Nuclear Receptor Research
Vol. 2 (2015), Article ID 101163, 9 pages
doi:10.11131/2015/101163

AgiAl Publishing House
http://www.agialpress.com/

Research Article

Activation of Nuclear Receptors RAR, RXR, and LXR Does Not Reduce Cuprizone-Induced Demyelination in Mice

Davina Kruczek[1], Tim Clarner[2], Cordian Beyer[2], Markus Kipp[2,3] and Jörg Mey[1,4,5]

[1] *Institut für Biologie II, RWTH Aachen, Germany*
[2] *Institut für Neuroanatomie, Universitätsklinikum Aachen, Germany*
[3] *Lehrstuhl II – Neuroanatomie, Ludwig-Maximilians-Universität München, Germany*
[4] *Laboratorio Regeneración Nerviosa, Hospital Nacional de Parapléjicos, Toledo, Spain*
[5] *EURON Graduate School of Neuroscience, Maastricht University, Netherlands*

Corresponding Authors: Jörg Mey; email: jmey@sescam.jccm.es and Markus Kipp; email: markus.kipp@med.uni-muenchen.de

Recieved 26 March 2015; Accepted 15 May 2015

Editor: Xiaoying Li

Abstract. Experiments with animal models of multiple sclerosis have shown that the expression of retinoid X receptors (RXR) increases during demyelination and that RXR is involved in the regulation of remyelination. After ligand binding RXRs form heterodimeric transcription factors with other nuclear receptor (NR) families including the retinoic acid receptors (RAR) and liver X receptors (LXR). We tested whether activation of these nuclear receptor complexes reduces pathological demyelination using the cuprizone mouse model. Cuprizone, which causes oligodendrocyte degeneration, was given for three weeks as a food additive. For the activation of nuclear receptors mice were treated with daily i.p. injections of agonists for RXR (9-*cis* RA), RAR (all-*trans* RA), and LXR (T0901317). Myelin status, oligodendrocyte survival, astrogliosis, microglial activation, and axon density were monitored with immunohistochemistry and evaluated quantitatively. Three weeks of cuprizone feeding caused severe demyelination and significantly raised the number of Iba1 immunoreactive microglia cells in the caudal corpus callosum. This increase of microglia activity was reduced with 9-*cis* RA treatment but was enhanced with all-*trans* RA and was not affected by T0901317. Nuclear receptor activation did not influence the degree of demyelination, oligodendrocyte survival, astrogliosis, or axonal preservation. We conclude that RXR activation, although affecting Iba1-positive microglia, does not protect oligodendrocytes from cuprizone toxicity and does not induce compensatory mechanisms in the initial phase of demyelination.

Keywords: cuprizone; retinoic acid; nuclear receptor; astrocyte; myelin; microglia

1. Introduction

Recently, ligand activated transcription factors of the retinoid X receptor family (RXR) have been shown to be involved in the endogenous responses to toxin induced demyelination [1, 2]. The RXRs form heterodimers with other nuclear receptor (NR) families, including retinoic acid receptors (RAR) and liver X receptors (LXR). Experimental administration of agonists of these receptors was suggested as a potential treatment option for Alzheimer's disease, multiple sclerosis, ischemia, and spinal cord injury [3]. Anti-inflammatory functions of retinoic acid (RA), the natural ligand of

