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Nuclear Receptor Control of Myeloid Cell Responses - Implications for CNS Autoimmunity

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Abstract

During autoimmunity of the central nervous system (CNS), such as Multiple Sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE), myeloid cells play a central role in shaping the local inflammatory milieu and significantly contribute to the extent of disease pathology. Myeloid cells contribute to local reactivation of encephalitogenic T cells and, through production of pro-inflammatory mediators, also promote neurotoxicity and demyelination. In contrast, myeloid cells that acquired an anti-inflammatory phenotype ameliorate CNS autoimmunity and significantly contribute to resolution of inflammation. In this review we will discuss the role of selected nuclear receptors in modifying myeloid cell immune responses during autoimmune inflammation of the CNS and their potential as new targets for treatment regimens in MS.

Keywords: Nuclear receptor; Myeloid cell; Autoimmunity; Multiple sclerosis; Experimental autoimmune encephalomyelitis (EAE)

Role of Myeloid Cells in CNS Autoimmunity

During CNS autoimmunity, i.e. Multiple Sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE), $CD4^+$ T cells play a central role in the induction of autoimmune processes [1]. After priming in peripheral lymphoid organs, autoreactive $CD4^+$ T cells infiltrate the CNS [1], where they, by interacting with local antigen-presenting cells, induce a chemokine cascade [2] that leads to further recruitment of immune cells including inflammatory monocytes, which crucially shape the inflammatory milieu within the CNS during the effector phase [3].

Within the CNS, myeloid cells represent a prominent constituent of local inflammatory infiltrates. In acute lesions of MS patients, myeloid cells can outnumber lymphocytes by 10-20 times [4], indicating that myeloid cells strongly contribute to the local inflammatory milieu and the pathological outcome of the disease. Indeed, macrophages and microglial cells within MS lesions express neurotoxic mediators such as inducible nitric oxide synthase (iNOS) and tumor necrosis factor alpha (TNFα) [5-7] as well as surface molecules involved in (re-) activation of autoreactive T cells, such as MHC-II, CD40, CD80, and CD86 [8]. Interestingly, blood monocytes from MS patients produce also more IL-6 and IL-12 compared to monocytes derived from healthy controls [9]. The contribution of myeloid cells to the extent of CNS autoimmunity could be clearly demonstrated in the animal model EAE where clodronate liposome-mediated depletion of macrophages results in disease amelioration [10]. Furthermore, CCR2-expressing, $Ly6C^{hi}$ blood monocytes infiltrate the CNS during EAE [3,11,12] and antibody-mediated depletion of $CCR2^+Ly6C^{hi}$ inflammatory monocytes significantly ameliorates clinical signs of EAE [3]. After infiltration into the CNS, monocytes differentiate into $CD11b^+CD45^{hi}$ macrophages [13] and acquire a pro-inflammatory phenotype [11]. This phenotype is characterised by upregulation of pro-inflammatory gene transcription, including genes of the characteristic macrophage pro-inflammatory response, like iNOS or TNFα, but also genes involved in local reactivation of encephalitogenic T cells, such as MHC-II [11]. After entering into the CNS, myeloid cells locally interact in an antigen-dependent manner with autoreactive $CD4^+$ T cells. This results not only in re-activation of the T cell but also in antigen-dependent reciprocal activation of the myeloid cell, a process termed "T cell-mediated licensing". Then, licensed myeloid cells release TNFα and nitric oxide (NO), which promotes neurpdegeneration, and induce astrocytic CCL2 production, which promotes further inflammatory monocyte recruitment and perpetuation of disease [13]. This cascade of events is summarised in Figure 1A.

Importantly, myeloid cells can acquire different phenotypes in response to environmental factors [14]. The pro-inflammatory phenotype (also termed "M1") is induced by toll-like receptor (TLR)-mediated activation or the presence of IFNγ and is characterised by expression of iNOS. In contrast, IL-4 induces an anti-inflammatory phenotype (also termed "M2"), which promotes wound-healing and is characterised by expression of arginase 1 (Arg-1). Importantly, macrophages retain their plasticity and can change phenotypes gradually [14]. During EAE, the phenotype of macrophages within the CNS changes from a M1 to a M2 phenotype. While the number of $iNOS^+$ CNS macrophages peaks at early stages of EAE and declines thereafter, numbers of $Arg\text{-}1^+$ macrophages increase during peak and remission stages of EAE [15]. Furthermore, although most macrophages express either iNOS or Arg-1, some macrophages express both molecules [15]. Also in MS lesions it has been observed that macrophages and microglia express markers characteristic of the M1, M2, or a mixed M1/M2 phenotype [8,16]. This suggests that during CNS autoimmunity, myeloid cells exhibit a pro-inflammatory phenotype during the early phases of inflammation whereas the acquisition of an anti-inflammatory phenotype at later stages might represent a contribution to resolution of inflammation and promotion of tissue repair. Indeed, in EAE studies it could be shown that anti-inflammatory myeloid cells contribute to amelioration of CNS autoimmunity as transfer of IL-4- or IL-33-treated macrophages results in amelioration of EAE [17,18]. In accordance with this, promoting a shift from M1 to M2 phenotype in

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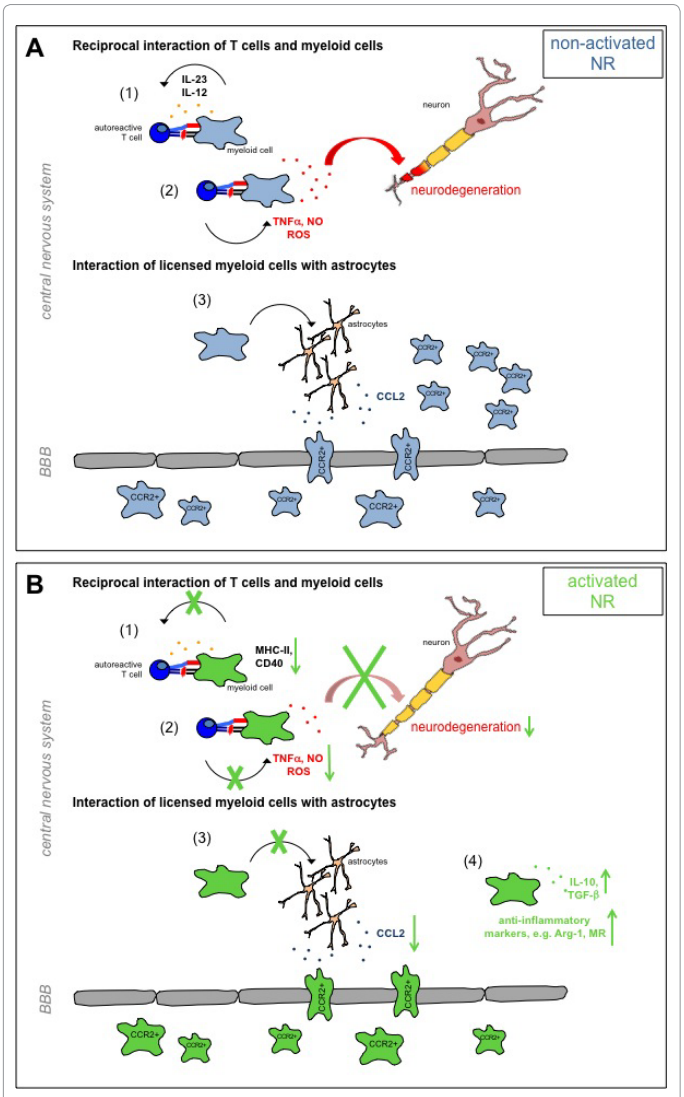


