the X chromosome, gene 1	DAX-1	NR0B1	
Estrogen related receptor α	ERR α	NR3B1	
Estrogen related receptor β	ERR β	NR3B2	
Estrogen related receptor γ	ERR γ	NR3B3	
Germ cell nuclear factor	GCNF	NR6A1	
Hepatocyte nuclear factor 4 α	HNF4 α	NR2A1	[fatty acids]
Hepatocyte nuclear factor 4 γ	HNF4 γ	NR2A2	[fatty acids]
Liver receptor homolog-1	LRH-1	NR5A2	[phospholipids]
Nerve-growth-factor-induced gene B	NGF1-B	NR4A1	
Neuron-derived orphan receptor 1	NOR-1	NR4A3	
Nur-related factor 1	NURR1	NR4A2	
Photoreceptor-cell-specific nuclear receptor	PNR	NR2E3	
RAR-related orphan receptor α	ROR α	NR1F1	[sterols]
RAR-related orphan receptor β	ROR β	NR1F2	[sterols]
RAR-related orphan receptor γ	ROR γ	NR1F3	[sterols]
Reverse-Erb α	REV-ERB α	NR1D1	[heme]
Reverse-Erb β	REV-ERB β	NR1D2	[heme]
Short heterodimeric partner	SHP	NR0B2	
Steroidogenic factor 1	SF-1	NR5A1	[phospholipids]
Tailless homolog orphan receptor	TLX	NR2E1	
Testicular orphan receptor 2	TR2	NR2C1	
Testicular orphan receptor 4	TR4	NR2C2	

Cross References

10001 IL-1 Family

10004 IL-6 Family

10012 IL-4/IL-13

10016 TGF β

04006 Transcriptional regulation of T-cell lineage development

04008 NKT cell development
04017 Natural Treg development (in thymus)
06017 CD1 (in mouse and man)
07003 Spleen structure
07006 Gut immune system: microanatomy
07015 Leukocyte adhesion molecules
11005 Cell Biology of T cell: APC interactions
11011 Signaling and phagocytosis
11017 Signaling pathways downstream of TLRs and IL-1 family receptors
15003 Systemic Lupus Erythematosus (SLE)
15012 Tolerance and autoimmunity