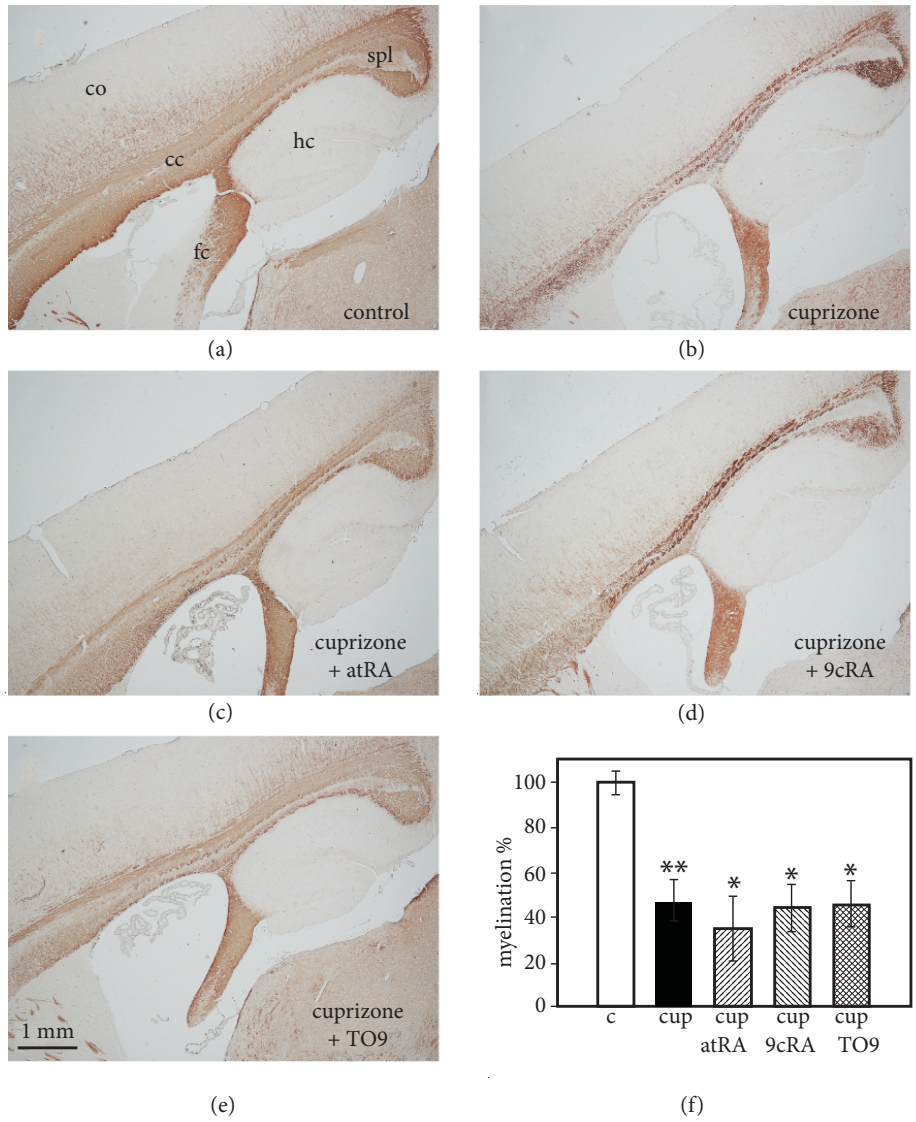

Figure 1: Myelination of central fiber tracts shown with immunohistochemistry of PLP. Parasagittal sections of the Cc area are shown three weeks after cuprizone treatment. (a) Control section of a mouse fed normal chow; (b) effect of cuprizone feeding; (c) of cuprizone and daily intraperitoneal injections of all-*trans* RA; (d) of cuprizone and 9-*cis* RA; (e) of cuprizone and T0901317. Reduced myelin staining in all cuprizone treated animals is visible as is dark granular myelin debris. The caudal end of the Cc is in the upper right corner of the photographs; co: cerebral cortex, cc: corpus callosum; spl: splenium; hc: hippocampus; fo: fornix. (f) Quantitative evaluation of myelin status in the Cc, based on PLP immunohistochemistry; myelin scale converted to % of control. Bars indicate means and standard error of the mean (SEM). Nonparametric H-test of treatment effect was significant and post hoc U-tests demonstrated a significant effect of cuprizone feeding compared to control (*$P < 0.05$, **$P < 0.01$) and no influence of the NR agonists.

RAR/RXR, have been described for astrocytes [4–6] and microglia [7, 8]. In three animal models of multiple sclerosis, that is, experimental allergic encephalomyelitis (EAE), systemic treatment with cuprizone, and focal application of lysolecithin, the oral administration of ligands of RAR [9-11], PPAR [12, 13], or n-3 polyunsaturated fatty acids (which activate RXR) [14] resulted in therapeutic benefits. The studies evaluated T-cell function, pro- and anti-inflammatory cytokines, histological signs of demyelination, and clinical scores of disease progression. Since LXR/RXR signaling regulates cholesterol homeostasis in oligodendrocytes [15] and modulates the activity of macrophages [16] and T-cells [17], their ligands are likely to be involved in the physiological processes during remyelination as well.

Using acute demyelination combined with laser capture microdissection the groups of Franklin, ffrench-Constant, and Chambon demonstrated that RXRγ controls the differentiation of oligodendrocyte precursor cells and promotes remyelination *in vivo* [1]. The involvement of RXRγ in the process of demyelination was demonstrated by us using the copper chelator cuprizone [2]. In this model 0.2% cuprizone is fed to young male mice. The treatment causes apoptosis