Figure 1: NR control of local myeloid cell responses during CNS autoimmunity. **(A)** (1) During CNS autoimmunity, myeloid cells interact with autoreactive T cells in an antigen-dependent manner, which results in local (re-)activation of T cells (2) but also in activation of myeloid cells ("licensing"). Licensed myeloid cells produce neurotoxic mediators that lead to neurodegeneration. (3) Furthermore, licensed myeloid cells induce CCL2 production by astrocytes, thereby promoting recruitment of further inflammatory monocytes from the blood stream and in consequence, perpetuation of disease. **(B)** Activation of NRs in myeloid cells results in interruption of this vicious circle at several steps: (1) Due to lower expression of MHC-II / CD40, reactivation of T cells is hampered. (2) NRs decrease the susceptibility towards T cell-mediated licensing, which results in reduced activation of myeloid cells and in consequence reduced neurodegeneration as well as (3) decreased astrocytic CCL2 production and hence, further monocyte recruitment. Moreover, some NRs were shown to induce an alternatively-activated phenotype, which contributes to resolution of local inflammation and potentially remyelination.

macrophages is beneficial for the disease course of EAE and results in amelioration of clinical signs as well as reduced demyelination within the CNS [19]. Furthermore, also CNS-resident microglial cells promote CNS remyelination if they have acquired an alternatively-activated status [20].

Hence, pro-inflammatory myeloid cells play a central role in establishment and maintenance of local inflammation during CNS autoimmunity whereas anti-inflammatory myeloid cells contribute to remission and restriction of inflammatory responses. Regulatory factors that dampen the pro-inflammatory while enhancing the anti-inflammatory phenotype of myeloid cells therefore represent an interesting therapeutic target for treatment of CNS autoimmunity.

Nuclear Receptors

Nuclear receptors (NRs) exhibit a broad spectrum of regulatory functions and are involved in almost all biological processes: metabolism, development, cell growth, and homeostasis but also in regulation of immune responses [21-23]. NRs belong to the superfamily of structurally conserved, ligand-dependent transcription factors, which are subdivided into three different classes: First, steroid and hormone receptors, for which ligands have been identified; examples are the glucocorticoid receptor (GR), vitamin D receptor (VDR), or the aryl hydrocarbon receptor (AHR). Second, orphan receptors for which no natural ligand is known or which might act ligand-independent, like the NR4A family members or tailless homolog. Third, the group of 'adopted' orphan receptors, which were originally identified as orphan receptors but for which ligands were identified afterwards; Peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), and retinoid X receptors (RXRs) belong to this group [24]. Structurally, all NRs contain three highly conserved domains: a N-terminal activation domain, a highly conserved DNA-binding domain (DBD), which is responsible for binding of the receptor to so-called response elements in the enhancer / promotor region of target genes, and a C-terminal ligand-binding domain (LBD), which plays an important role in NR function as the conformational change upon ligand-binding influences homo- and heterodimerisation, subcellular localisation, and transcriptional activation / repression of the NR [25]. Most NRs, e.g. PPARs, LXRs and VDR, form heterodimers with the retinoid X receptor (RXR) and bind to the DNA as heterodimers. RXRs can also bind to the DNA as homodimer or homotetramer [23,25,26]. NRs regulate gene expression by different mechanisms: ligand-dependent transactivation, ligand-independent transrepression, or ligand-dependent transrepression [25], which are described in detail in the review by Glass and Saijo [24].

In this review, we focus on selected members of the group of NRs which are known to regulate myeloid cell immune responses. Table 1 summarises their endogenous and synthetic ligands and gives examples for the modulation of myeloid cell responses *in vitro* and *in vivo* during CNS autoimmunity.

Influence of NRs on Pro-/anti-inflammatory Myeloid Cell Responses *In vitro*

Several NRs have been shown to negatively regulate pro-inflammatory immune responses mediated by human and murine myeloid cells, i.e. monocytes, macrophages, and CNS-resident microglial cells, which make these receptors interesting targets to limit excessive myeloid cell responses during autoimmune diseases such as MS.

Peroxisome proliferator-activated receptor gamma (PPARγ)

PPARs a, b/d, and γ play a role in regulation of lipid and glucose metabolism in various tissue cells. Agonists include a diverse range of endogenously produced fatty acids and synthetic ligands such as thiazolidinediones (TZD), which target PPARα and PPARγ, and are commonly used as insulin sensitizers in treatment of diabetes type II [26]. As PPARγ is best characterised for its function in regulation of myeloid cell responses, we focus on this PPAR family member. PPARγ is expressed by monocytes [27], macrophages [28], and microglial cells [29], and influences pro- as well as anti-inflammatory responses [22,26].

Nuclear receptor (abbreviation)	**Aryl hydrocarbon receptor (AHR)**	**Glucocorticoid receptor (GR)**	**Liver X receptors (LXRs)**	**Peroxisome proliferator activated receptors (PPARs)**	**Retinoid X receptors (RXRs)**	**Vitamin D receptor (VDR)**
Isoforms			**LXRα, LXRβ**	**PPARα, PPARβ/δ, PPARγ**	**RXRα, RXRβ, RXRγ**	
References	[100,101]	[44,102]	[24-26]	[24-26]	[52]	[26,52]
Systematic name			NR1H3, NR1H2	NR1C1, NR1C2, NR1C3	NR2B1, NR2B2, NR2B3	NR1I1
Binding partner (examples)	Aryl hydrocarbon nuclear translocator	GR	RXR	RXR	LXRs, PPARs, VDR, RXRs	RXR
General function	Proliferation, differentiation, cytokine production	Stress response	Cholesterol metabolism	Lipid and glucose metabolism, adipocyte differentiation	Binding partner of other NRs	Calcium metabolism, bone homeostasis
Endogenous ligands (examples)	2-(1'H-indolo-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester	Corticosterone	Oysterols e.g. 24S-hydroxycholesterol and 22R-hydroxycholesterol, intermediates of the biosynthetic cholesterol pathway	polyunsaturated and oxidised fatty acids, prostaglandin-derivates (e.g. 15d-PGJ2), components of oxLDL (e.g. linoleic acid metabolites 13-HODE and 15-HODE)	Retinoids, e.g. vitamin A derivate 9-cis retinoic acid, fatty acids e.g. docosahexaenoic acid, oleic acid and phytanic acid	$1,25(OH)_2D_3$
Synthetic ligands (examples)	polycyclic aromatic hydrocarbons, 2,3,7,8-Tetrachloro-dibenzo-p-dioxin, 3-methyl-cholanthrene	Dexamethasone, methylprednisolone	GW3965, T0901317	Thiazolidinediones, e.g. Pioglitazone and Rosiglitazone	Rexinoids	MC903
Modulation of myeloid cell responses (*in vitro*)	TLR-stimulation induces AHR expression [59]; *AHR activation*: oxidatitive burst ↑ [62] *AHR-deficiency*: pro-inflammatory cytokines ↑, IL-10 ↓ [59]; *AHR-overexpression* cytokine production ↓ [59,60]	TLR-stimulation increases GR expression [28] *GR activation*: pro-inflammatory cytokines ↑ (low dose GC) / ↓ (high dose GC) [45] susceptibility to apoptotic stimuli ↓ [49] anti-inflammatory properties ↑ [47,48]	*LXR activation*: metabolic genes ↑, pro-inflammatory genes ↓ [38]; pro-inflammatory cytokines and NO ↓ [38-40]; inhibition of NF-kB [40]; SUMOylation [41]; Arg-1 expression ↑ [42] *LXR deficiency*: pro-inflammatory genes ↑ [38]	*PPARγ-activation*: pro-inflammatory cytokines and NO ↓ [13,29,30]; repression of pro-inflammatory transcription factors [22]; SUMOylation of PPARγ [31]; alternative activation [33-36] *PPARγ-deficiency*: pro-inflammatory cytokines and NO ↑ [13]	*RXR activation*: pro-inflammatory cytokines and NO ↓ [40,53]; Arg-1 expression ↑ [35]; *RXR-deficiency*: impaired phagocytosis capacity [51]	*VDR activation*: pro-inflammatory cytokines and NO ↓ [55-57]; antigen-specific T cell stimulatory capacity ↓ (IL-10-dependent) [55]; induction of MAPK phosphatase-1 and reduced p38 phosphorylation [56]
Modulation of myeloid cell responses in CNS autoimmunity (*in vivo*)	*AHR activation* ameliorates EAE [64,97,98]; infiltrates ↓ [64,97]; cytokines in serum ↓ [97]; EAE aggravated in *AHR*^{d} mice [64]	*GR activation* ameliorates EAE in therapeutic setting [75, 76]; different outcomes in preventive treatment [75,76]; infiltrates ↓ [79]; MHC-II expression ↓ [78,79]	*LXR activation* ameliorates EAE [65]; infiltrates ↓, activation status ↓ [65]; myelin-ingestion induces LXR expression and reduces pro-inflammatory cytokine production [72] *LXR deficiency* aggravates EAE [65]	*PPARγ-activation* ameliorates EAE [13,63,67,83]; activation status of CNS myeloid cells ↓ [13] *myeloid-cell-specific PPARγ-deficiency* aggravates EAE during effector phase [13]; increased activation status of CNS myeloid cells [13]	RXR is expressed by myeloid cells in active MS lesions [85] *RXR-activation* ameliorates EAE [83]	VDR is expressed in myeloid cells in MS lesions [86] *VDR activation* ameliorates EAE [87-90] dependent on VDR [87,88] and IL-10 [89]; IL-4 and TGFβ in CNS and lymph nodes ↑ [92]; MHC-II and iNOS expression in CNS ↓ [90]