Figure 1. Domain structure of nuclear receptors

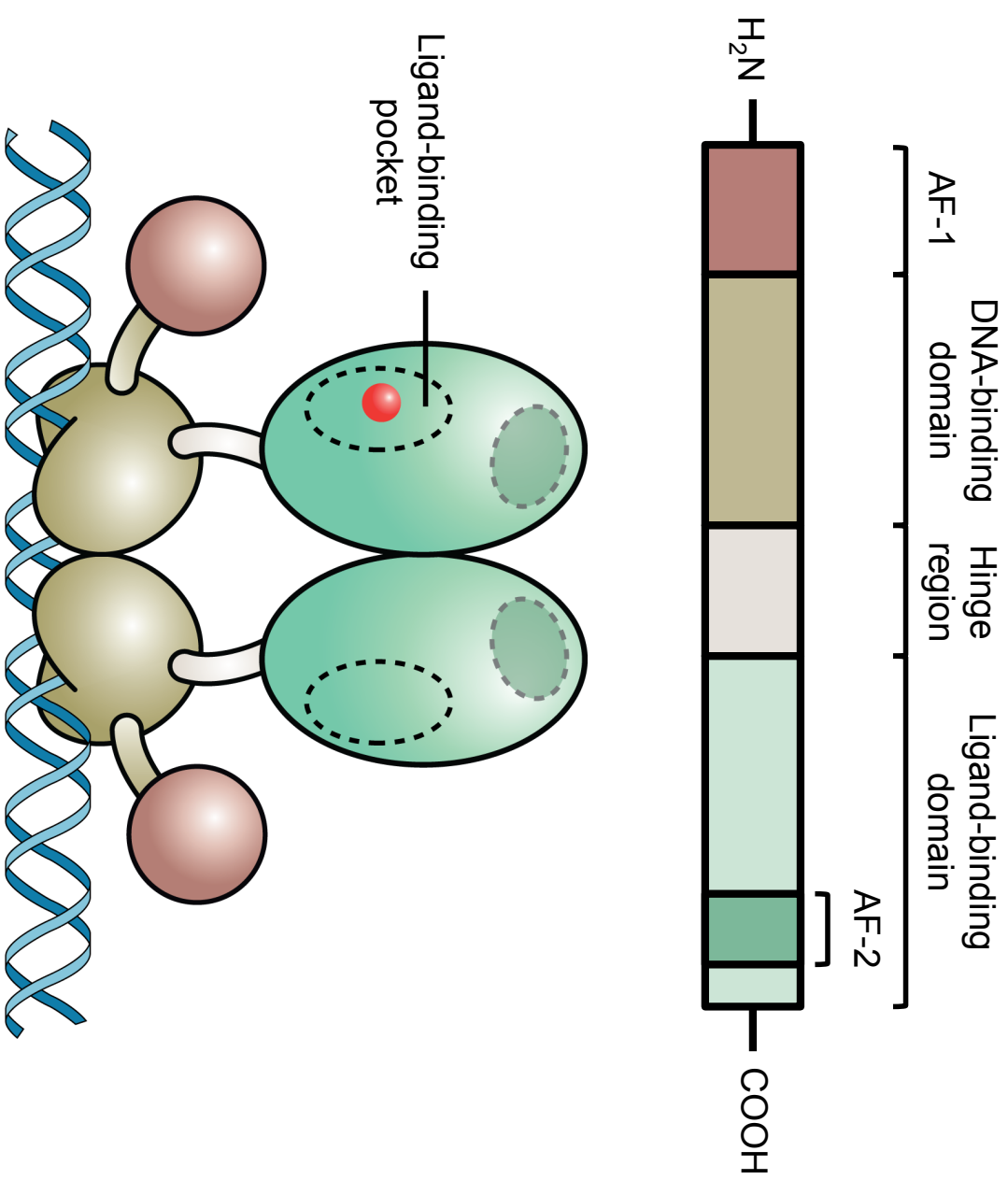


Figure 2. Distinct ways of transcriptional activation by different classes of nuclear receptors