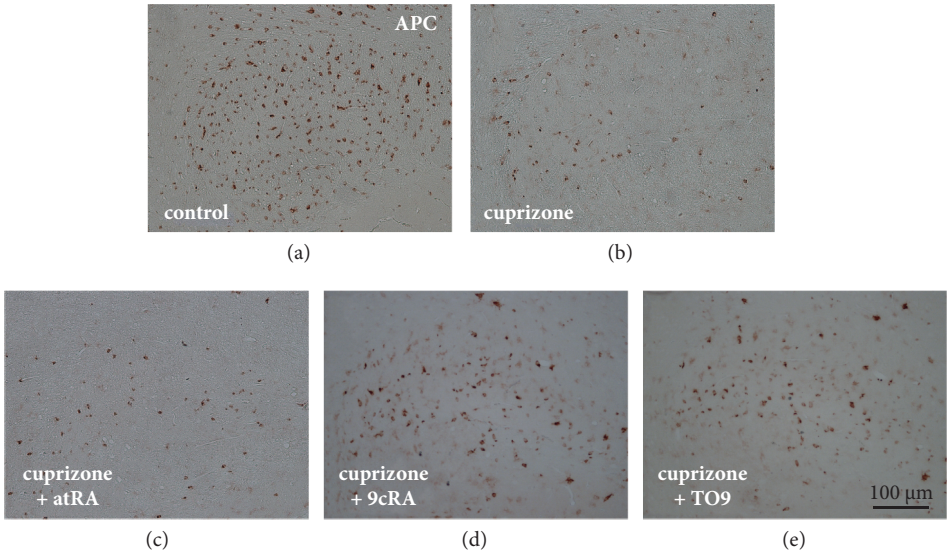

Figure 2: Examples of immunohistochemical staining illustrate the effect of cuprizone and NR agonists on oligodendrocytes. Shown are sections of the caudal Cc following treatments; (a) control condition, normal chow; (b) after three weeks of cuprizone feeding; (c) combined cuprizone feeding with injection of all-*trans* RA; (d) combined treatment of cuprizone with 9-*cis* RA; and (e) combined treatment of cuprizone with T0901317. Oligodendrocytes were marked with an antibody against adenomatous polyposis coli (APC). Scale bar 100 µm in panel e is valid for all photographs.

of mature oligodendrocytes, which is closely followed by microglia and astrocyte activation. The blood-brain barrier remains intact and a hematogenic immune response with cerebral T-cell infiltration is not elicited [18]. In the present work we investigated whether activation with ligands of RAR (all-*trans* RA), RXR (9-*cis* RA), and LXR (T0901317) affected the process of demyelination in this animal model. Since we observed that three weeks of cuprizone treatment led to a strong degree of demyelination of the *Corpus callosum* (Cc) this treatment period and fiber tract were selected in the present study.

2. Materials and Methods

2.1. The cuprizone animal model. To induce demyelination of central fiber tracts, 8-week-old male C57BL/6 mice, 20–25 g body weight (Charles River, Germany), were treated with the copper chelator cuprizone (Sigma-Aldrich, Germany). Demyelination was induced by feeding a diet containing 0.2% cuprizone mixed into a ground of standard rodent chow for three weeks. Animals were maintained in a pathogen-free environment, underwent routine cage maintenance once a week and microbiological monitoring. Food and water were available *ad libitum*. Research and animal care procedures were in accordance with national regulations and guidelines for the care and use of laboratory animals and were approved by the Review Board for the Care of Animal Subjects of the district government in Nordrhein-Westfalen, Germany.

2.2. Nuclear receptor activation in vivo. To activate RAR, RXR, or LXR transcription factors, mice received daily intraperitoneal (i.p.) injections of either 20 µg all-*trans* RA (Sigma-Aldrich), 20 µg 9-*cis* RA (Sigma-Aldrich) diluted in 100 µl of 50/50 DMSO/PBS, or 0.5 mg T0901317 (Cayman Chemicals) dissolved in 100 µl of 10/90 DMSO/PBS. Animals in the cuprizone-treated control group received daily i.p. injections of 100 µl 50/50 DMSO/PBS. The efficacy of NR activation with i.p. application of these listed agonists had been established in previous studies [19–21]. Following an accommodation period and the three weeks of experimental drug application, all animals were sacrificed and further processed.

2.3. Tissue preparation and demyelination scoring. For the histological evaluation, mice were transcardially perfused with 4% paraformaldehyde containing saturated picric acid. After decapitation and post fixation overnight, brains were dissected and embedded in paraffin. Best staining results were achieved with dehydration for seven days in 70% ethanol followed by 96%, 100% ethanol, acetone, and paraffin embedding. Parasagittal sections of 5 µm covered the lateral 0.48 mm–0.72 mm area according to the stereotaxic atlas [22]. The degree of demyelination was evaluated immunohistochemically and by staining with Luxol fast blue/cresyl violet following the protocol previously described [18]. The myelin status was staged independently by three blinded investigators using a scale between 0 and 100, where a score of 100 was given to the myelin status of reference mice, not treated with cuprizone, whereas zero corresponded to a totally demyelinated corpus callosum.