Table 1: Features of the NRs in the focus of this review.

Lack of PPARγ results in enhanced production of pro-inflammatory mediators, such as NO and cytokines, e.g. TNFα, upon LPS stimulation [13], which indicates that PPARγ mediates anti-inflammatory effects and restricts myeloid cell immune responses. In contrast, activation of PPARγ in myeloid cell restricts iNOS expression and NO production as well as secretion of pro-inflammatory cytokines, including TNFα and IL-6, in response to LPS-treatment [13,29,30] further underlining the anti-inflammatory role of this NR in macrophages.

PPARγ-mediated inhibition of myeloid cell activation results from repression of pro-inflammatory transcription factors like nuclear factor-κB (NF-κB), signal transducer and activator of transcription (Stat), or activator protein-1 (AP-1) [22]. Furthermore, upon ligand binding, PPARγ undergoes SUMOylation by PIAS1 and SUMO1 which allows PPARγ to interact with and bind to co-repressor complexes formed by NCoR and SMRT (nuclear receptor corepressor and silencing mediator for retinoid and thyroid hormone receptors, respectively) that silence pro-inflammatory genes in the absence of stimulation. Binding of SUMOylated PPARγ inhibits LPS-induced removal of the NCoR/SMRT complex from the promotor of pro-inflammatory genes, which then remain in a repressed state – even in the presence of pro-inflammatory signals [31].

With regard to therapeutic interventions, inhibition of pro-inflammatory responses by myeloid cells is desirable in the context of CNS autoimmunity, where local myeloid cell responses promote ongoing tissue-destruction. However, additional induction of anti-inflammatory properties of myeloid cells would further contribute to resolution of inflammation and tissue-repair. Interestingly, treatment of macrophages with IL-4, the classical inducer of alternative

activation in macrophages [32], induces expression of the enzyme 12/15-lipoxygenase (12/15-LOX), which generates endogenous PPARγ ligands from linoleic and arachidonic acids, and also PPARγ [33], which suggests that PPARγ might be involved in promoting an M2 phenotype in macrophages. Indeed, several studies demonstrate a role for PPARγ in alternative activation of macrophages: PPARγ physically interacts with Stat6, the transcription factor responsible for M2 polarisation, to bind to DNA [34], PPARγ agonists induce expression of Arg-1 [35], and PPARγ-deficiency impairs the capacity of macrophages to acquire an anti-inflammatory phenotype [36]. In line with this, in human monocytes PPARγ activation also induces Arg-1 [13] and mannose receptor [37] expression. Moreover, supernatants of PPARγ agonist-treated monocytes inhibit LPS-induced TNFα production of M1 macrophages [37] suggesting that upregulation of M2 markers also correlates to suppressive function.

Taken together, PPARγ activation in myeloid cells restricts pro-inflammatory responses and concomitantly promotes the acquisition of an anti-inflammatory phenotype (Figure 2).

Liver X receptors (LXRs)

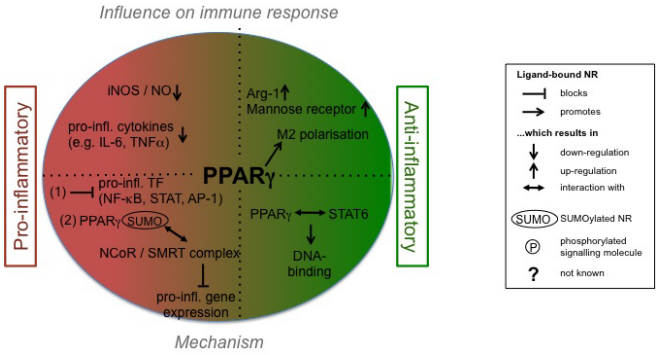
LXRs, i.e. the tissue-specific LXRα and the broadly-expressed LXRβ, play a central role in the regulation of cholesterol metabolism: LXRs are activated in response to increased levels of cholesterol, leading to activation of cholesterol efflux pathways and restoration of homeostatic cellular cholesterol levels. Ligands of LXRs include endogenous as well as synthetic agonists [25] (Table 1). In LPS-activated macrophages, LXRs reciprocally regulate genes of lipid metabolism and the innate immune response, i.e. metabolic genes are induced, whereas pro-inflammatory gene expression is reduced [38]. Treatment of macrophages with synthetic LXR agonists results in reduced expression of several pro-inflammatory mediators, such as iNOS, IL-6, IL-1b, and other pro-inflammatory cytokines, as well as chemokines, such as CCL2. In contrast, lack of LXRs – either LXRα, LXRβ, or both – enhances pro-inflammatory responses to LPS [38]. Similarly, LXR activation in monocytes [39] and microglial cells [40] limits production of pro-inflammatory cytokines and NO in response to LPS stimulation. Several mechanisms have been proposed by which LXRs regulate innate immune responses: activation of LXR has been shown to inhibit NF-κB DNA binding activity after LPS stimulation [40]. Furthermore, ligand-binding results in SUMOylation by HDAC4 E3 ligase and SUMO2/3. SUMOylated LXR binds to and stabilises repressor complexes in the promotor regions of pro-inflammatory genes, which inhibits LPS-induced gene transcription [41]. Interestingly, besides limiting pro-inflammatory responses by myeloid cells, LXRα activation induces Arg-1 expression in macrophages by promoting the interaction of the transcription factors PU.1 and interferon regulatory factor 8 (IRF8) and their binding to the Arg-1 promotor [42]. In addition, LXRα activation enhances IL-4 induced Arg-1 expression [42] showing that LXR can act in concert with IL-4 to enhance anti-inflammatory responses.