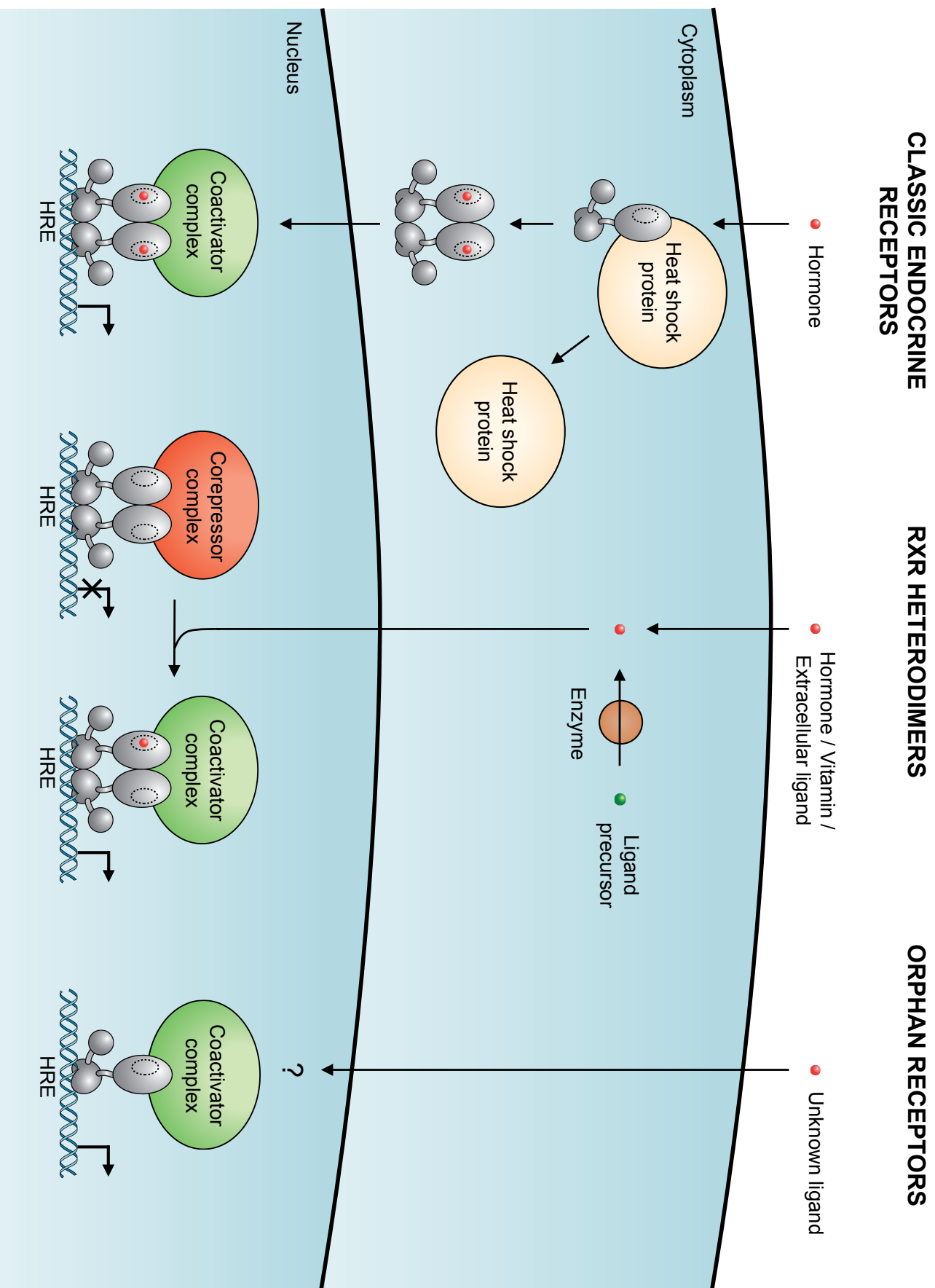


Figure 3. Mechanisms of transcriptional repression by nuclear receptors

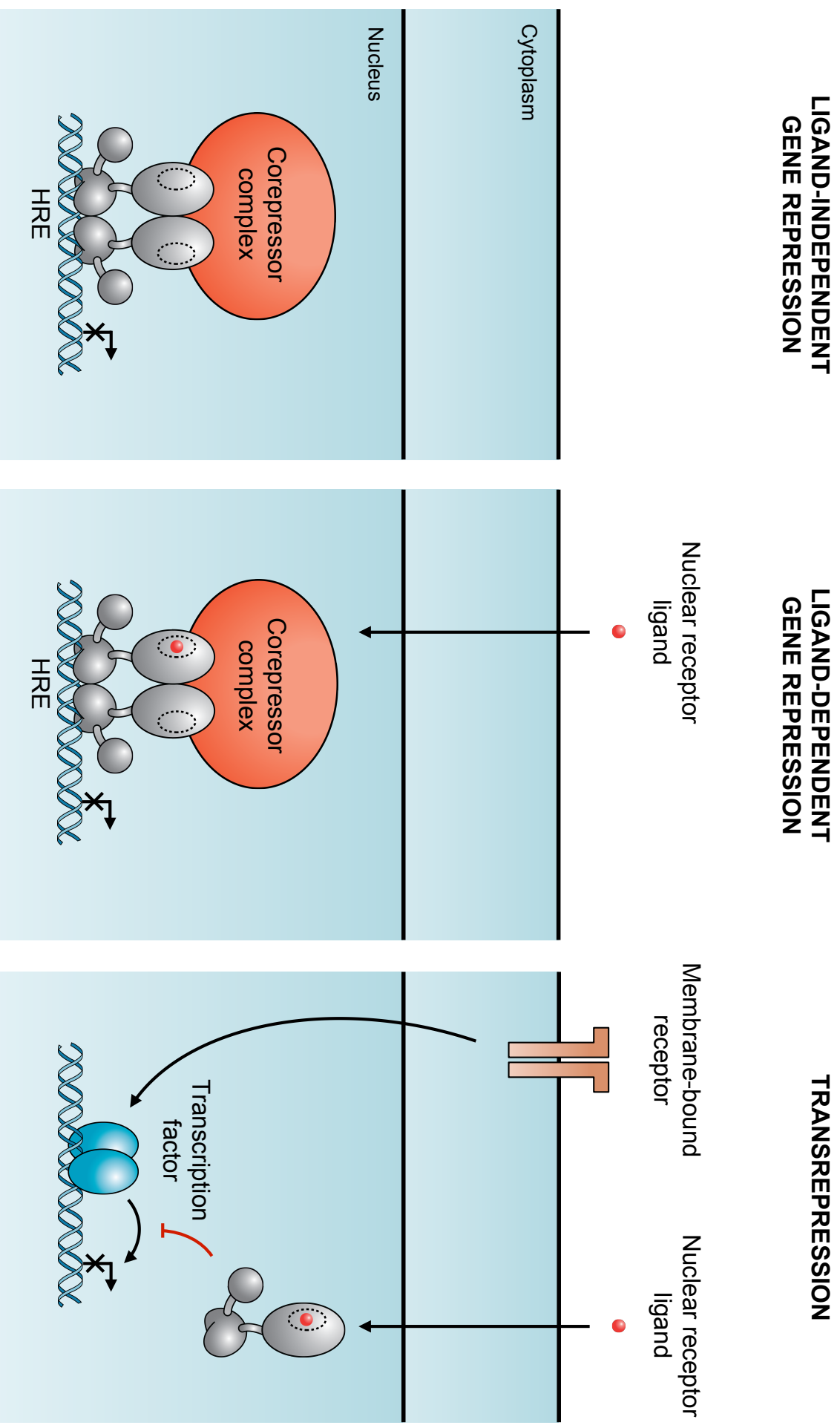


Figure 4. Effects of glucocorticoids on