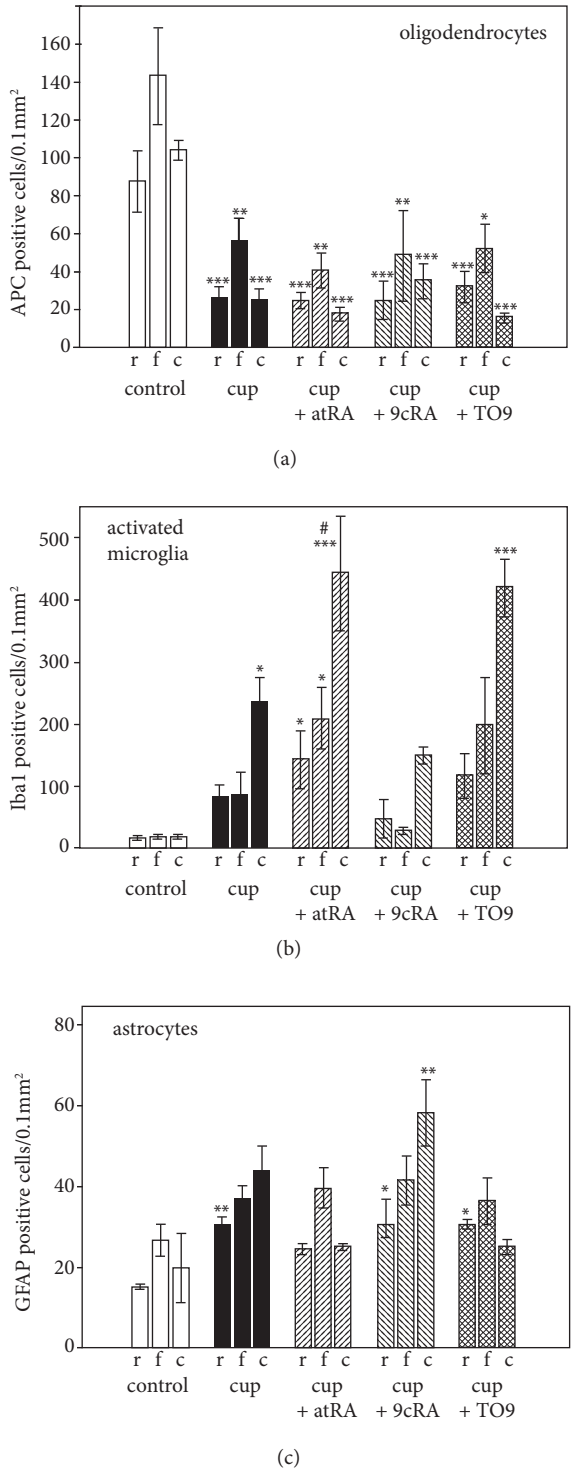

Figure 3: Quantification of the effect of cuprizone and NR agonists on cell numbers of oligodendrocytes, microglia, and astrocytes. (a) After three weeks of cuprizone feeding APC positive cell counts were significantly reduced in all regions of the Cc while treatment with the NR agonists had no mitigating or aggravating effect. (b) Quantification of microglia activation and (c) of GFAP positive cell numbers. Bars indicate means ± SEM. Following ANOVA, Dunnett's test comparisons vs. control condition are marked with asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$), comparison vs. cuprizone condition with the pound symbol (#$P < 0.05$). Regions of the Cc are abbreviated r: rostral, f: central near fornix, and c: caudal.

2.4. Analysis of nuclear receptor expression. The expression of nuclear receptors under conditions of cuprizone induced demyelination was assessed with the GeneChip Mouse Gene 1.0 ST Array (Affymetrix, USA) using the Agilent 2100 Bioanalyzer (Agilent, USA). The detailed procedure and treatment regimen have been published before [18]. Effects on nuclear receptors that are likely to mediate RA signaling were confirmed with quantitative RT-PCR using iQ5 real-time PCR detection (BioRad) using the same primers as listed previously [2].

2.5. Immunohistochemistry. Paraffin-embedded sections on silane-coated slides were rehydrated, heat-unmasked in citric acid buffer (pH 6.0; 20 min, microwave 180 W), and then processed using the Vectastain Elite ABC-Kit (avidin-biotinylated enzyme complex, Vector Laboratories, PK4010). Sections were blocked with 2% normal serum and incubated overnight with mouse monoclonal antibodies against myelin proteolipid protein (PLP; 1/500; Abd Serotec, Germany, catalogue number: MCA839G), adenomatous polyposis coli (APC; 1/250; Calbiochem, Germany, OP80), and pan-axonal neurofilament (smi312, 1/1000; Covance, UK, SMI312-R), with rabbit polyclonal sera against glial fibrillary acidic protein (GFAP; 1/1000; EnCor, USA, RPCA-GFAP) and against ionized calcium binding adaptor molecule-1 (Iba1; 1/200; Wako, Germany, 019-19741). In Iba1 labeling experiments, counterstaining with hematoxylin/eosin was used. Biotinylated secondary antibodies, included in the Vectastain kit, were goat anti-rabbit IgG and horse anti-mouse IgG (both from Vector Laboratories, BA-1000, BA-2000). For visualization of peroxidase labeling a substrate kit with aminoethyl carbazole and a chromophore was used (Zymed, AEC Red Substrate Kit, 00-2007; Invitrogen). Sections were viewed and documented using a Nikon Eclipse 80i microscope equipped with a Nikon Y-TV55 camera.