Thus, similar to PPARγ, LXRs regulate immune responses of myeloid cells by restricting pro-inflammatory responses and inducing / enhancing alternative activation (Figure 3).

Glucocorticoid receptor (GR)

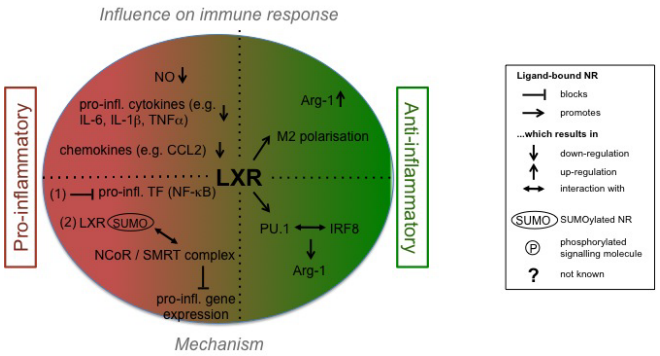
The glucocorticoid receptor (GR) is ubiquitously expressed and binds glucocorticoids (GCs), which are produced as part of the stress response [43] but also synthetic derivates, which are in use for treatment of acute inflammatory bouts, such as in MS and rheumatoid arthritis [44] (Table 1). GCs can either bind to membrane-bound GR or interact with GR in the cytosol after passing the cell membrane. Interaction within the cytosol results in release of the GR from a heat-shock protein complex and allows the receptor to interact with signaling molecules in the cytosol or to translocate into the nucleus, where it modulates gene transcription by either direct DNA-binding as a homodimer or by repressing the activity of pro-inflammatory transcription factors, such as NF-κB or AP-1 [44].

Macrophages express the GR and its expression is further increased after macrophage activation [28]. Immune responses by macrophages can be modulated through the addition of GCs, however, the outcome depends on the administered GC concentration [45]. Macrophages treated with low doses of GCs express increased levels of iNOS, pro-inflammatory cytokines, and chemokines in a GR-dependent manner [45], whereas addition of high GC doses limits pro-inflammatory gene expression [45,46]. Interestingly, GR-mediated repression of pro-inflammatory genes occurs at distinct steps of the transcription cycle [46]. In addition, GC-treatment induces an anti-inflammatory phenotype in human and murine monocytes [47-49], which is characterised by distinct gene regulation [47], higher migratory capacity, and induction of IL-10 production [47,48]. Furthermore, GC-treated monocytes exhibit decreased susceptibility to apoptotic



Activation of PPARγ by its ligands results in modulation of the myeloid cell immune response by restriction of pro-inflammatory and promotion of anti-inflammatory responses. For some of these effects, mechansims have been identified

Figure 2: Schematic overview of PPARγ-mediated modulation of myeloid cell responses *in vitro*.



Activation of LXR by its ligands results in modulation of the myeloid cell immune response by restriction of pro-inflammatory and promotion of anti-inflammatory responses. For some of these effects, mechansims have been identified

Figure 3: Schematic overview of LXR-mediated modulation of myeloid cell responses *in vitro*.

stimuli, which is mediated by upregulation of A3 adenosine receptor, subsequent activation of extracellular signal regulated kinase (ERK)1/2, and finally inhibition of caspase activity [49]. Decreased susceptibility towards apoptosis stimuli promotes survival of anti-inflammatory myeloid cells within the inflamed tissue, which allows more efficient down-regulation of inflammation [49]. Interestingly, treatment of macrophages with GCs enhances their capacity to phagocytose apoptotic cells [50], which plays an important role in resolution of inflammation and has been associated with the aquisition of an anti-inflammatory phenotype in macrophages [51]. Interestingly, there are hints that *in vivo* macrophage responses are kept in check by endogenous GCs as macrophages isolated from adrenalectomised rats, which have reduced levels of endogenous GCs, produce elevated amounts of NO and TNFα even in the absence of stimulation [45].

Hence, GC-mediated GR activation modulates myeloid cell immune responses in different ways. In contrast to other NR ligands, GCs do not inhibit myeloid cell pro-inflammatory responses *per se* but the inhibition of pro-inflammatory responses depends on the GC dosis. Importantly, GCs promote anti-inflammatory properties of myeloid cells, which contribute to resolution of inflammation (Figure 4).

Retinoid X receptors (RXR)

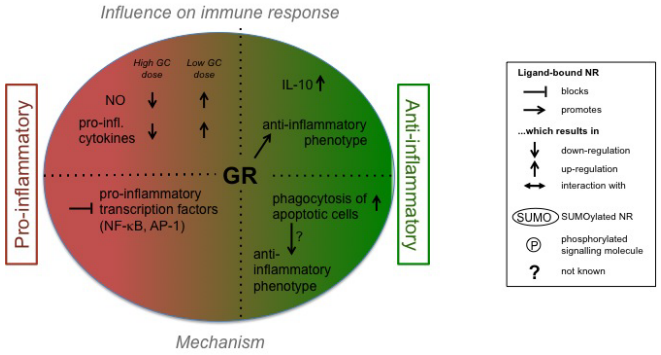
The retinoid X receptors (RXR) play a central role in NR-mediated immune regulation as they are heterodimeric binding partners of a range of NRs. These heterodimers are specified into either permissive heterodimers, which can be activated by agonists for RXR and for its NR partner (e.g. RXR-PPAR or RXR-LXR), or nonpermissive heterodimers, which are only activated by ligands of the selective RXR binding partner (e.g. RXR-VDR) [26,52]. In addition, *in vitro* RXRs also regulate gene expression as homodimers or –tetramers, although the relevance still has to be confirmed *in vivo*. RXRs are activated by several agonists, such as the vitamin A derivative 9-cis retinoic acid (9-cis RA) [26] (Table 1).

So far, only few studies have investigated the influence of RXR itself on innate immune responses mediated by myeloid cells. Microglial treatment with the RXR agonist 9-cis RA restricts LPS-induced production of NO [40,53] and pro-inflammatory cytokine production TNFα, IL-1β, and IL-12p40 in a dose-dependent manner [53]. Such suppression is further enhanced when microglia are additionally treated with a LXR agonist [40] indicating that ligand-binding to both partners of a RXR-LXR heterodimer further increases the potential to inhibit pro-inflammatory responses. RXRα plays an important role in phagocytosis of apoptotic cells by macrophages as RXRα-deficient macrophages display deficits in phagocytosis whereas the addition of RXR agonists enhances phagocytosis [51]. Phagocytosis of apoptotic cells induces an anti-inflammatory response in macrophages that is characterised by upregulation of anti-inflammatory mediators, i.e. IL-10 and transforming growth factor beta (TGF-β), as well as downregulation of pro-inflammatory mediators such as iNOS and IL-1β [51]. As RXRα-deficient macrophages were impaired in their capacity to take up apoptotic cells, an anti-inflammatory phenotype was not induced in these cells [51]. This suggests that during clearance of tissue damage, RXR activation in myeloid cells promotes uptake of apoptotic cell bodies and induces an anti-inflammatory phenotype which contributes to resolution of inflammation. Importantly, RXR activation polarises macrophages towards an alternatively-activated phenotype. 9-cis RA induces Arg-1 expression in macrophages and furthermore, additional activation of a PPARγ agonist further enhances Arg-1 expression [35].

Thus, immune responses by myeloid cells can be modulated by RXR-activation and the combination of agonists targeting both partners of a permissive RXR heterodimer further enhances RXR-mediated modulation (Figure 5).