major immune cell populations contributing to inflammation

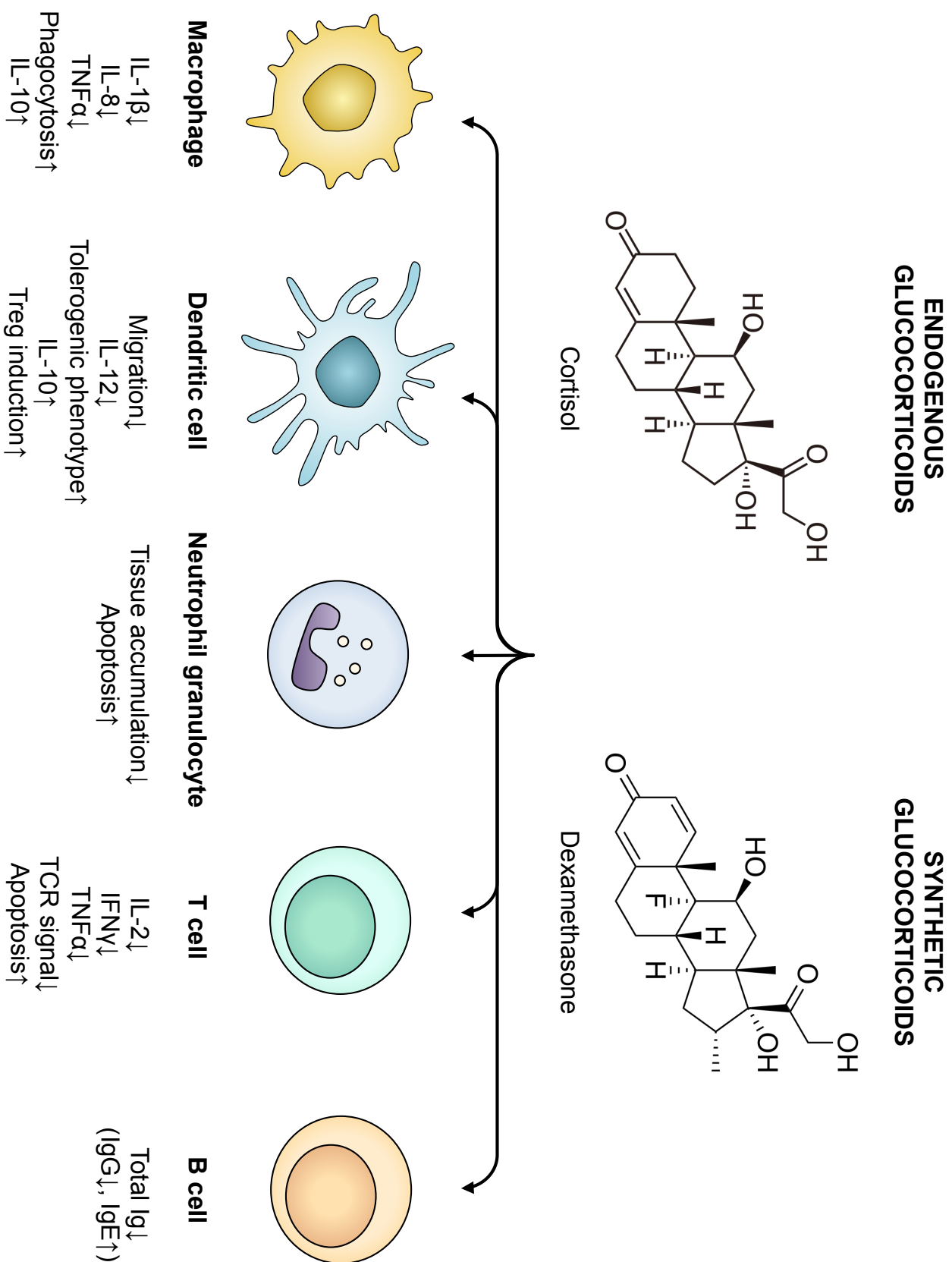


Figure 5. Regulation of macrophage cholesterol homeostasis by the cross-talk of nuclear receptors