2.6. Quantification and statistical analysis. Microphotographs were captured and evaluated with Nikon NIS Element D program and NIH *ImageJ*. Immunoreactive cells were manually selected and counted automatically. Three regions of the Corpus callosum (Cc), caudal (splenium), central (adjacent to fornix), and rostral Cc, were assessed independently. Cell densities were normalized to 0.1 mm^2 tissue area. Using a 40x objective (Iba1) or 20x objective (GFAP, APC) the numbers of positive cells were counted by two blinded observers in four animals per condition except for the group treated with cuprizone only, which contained eight animals. Two sections per mouse were averaged.

Non-parametric data of myelin staging were evaluated with the H-Test after Kruskal and Wallis followed by Wilcoxon/Mann/Whitney post hoc U-test with correction for ties. Cell density data were evaluated with ANOVA and post hoc Dunnett's test. Statistical tests were done using GraphPad

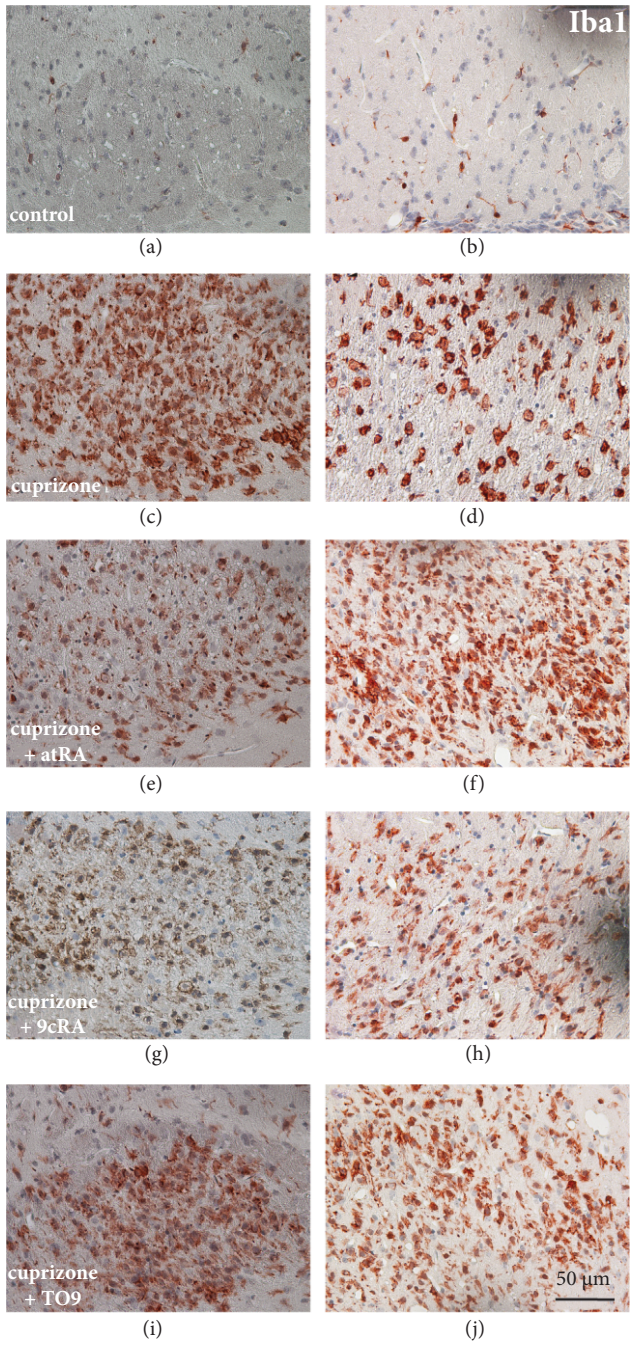

Figure 4: Examples of immunohistochemical staining illustrate the effect of cuprizone and NR agonists on microglia cells. Shown are sections of Cc following treatments; (a), (b) control condition, normal chow; (c), (d) after three weeks of cuprizone feeding; (e), (f) combined cuprizone feeding with injection of all-*trans* RA; (g), (h) combined treatment of cuprizone with 9-*cis* RA; and (i), (j) combined treatment of cuprizone with T0901317. Panels (a), (c), (e), (g), and (i) represent areas in the caudal, panels (b), (d), (f), (h), and (j) in the rostral Cc. Microglia cells were marked with an antiserum against ionized calcium binding adapter molecule-1 (Iba1). Scale bar 50 µm in panel j is valid for all photographs.

Prism 5.01, the U-test manually with Excel. In all graphs means ± standard error of the mean are shown.