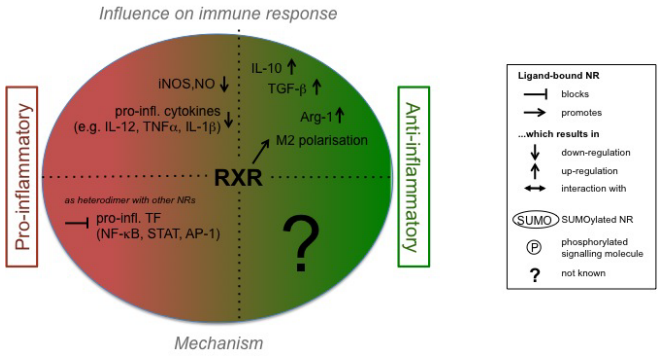
Vitamin D receptor (VDR)

The vitamin D receptor (VDR) binds the active vitamin D metabolite 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and is best characterised for its role in regulation of bone health [54] (Table 1). Macrophages constitutively express the VDR and the addition of its ligand $1,25(OH)_2D_3$ further increases VDR gene expression [55]. Importantly, the VDR plays a role in regulation of immune responses by macrophages as $1,25(OH)_2D_3$-treated macrophages produce less pro-inflammatory cytokines, e.g. TNFα and IL-12p40, and express reduced levels of iNOS upon LPS stimulation. Furthermore, VDR activation in macrophages reduces their capacity to antigen-specifically activate T cells [55]. Interestingly, the modulatory effects are mediated - at least in parts - by IL-10 as effects of $1,25(OH)_2D_3$-treatment in macrophages are (partially) abrogated in IL-10-deficient macrophages [56]. In line with this, also $1,25(OH)_2D_3$-treated, LPS-stimulated human $CD14^+$ monocytes produce reduced amounts of pro-inflammatory cytokines



Activation of GR by its ligands results in modulation of the myeloid cell immune response by restriction of pro-inflammatory and promotion of anti-inflammatory responses. For some of these effects, mechansims have been identified / proposed

Figure 4: Schematic overview of GR-mediated modulation of myeloid cell responses *in vitro*.



Activation of RXR by its ligands results in modulation of the myeloid cell immune response by restriction of pro-inflammatory and promotion of anti-inflammatory responses. For some of these effects, mechansims have been identified / proposed

Figure 5: Schematic overview of RXR-mediated modulation of myeloid cell responses *in vitro*.

[56]. It was revealed that VDR activation results in modulation of the signalling pathway induced after LPS-binding to TLR4 in human and murine myeloid cells, i.e. $1,25(OH)_2D_3$-treatment induces expression of MAPK phosphatase-1 (MKP-1) in myeloid cells by binding of the activated VDR to a response element in the MKP-1 promotor [56]. In consequence, MKP-1 inhibits LPS-induced phosphorylation of p38, which, in turn, prevents induction of pro-inflammatory cytokine expression [56]. Similarly, also microglia express the VDR and are susceptible to $1,25(OH)_2D_3$-mediated restriction of inflammatory responses [57].

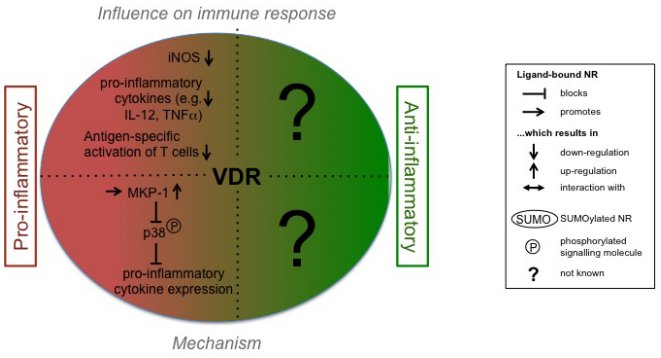
Taken together, it has been shown that the VDR restricts immune responses of activated myeloid cells. However, despite a role for IL-10 in mediating anti-inflammatory effects, so far no studies have been conducted to unravel the potential of VDR to polarise myeloid cells towards an anti-inflammatory M2 phenotype (Figure 6).

Aryl hydrocarbon receptor (AHR)

The AHR is a transcription factor that binds a broad range of ligands and is abundantly expressed by most immune cells including macrophages [58] (Table 1). Upon stimulation with TLR-ligands, macrophages upregulate AHR expression [59] which suggests that AHR might be induced after TLR-stimulation to limit excessive pro-inflammatory responses by myeloid cells. Indeed, the anti-inflammatory role of AHR in macrophages has been demonstrated: overexpression of AHR in the macrophage-like cell line RAW restricts pro-inflammatory cytokine production, i.e. TNFα, IL-6, and IL-12, upon LPS-stimulation, whereas LPS-stimulated AHR-deficient macrophages produce enhanced amounts of cytokines [59]. A different study showed that AHR activation in macrophages inhibits upregulation of histidine decarboxylase and hence histamine production, which is known to enhance immune responses. In consequence, IL-6 production is reduced [60]. Both studies further unraveled the underlying mechanism of AHR-mediated suppression of IL-6 production and described two independent mechanisms. First, ligand-bound AHR interacts with Stat1 to inhibit NF-κB and prevent NF-κB-mediated induction of IL-6 transcription [59]. Second, AHR interacts with the transcription factor Sp1 that is activated following LPS-stimulation. AHR-Sp1 interaction inhibits LPS-induced phosphorylation of Sp1, which reduces Sp1-binding to the promotor region of the histidine decarboxylase gene and, in consequence, results in reduced histamine production and IL-6 [60]. In line with this, AHR activation by resveratrol (*trans*-3,5,4`-Trihydroxystilbene) inhibits NO and TNFα production by LPS-activated microglia [61]. In addition, activation of AHR in macrophages might mediate anti-inflammatory functions in the LPS-TLR4 signaling pathway by promoting IL-10 production as AHR-deficient macrophages produce reduced levels of IL-10 after LPS-stimulation when compared to wild type macrophages [59].

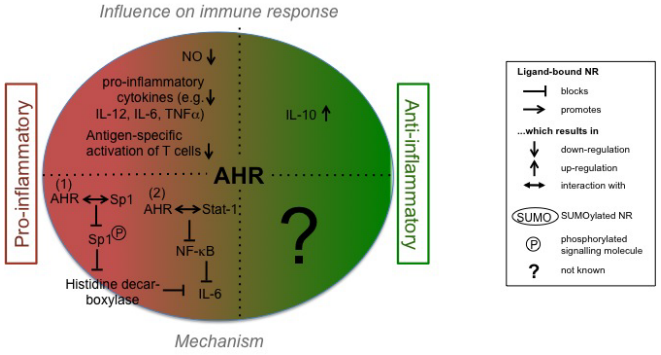
In contrast to the limiting function regarding pro-inflammatory cytokine production, treatment of human blood-derived monocytes with the AHR agonist benzo(α)pyrene enhances expression of a NADPH-oxidase complex subunit and induces its localisation to the plasma membrane, which is a prerequisite for the final assembly of the NADPH-oxidase complex. However, without additional stimulation, increased expression of the NADPH-oxidase complex subunit does not correlate to changes in oxidative burst, whereas additional stimulation by PMA-treatment induces an increased oxidative burst in benzo(α) pyrene-treated compared to untreated monocytes [62].