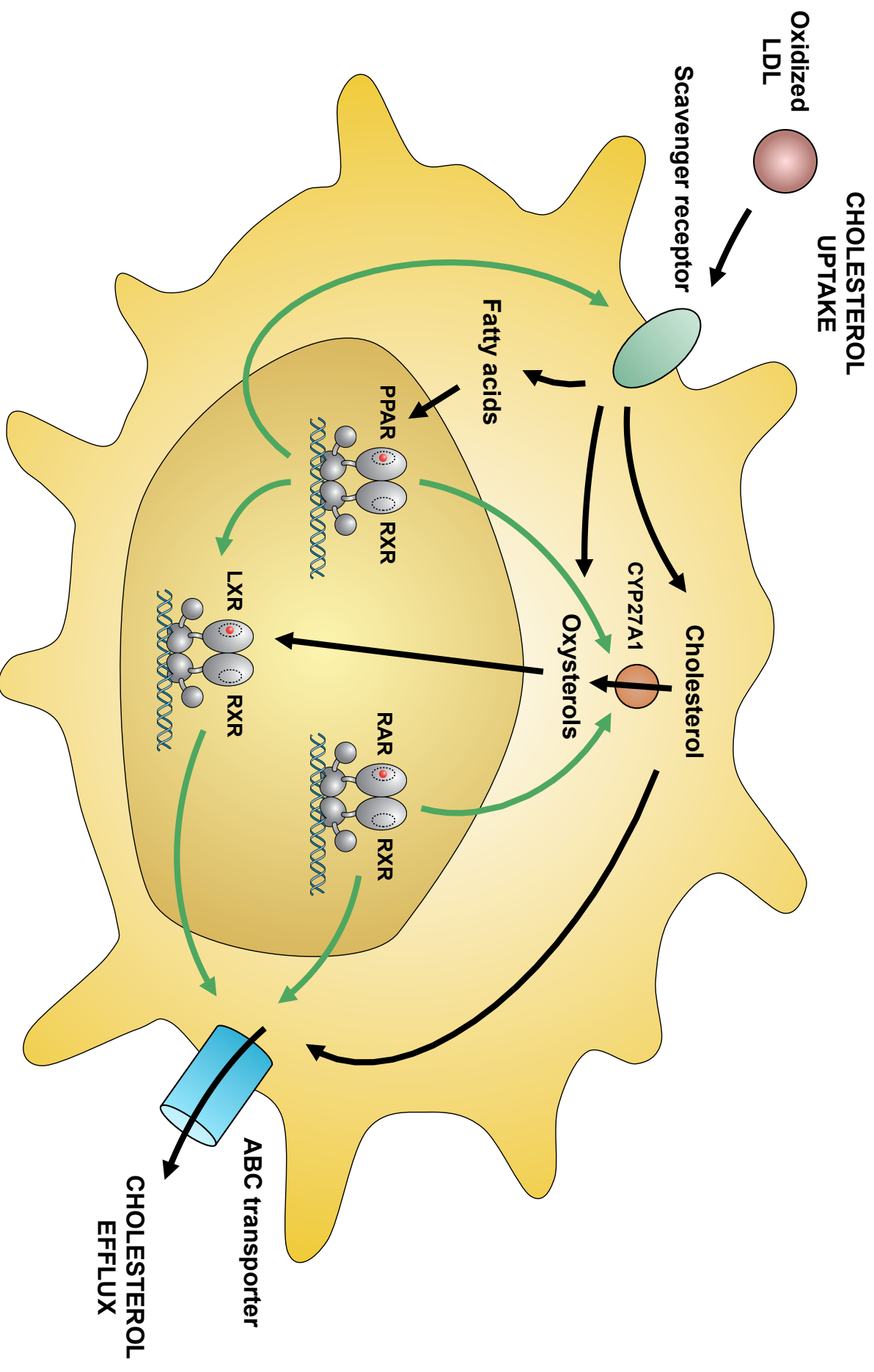


Figure 6. Positive and negative regulation of Th17 development by nuclear receptors