3. Results

3.1. Cuprizone-induced demyelination is not affected by retinoic acid or T0901317. Three weeks of oral cuprizone treatment caused severe demyelination of the Cc, as shown with histological staining (Luxol fast blue) and PLP immunohistochemistry (Figure 1(a), 1(b)). In addition to the reduced staining, dark granular myelin debris was visible, as illustrated in the Cc and adjacent cingulum. In contrast to these fiber tracts, myelination of the fornix was not affected. To test whether pharmacological activation of NR influences demyelination, the cuprizone treatment was combined with daily i.p. injections of ligands for RARs (all-*trans* RA), RXRs (9-*cis* RA), and LXRs (T0901317; Figure 1(c)–1(e)). Previous studies have established that this treatment alters gene expression in the CNS [19–21]. Immunoreactivity of RARα and RXRβ is present in the corpus callosum [2]. The cuprizone-induced demyelination was not affected by any of the NR agonists (LFB staining). Quantification of PLP immunoreactivity corroborated the signal reduction due to cuprizone administration (Figure 1(a), 1(b)). Although myelin degradation appeared to be somewhat attenuated after treatment with all-trans RA (Figure 1(c)) and T0901317 (Figure 1(e)), the quantification showed no significant effect of any of the NR ligands (Figure 1(f)).

Mature oligodendrocyte cell bodies were marked with an antibody against APC (Figure 2). Numerous oligodendrocytes were homogeneously distributed in the Cc of control mice, and no difference was apparent between caudal, middle, and rostral parts of the Cc. In cuprizone-treated animals the numbers were strongly reduced. After three weeks we found the decline to be most pronounced in the caudal area, where the APC positive cell population was reduced to 24% (Figure 2(a), 2(b)). The administration of all-*trans* RA, 9-*cis* RA, or T0901317 did not affect oligodendrocytes (Figure 2(b)–2(e)), as demonstrated by the quantification of cell numbers (Figure 3(a), no statistical differences between cuprizone-treated animals and the groups that received additional injections of NR ligands). Across the rostrocaudal extent of the Cc and treatment conditions the decline in oligodendrocytes paralleled demyelination.

At three weeks of continued cuprizone treatment the density of nerve fibers, quantified with smi312 immunohistochemistry, was not yet affected. Compared to the neurofilament signal intensity in the Cc of control animals (normalized mean 100 ± SEM 1.7), cuprizone treatment caused no change (100 ± 2.6), and none of the NR agonists affected fiber density either (cuprizone with all-*trans* RA: 99 ± 1.5; cuprizone with 9-*cis* RA: 99 ± 3.6; cuprizone with T0901317: 96 ± 1.0). These are data from the rostral Cc, and similar results were obtained from the central and caudal areas of the fiber tract.

3.2. Effect of NR agonists on microglial activation and astrogliosis. Although the cuprizone-induced demyelination differs from the EAE model by not activating the immune system systemically, the tissue damage caused a local inflammatory reaction. In cuprizone treated mice we noted the appearance of a large population of Iba1 expressing microglia (Figure 4), which was particularly strong in the caudal Cc. The quantification is shown in Figure 3(b). Additional treatment with 9-*cis* RA reduced this increase (Figure 4(g), 4(h)), such that the difference with respect to the control condition without cuprizone was no longer significant; however the comparison between 9-*cis* RA treated and non-treated animals under conditions of demyelination fell short of the required level of significance ($0.05 < P < 0.1$). On the other hand, injections of all-*trans*-RA caused a significantly stronger Iba1 immunoreactivity than cuprizone feeding alone ($P < 0.05$). The LXR agonist T0901317 did not modify the response (Figures 3(b) and 4(c)–4(j)).

Cuprizone-induced damage was accompanied with mild signs of local astrogliosis. Immunohistochemical staining against GFAP revealed a moderate increase in the size and staining intensity of astrocytes in the Cc (Figure 5(a), 5(b)). The strongest signal was apparent in the caudal area, although it reached significance only in the rostral Cc (Figure 3(c)). The quantitative evaluation revealed no influence of all-*trans* RA, 9-*cis* RA and T0901317 under conditions of demyelination ($P > 0.05$ in all cases; Figures 3(c) and 5(b)–5(e)).

3.3. Nuclear receptors of the RAR, RXR, and LXR families are expressed in the murine corpus callosum under conditions of demyelination. The expression of nuclear receptors that form functional dimers with RXR and would provide the basis for physiological effects of the investigated ligands was detected in the Cc of untreated and cuprizone-fed mice. An Affymetrix GeneChip screen indicated high levels of expression of RARβ, RXRγ, RORα, LXRα and PPARβ and a cuprizone-induce increase of the transcripts for RXRβ and RORβ (Table 1). The observed effects of RA on microglia are likely to be mediated by RAR/RXR heterodimers.