Thus, although AHR activation in myeloid cells limits pro-inflammatory cytokine production, it supports the oxidative burst of



Activation of VDR by its ligands results in modulation of the myeloid cell immune response by restriction of pro-inflammatory for which mechanisms have been identified / proposed. Whether the VDR also promotes anti-inflammatory properties of myeloid cells has to be investigated

Figure 6: Schematic overview of VDR-mediated modulation of myeloid cell responses *in vitro*.



Activation of AHR by its ligands results in modulation of the myeloid cell immune response by restriction of pro-inflammatory immune responses for which mechanisms have been identified / proposed. How AHR promotes anti-inflammatory properties of myeloid cells remains to be investigated

Figure 7: Schematic overview of AHR-mediated modulation of myeloid cell responses *in vitro*.

monocytes (Figure 7).

Taken together, all introduced NRs exhibit myeloid cell modulatory function which is most often characterised by restriction of pro-inflammatory responses. Additionally, some NRs promote the polarisation towards an anti-inflammatory phenotype which is important with regard to resolution of local inflammation in CNS autoimmunity.

NR control of myeloid cell responses influence CNS autoimmunity

In the last part of this review we will focus on NR control of myeloid cell responses during CNS autoimmunity. Myeloid cells play a central role in promoting and maintaining local inflammation and contribute to perpetuation of disease [3,11,13]. However, if alternatively activated, they also contribute to resolution of inflammation and induction of remyelination, which, in consequence, ameliorates clinical signs [17,20]. With regard to treatment of CNS autoimmunity, NRs in myeloid cells represent an interesting therapeutic target as their activation often limits pro-inflammatory and concomitantly increases anti-inflammatory properties of myeloid cells, which would not only

contribute to amelioration of local inflammation but also promote CNS repair mechanisms. Importantly, with regard to potential treatment of MS patients, aiming at modulating myeloid cell responses has the advantage that such treatment regimens also prove to be effective after disease onset and do not depend on antigen-specificity. Many studies investigated the influence of NR activation on CNS autoimmunity with a special focus on T_H17 responses [63-65] whereas only few studies addressed the role of NR activation in myeloid cells. Here we summarise findings that underline the potential of NRs as pharmaceutical targets to control and modulate myeloid cell responses in CNS autoimmunity.

Peroxisome proliferator-activated receptor gamma (PPARγ)

The role of PPARγ in myeloid cells during CNS autoimmunity was unraveled in the context of EAE. Mice heterozygous for PPARγ-deficiency show an exacerbated EAE disease course [66], whereas daily administration of synthetic PPARγ-agonists results in amelioration of disease during the initiation as well as the effector phase [63,67]. By employing a conditional knockout model, i.e. LysM-PPARγ^{KO} mice, it was shown that PPARγ-deficiency in myeloid cells aggravates EAE specifically during the effector phase, whereas the T cell-mediated-initiation phase was not altered when compared to wildtype mice [13]. Furthermore, it was revealed that inflammatory CCR2^{+}Ly6C^{hi} monocytes represent the central myeloid cell population, which is regulated by PPARγ [13]. Within the CNS, myeloid cells are activated upon antigen-specific interaction with autoreactive T cells, which induces NO and TNFα production in myeloid cells. In this process of "T cell-mediated licensing", PPARγ plays a central role in restricting the reciprocal activation of macrophages as PPARγ-deficient macrophages exhibited increased activation, whereas PPARγ-activated macrophages showed reduced activation upon antigen-specific interaction with activated T cells [13].

The finding that the effector phase of EAE is aggravated due to PPARγ-deficiency in myeloid cells suggests that in the wildtype situation endogenous ligands bind to PPARγ and keep it constantly activated. This basal level of activation in myeloid cells contributes to restriction of local inflammation during CNS autoimmunity. In line with this, mice deficient in 12/15-LOX, the enzyme that produces endogenous PPARγ ligands, exhibit an aggravated EAE disease course, which shows the important contribution of endogenous PPARγ ligands to limit CNS autoimmunity [68]. Importantly, the administration of conjugated linoleic acid (CLA), a substrate for 12/15-LOX and precursor of PPARγ ligands, ameliorates autoimmunity as demonstrated in animal models of colitis. CLA-mediated amelioration was due to up-regulation of PPARγ and a reduction of NF-κB-mediated inflammation [69,70]. It will be interesting to investigate whether the administration of conjugated linoleic acid will also prove beneficial for treatment of CNS autoimmunity and how this affects myeloid cell responses.

Inflammatory stimulation of human peripheral blood mononuclear cells (PBMCs), which include also monocytes, leads to down-regulation of PPARγ expression [71]. Interestingly, PBMCs derived from MS patients exhibit reduced PPARγ expression levels compared to PBMCs from healthy donors and PPARγ expression inversely correlates to disease activity. Importantly, despite reduced levels of PPARγ, PBMCs from MS patients not undergoing an acute relapse were still responsive to PPARγ-mediated restriction of pro-inflammatory cytokine production, such as TNFα [71] suggesting that PPARγ activation could indeed interfere with excessive myeloid cell immune responses during MS. However, in PBMCs derived from patients with an acute relapse, PPARγ-activation does not prevent pro-inflammatory cytokine production, probably due to pre-activation of these PBMCs *in vivo* [71].

Importantly, also *in vivo*, PPARγ activation by administration of an agonist induces M2 polarisation of monocytes. Myeloid cell populations isolated from spleen and blood of mice treated with the PPARγ agonist pioglitazone expressed higher levels of M2 markers including Arg-1 [13] and furthermore, monocytes derived from rosiglitazone-treated patients expressed higher levels of diverse M2 markers than monocytes isolated from non-treated controls [37].

Liver X receptors (LXRs)

LXRs limit the extent of CNS autoimmunity as LXR^{KO} mice exhibit an aggravated EAE disease course during the initiation as well as during the effector phase and exhibit more severe demyelination and larger immune cell infiltrates in the spinal cord [65]. Conversely, administration of LXR agonists ameliorates EAE in a LXR-mediated fashion. This is accompanied by reduced myeloid cell MHC-II expression in the CNS [65], which indicates that LXR activation reduces the activation status of myeloid cells.

During ongoing CNS autoimmunity, macrophages and microglia phagocytose myelin that is abundantly present within active MS lesions. Interestingly, it was observed that macrophages upregulate LXRβ in response to myelin-ingestion and that this upregulation results in reduced LPS-induced IL-6 production [72]. In line with this, myelin ingestion by human macrophages results in inhibition of diverse pro-inflammatory cytokines, i.e. IL-12p35 IL-12 subunit, IL-12p40 (IL-12 and IL-23 subunit), and TNFα [16]. Furthermore, myelin-containing CNS macrophages in the centre of MS lesions express markers associated with the M2 phenotype: CD163, mannose receptor, IL-1 receptor α, and CCL18 whereas pro-inflammatory cytokine expression is not detected [16]. Thus, myelin ingestion induces upregulation of LXR in myeloid cells [72] which might contribute to the observed restriction of pro-inflammatory cytokine responses and induction of M2 phenotype in myeloid cells present in MS lesions [16].

In contrast to PPARγ, LXRβ mRNA levels are increased in PBMCs derived from MS patients when compared to PBMCs from healthy controls [73]. One explanation might be the low levels of the LXRβ agonist 24S-hydroxycolesterol present in the sera of MS patients [73]. However, higher LXRβ expression could also represent a counter-regulatory mechanism to limit excessive autoimmune responses in a cell-intrinsic fashion. Interestingly, LXR activation in human monocytes leads to upregulation of specific surface markers, i.e. CD82, CD226, and CD244 *in vitro*, which is easily detectable by flow-cytometry [74]. If this can be proven in the *in vivo* situation, it would be possible to monitor the efficacy of LXR activation in human monocytes by an administered agonist, as LXR-activation in monocytes can be identified by expression of the above mentioned surface markers.

Glucocorticoid receptor (GR)

Application of high-dose GCs is a prominent therapeutic approach in treatment of patients with relapsing-remitting MS undergoing an acute relapse. GC-mediated amelioration of disease is mediated by several mechanisms including limitation of pro-inflammatory and enhancement of anti-inflammatory activities of myeloid cells [44]. In EAE, treatment with dexamethasone ameliorates clinical signs if administered before (preventive setting) or after onset of disease (therapeutic setting) depending on sufficient GR expression levels [75]. In line with this, therapeutic treatment with methylprednisolone (MP) also ameliorates EAE [76]. In contrast to dexamthasone-treatement however, preventive treatment with MP exacerbates EAE [76] indicating that GC-administration does not generally prove beneficial but that timing of therapy as well as the choice of the right GC might be important.

GC-treatment during EAE primarily targets T cells, which was demonstrated by comparing GC-treatment efficiency in mice heterozygous for GR-deficiency in T cells or myeloid cells ($GR^{LysMCre}$ mice) [75]. Interestingly, the clinical score of $GR^{LysMCre}$ mice was slightly aggravated at the beginning of the effector phase (d14 – d17 post immunisation) when compared to wildtype controls, while the induction phase was not altered [75]. These data suggest that endogenously-produced GCs could bind to myeloid cell GR and restrict myeloid cell responses during EAE. Further investigation needs to be carried out to confirm this hypothesis, which would necessarily include the assessment of the clinical score longer than 17 days after immunisation, when the effector phase is just at its beginning.

In line with this, corticosterone, an endogenous GC, is involved in susceptibility and severity of CNS autoimmunity [77]. After induction of EAE, corticosterone levels rise and peak at the maximum of disease, when animals start to recover. Subsequently corticosterone levels decline with resolution of inflammation. Furthermore, adrenalectomised animals, which cannot produce GCs, show earlier signs of EAE and exhibit a strongly aggravated disease [77].

Importantly, GCs can cross the blood-brain barrier to act on myeloid cells present in the CNS [43] as shown in different models of CNS inflammation [43,78,79]. GC-treatment limits upregulation of MHC-II on CNS-infiltrating macrophages during EAE [79] and on microglial cells following facial nerve axotomy [78]. Moreover, determination of gene expression revealed that leukocytes isolated from the spinal cords of GC-treated EAE-diseased mice express reduced levels of myeloid cell-associated pro-inflammatory cytokines, such as IL-1β, and chemokines, like IP-10, whereas M2-associated genes are significantly increased. This suggests that also *in vivo*, GC-treatment could promote polarisation of myeloid cells towards an anti-inflammatory phenotype [79]. In contrast to these beneficial effects, GC-treatment resulted in worsening of CNS inflammation in models of excitotoxicity and stroke, which was mediated by increased activation of myeloid cells and depended on the temporal context of GC-administration [43]. Hence, GCs can promote both, stimulatory and anti-inflammatory responses of myeloid cells *in vivo* [45,79].

GC-treated PBMCs produce reduced amounts of pro-inflammatory cytokines such as IL-1β or IL-12 [80], whereas IL-10-secretion is increased in whole PBMCs as well as in isolated monocytes [47]. In line with this, PBMCs isolated from steroid-treated MS patients express reduced levels of TNF-α and increased levels of IL-10 [81]. Importantly, the induction of anti-inflammatory myeloid cells is beneficial for treatment of CNS autoimmunity as myeloid cells with suppressive properties efficiently ameliorate EAE [82].

Taken together, during CNS autoimmunity GR activation by administration of GCs results (mostly) in amelioration of clinical signs, which is characterised also by modulation of myeloid cell responses. However, as long-term treatment with GCs has severe side effects, including diabetes, osteoporosis, and muscle atrophy, improved synthetic GCs are needed, which act anti-inflammatory while causing less metabolic side effects [44]. The discovery of so-called dissociating ligands, which act mainly by mechanisms independent of DNA-binding and hence, not by induction of gene-transcription, is a major breakthrough in the development of new treatment regimes targeting the GR and might represent a promising approach to reduce unwanted side effects of GCs while promoting their anti-inflammatory properties [44].

Retinoid X receptors (RXRs)

Despite the central role as heterodimeric partner for other NRs, the role of RXRs during CNS autoimmunity has not been the area of intensive research. Administration of 9-cis RA results in amelioration of clinical signs during EAE. As this effect is most pronounced during the effector phase of EAE, 9-cis RA probably also modulates myeloid cell responses and indeed, 9-cis RA-treated animals exhibit reduced numbers of macrophages in the CNS [83]. In line with *in vitro* findings, co-administration of a PPARγ agonist further ameliorates EAE and dual-treated mice showed also less macrophage infiltrates compared to animals which were only treated with 9-cis RA. This demonstrates that also *in vivo*, targeting both partners of the permissive RXR-PPARγ heterodimer has additive effects [83].

Inflammatory signals induce RXR expression in microglia and macrophages as demonstrated in a model of spinal cord injury [84], which suggests that also during EAE, RXR might be upregulated in myeloid cells. Importantly, in the active borders of MS lesions, RXRγ expression was indeed detected in MHC-II-expressing cells, which represent infiltrating macrophages and locally activated microglia [85]. Moreover, RXR expression was primarily detectable in active lesions and peri-plaque white matter around the lesion, whereas only low numbers of RXR^+ cells were detected in chronic inactive core lesions. This observation indicates that in areas of high cellular activity, i.e. in active MS lesions, RXR activation might be correlated to tissue repair whereas areas of chronic, inactive lesions contain only reduced numbers of RXR^+ cells, which might correlate to inability of remyelination [85]. As it was shown that RXRα is involved in phagocytosis by macophages [51], it is conceivable that RXR upregulation by myeloid cells within the CNS might represent an important step for subsequent phagocytosis and clearance of debris from degenerating cells and myelin. Furthermore, as RXR activation induces polarisation towards an M2 phenotype in macrophages [35] and the ingestion of apoptotic cells results in increased IL-10 and TGFβ expression [51], the observed RXR expression by myeloid cells in MS lesions might also represent alternative activation of myeloid cells, which would further contribute to resolution of inflammation and subsequent tissue repair.

Vitamin D receptor (VDR)

Low vitamin D levels are associated with an increased risk to develop MS and conversely, administration of vitamin D decreases prevalence and severity of MS [54]. The VDR is expressed in CNS lesions by several cell types including microglia and macrophages, which indicates that myeloid cells can be targeted by VDR agonists to restrict CNS autoimmunity. Furthermore, in normal appearing white matter (NAWM) derived from MS patients, VDR expression is increased when compared to NAWM from healthy individuals [86]. Several studies demonstrate that administration of the active vitamin D metabolite $1,25(OH)_2D_3$ reduces clinical signs of EAE [87-90], which is dependent on the presence of VDR [87,88] and IL-10 signalling [89]. Although the modulation of T cell responses was focus of these studies, there are hints that $1,25(OH)_2D_3$-treatment during EAE alters the activation status of myeloid cells. In the CNS of $1,25(OH)_2D_3$-treated animals, MHC-II expression in inflammatory lesions is drastically reduced and virtually absent compared to control animals [90]. As infiltrating macrophages represent the predominant cell type within MS lesions [4], this finding suggests that myeloid cells in $1,25(OH)_2D_3$-treated animals exhibit a reduced activation status. Furthermore, expression of iNOS, a characteristic pro-inflammatory gene expressed by myeloid cells, is strongly reduced in the CNS of $1,25(OH)_2D_3$-treated animals [90]. In line with this, in a model of brain inflammation that involves LPS injection into the hippocampus of rats, LPS-induced iNOS expression in myeloid cells was significantly reduced when $1,25(OH)_2D_3$ was co-injected with LPS [91], which suggests that also in

the EAE model, $1,25(OH)_2D_3$ could directly regulate iNOS expression in CNS myeloid cells via activation of VDR.