Their expression was confirmed with RT-PCR (Figure 6). During demyelination we measured a significant increase of RXRγ (t-test, $P < 0.05$) and a tendency to increase for RXRβ ($P = 0.059$).

4. Discussion

After three weeks of cuprizone feeding we found that activation of RAR, RXR, or LXR had no effect on the degree of demyelination, oligodendrocyte survival, or astrogliosis. Cuprizone feeding raised the number of activated microglia cells in the Cc, as shown with Iba1 IR. This increase was reduced with 9-*cis* RA treatment. On the other hand, after exposure to all-*trans* RA microglia activity was enhanced,

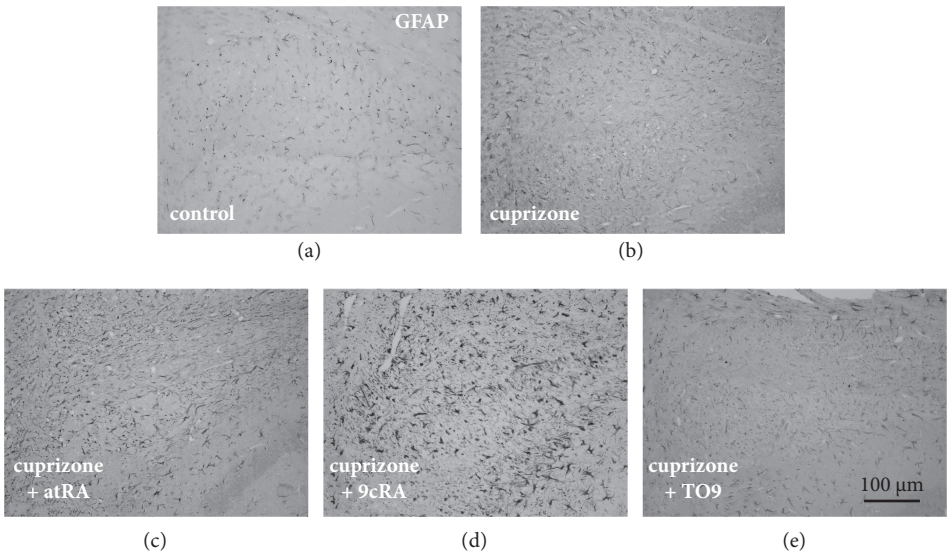

Figure 5: Examples of immunohistochemical staining illustrate the effect of cuprizone and NR agonists on astrocytes. Shown are sections of caudal Cc following treatments; (a) control condition, normal chow; (b) after three weeks of cuprizone feeding; (c) combined cuprizone feeding with injection of all-*trans* RA; (d) combined treatment of cuprizone with 9-*cis* RA; and (e) combined treatment of cuprizone with T0901317. Astrocytes were marked with an antiserum against glial fibrillary acidic protein (GFAP). Scale bar 100 µm in panel (e) is valid for all photographs.

Table 1: Effect of cuprizone treatment on the expression of nuclear receptors in the murine *corpus callosum* (Affimetrix gene chip).

probe set	gene	control	2 w Cu	5 w Cu	9 w Cu	13 w Cu
10381082	RARα (NR1B1)	present (7.55)	0.86	0.85	0.67	0.76
10417713	RARβ (NR1B2)	high (9.34)	0.91	1.14	0.74	0.79
10432972	RARγ (NR1B3)	present (7.27)	1.01	1.17	1.06	1.11
10470446	RXRα (NR2B1)	present (7.87)	1.01	0.95	0.97	0.93
10444137	RXRβ (NR2B2)	present (7.85)	1.14	**1.51**	1.41	1.34
10351430	RXRγ (NR2B3)	high (8,75)	0.78	0.94	0.60	0.60
10562847	LXRα (NR1H2)	high (8.77)	1.36	1.26	1.28	1.26
10484987	LXRβ (NR1H3)	present (6.95)	1.05	1.12	1.13	1.01
10425987	PPARα (NR1C1)	low (6.68)	1.00	0.94	0.89	0.96
10443332	PPARβ/δ (NR1C2)	high (8.93)	0.93	0.56	0.55	0.60
10540897	PPARγ (NR1C3)	present (7.84)	1.13	0.59	0.83	0.74
10586700	RORα (NR1F1)	high (8.80)	1.06	1.10	1.11	1.16
10466587	RORβ (NR1F2)	high (8.13)	1.19	**1.63**	**2.25**	**1,72**
10494023	RORγ (NR1F3)	present (7.45)	1.29	0.79	0.85	0.94
10585438	CRABP-I	present (8.31)	0.95	0.73	0.61	0.62
10493108	CRABP-II	low (6.41)	1.12	1.03	1.07	1.06
10585699	FABP-5	high (8.57)	0.76	**1.77**	**1.62**	**1.58**
10363224	FABP-7	low (6.95)	1.27	**3.32**	**2.05**	1.38

Expression after 2, 5, 9, and 13 weeks cuprizone treatment are expressed as fractions of the levels of non-treated controls. Changes of more than 0.5 are highlighted in bold font.

whereas T0901317 had no effect above the toxin-induced changes.