Furthermore, as the course of EAE is not altered when $1,25(OH)_2D_3$ was administered to IL-$10^{-/-}$ and IL-$10R^{-/-}$ mice, IL-10 signalling plays an essential role in mediating the ameliorative effect of $1,25(OH)_2D_3$ on EAE clinical signs [89]. Similarly, *in vitro* effects of $1,25(OH)_2D_3$-mediated modulation of macrophage responses are abrogated in IL-10-deficient macrophages [55]. Thus, $1,25(OH)_2D_3$-induced restriction of macrophage pro-inflammatory responses *in vivo* could also be mediated in an IL-10 dependent manner.

Interestingly, administration of $1,25(OH)_2D_3$ during EAE results in increased expression levels of IL-4 as well as TGFβ in the peripheral lymph nodes and within the CNS [92]. As IL-4 plays a central role in polarising macrophages towards an anti-inflammatory M2 phenotype [32], $1,25(OH)_2D_3$ could modulate macrophage responses indirectly, i.e. by creating an environment which promotes anti-inflammatory properties of macrophages.

As several studies have revealed the beneficial effects of vitamin D in immunmodulation during CNS autoimmunity, clinical trials are underway to study whether vitamin D supplementation reduces clinical signs of MS. Importantly, MS patients receiving high doses of vitamin D do not show signs of hypercalcemia or hypercalciuria and it was proposed that administration of high doses of vitamin D is safe [93]. Furthermore, although disease progression and activity was not altered due to vitamin D-treatment, the number of gadolinium-enhancing lesions per patient decreased [93]. In a one-year study comprising 66 MS patients the efficacy of vitamin D as add-on therapy to interferon beta-treatment was evaluated. Although additional vitamin D did not influence the annual rate of relapses, MRI disease activity in vitamin D-receiving patients was reduced, i.e. these patients had fewer new T2 lesions and significantly lower numbers of T1 enhancing lesions. Moreover, these patients exhibited a tendency to reduced disability accumulation and improved timed tandem walk [94]. Two ongoing, high quality phase II trials are currently being conducted, namely SOLAR [95] and EVIDIMS [96], which will significantly contribute to understanding whether high-dose vitamin D supplementation is efficient in ameliorating MS.

Aryl hydrocarbon receptor (AHR)

In EAE studies it was shown that administration of two different AHR agonists, i.e. resveratrol [97] and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) [64] as well as the endogenous ligand 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) [98] results in amelioration of clinical signs during initiation as well as the effector phase. These effects were mediated by AHR activation as TCDD and ITE did not prove to be effective when administered to AHR^d mice, which express AHR with reduced affinity for its ligands [64,98]. Again, primarily T cell responses were characterised but some results suggest that also myeloid cell responses were modulated by AHR-activation / -deficiency. Histological analysis showed reduced immune cell infiltration in the CNS of agonist-treated mice [64,97]. Although the exact cell types within the infiltrate were not determined, the reduction in infiltrating cells is probably due to a reduction in myeloid cell numbers as macrophages represent the dominant cell population within lesions [4]. Furthermore, resveratrol-treated animals show decreased serum levels of pro-inflammatory cytokines such as TNFα and IL-12 [97]. As these are cytokines produced by myeloid cells during CNS inflammation, reduced cytokine levels could correlate to AHR-mediated restriction of myeloid cell activation. In contrast, in mice expressing the AHR^d variant the clinical course of EAE is aggravated compared to wildtype mice [64] which indicates that binding of endogenous ligands and basal AHR activation restricts CNS autoimmunity, most probably also in myeloid cells.

Monocytes significantly contribute to the extent of CNS autoimmunity during the effector phase of EAE [3,11,13]. As the effector phase is ameliorated under AHR agonist-treatment and aggravated in AHR-deficient mice, respectively, one can assume that besides the described effects on T cell differentiation [64,97,98], AHR modulates also myeloid cell responses during EAE. Interestingly, in a study of sepsis it could be shown that AHR-deficient mice are more susceptible to inflammation in the model of LPS-induced toxicity [59]. LPS injection resulted in death of all AHR^{KO} animals within 60h whereas all wildtype mice survived. LPS-activated AHR^{KO} macrophages showed enhanced pro-inflammatory cytokine production *in vitro* and AHR^{KO} mice had higher serum levels of IL-6 and TNFα after LPS-injection [59]. This indicates that macrophage responses in AHR^{KO} mice were enhanced *in vivo* and furthermore, that AHR is involved in negative regulation of inflammatory responses mediated by myeloid cells. In line with this, it can be speculated that also in sterile inflammation, like in CNS autoimmunity, AHR limits myeloid cell responses and it will be interesting to further unravel the role of AHR in myeloid cells.

In summary, several studies have investigated the role of NRs in MS and its animal model EAE. However, despite promising *in vitro* data that clearly demonstrate regulatory function of diverse NRs in myeloid cells, most *in vivo* studies investigated the role of NRs in T cells and the effect on CNS autoimmunity. Future studies should aim at investigating the regulatory role of NRs in myeloid cells as these cells significantly shape the local pro-inflammatory milieu within the CNS during the effector phase but can also act in a beneficial way to support tissue repair and remyelination. Figure 1B indicates at which steps of the inflammatory cascade within the CNS, NR activation in myeloid cells can potentially prove beneficial for the outcome of disease.

Concluding Remarks

Myeloid cells express a diverse range of NRs that can strongly influence their inflammatory responses. Numerous *in vitro* studies have shown that NRs restrict pro-inflammatory responses and have also the potential to promote polarisation of myeloid cells towards an anti-inflammatory phenotype. However, the detailed analysis of NR influence on myeloid cell responses during CNS autoimmunity has been hampered by the availability of mouse models. Especially the generation of new mouse strains with a selective NR-deficiency in myeloid cells, similar to myeloid cell-specific $PPARγ^{KO}$ mice described in [13], will help to further clarify the role of NRs in regulation of myeloid cell responses.

Myeloid cells critically shape the inflammatory environment during CNS autoimmunity e.g. by production of pro-inflammatory mediators [3,4] but also by supporting remyelination [20]. Therefore, modulation of myeloid cell responses by targeting NRs represents a promising treatment approach as NR activation would not only limit excessive pro-inflammatory immune responses but at the same time promote an anti-inflammatory phenotype in myeloid cells. Furthermore, targeting myeloid cells during CNS autoimmunity has several advantages, i.e. modulation of myeloid cell responses also proves beneficial after disease onset and does not depend on antigen-specificity.

Although some NR agonists have already been approved for treatment of human diseases and exhibit a good safety profile, they show unwanted side effects due to their regulatory functions in metabolism, e.g. the PPARγ agonist Pioglitazone which is approved

for treatment of diabetes type II has side effects like oedema, fluid retention, and heart failure [99]. Hence, the development of new agonists that specifically promote the regulatory function of NRs on immune responses will be of critical importance. In addition, it would be very useful to reveal the identity and function of endogenous NR ligands, as dietary supplementation of such ligands or their precursors might be an alternative promising approach to ameliorate myeloid cell responses in the context of CNS autoimmunity.

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