Previous experiments suggested that tissue damage in the nervous system causes local production of all-*trans* RA, and we hypothesized that this constitutes a neuroprotective feedback via activation of RAR/RXR [23]. Recent experiments based on RXRγ knockdown and the application of RXRγ agonists provide convincing data that this receptor is an endogenous regulator of remyelination [1]. In another organ system, cholesterol homeostasis was disrupted in Sertoli cells of RXRβ deficient mice [24]. Therefore, we speculated that the regulation of lipid turnover is one of the functions

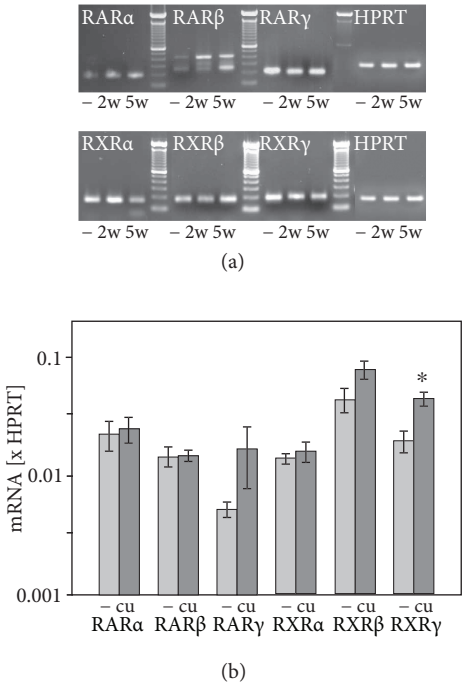

Figure 6: Gene expression analysis RARs and RXRs in the murine corpus callosum. (a) Agarose gels with RT-PCR products under control conditions, and after 2 and 5 weeks cuprizone feeding. (b) Quantification with qRT-PCR was normalized to the expression of hypoxanthine phosphoribosyltransferase (HPRT). Error bars indicate SEM, controls $n = 4$–8 animals/group; t-test: $*P < 0.05$.

of this receptor in the brain, at least under pathological circumstances with an effect on myelin [2]. In light of the results with RXRγ the absence of a treatment effect in the present experiments is surprising. Different experimental models were employed in the two approaches with RA treatment, focal demyelination with lysolecithin in one study [1] versus long-term systemic exposure to cuprizone in the other. While this divergence may explain some difference in the outcome, the more likely explanation is that RXRγ activation controls differentiation of oligodendrocytes but does not affect primary demyelination. Using the time period of three weeks we were able to disentangle demyelination from the compensatory processes regulated by RXRγ.

A second interesting observation is the opposite effect of 9-*cis* RA and all-*trans* RA on macrophage activation. So far, mainly anti-inflammatory activity of both isomers has been reported [4, 5, 8, 23, 25]. In the EAE mouse model, LXR activation reduced pathological symptoms via a reduction of Th17 mediated autoimmunity [17]. Thus, the lower number of Iba1 positive cells following injections with 9-*cis* RA was to be expected. However, Iba1 immunoreactivity was elevated in the caudal Cc when we combined cuprizone treatment with all-*trans* RA. In the brain, Iba1 is a specific marker of microglia which upregulate that gene during inflammation and when they assume a phagocytotic phenotype [26]. Although most studies focus on the destructive aspect of excessive microglia activation, it is crucial to bear in mind that different physiological phenotypes of microglia are not adequately described with a linear scale from resting state to inflammation [27]. In experimental models of demyelination, the role of macrophages is controversial [28]. Many studies suggest that the proinflammatory cytokines released by these cells are detrimental. Suppression of macrophages prevented the development of EAE. On the other hand, experiments with monocyte depletion indicate that microglia/macrophages have the potential to clear myelin debris, promote the recruitment of OPC, and release neurotrophic factors. A detailed discussion of these aspects is provided in a recent review [29]. The differential regulation of Iba1 described hereafter prolonged exposure to all-*trans* and 9-*cis* RA indicates that RAR and RXR modulate the activity of microglia cells. Stimulation by all-*trans* RA might foster phagocytosis and clearance of myelin debris and by this mechanism facilitate remyelination. It will be important in the future to study the differential effects of RXR-binding NR families with respect to the multiple physiological functions of microglia cells.

5. Conclusion

Experimental activation of the transcription factors RAR, RXR, and LXR does not directly affect toxin-induced demyelination. Ligands of these three NR families have differential effects on microglia activation.

Acknowledgments

The study was financed by intramural funding of the Institutes of Neuroanatomy and Biology II, RWTH Aachen. MK was supported by the Hertie-Foundation. We declare no conflict of interests. We thank Katherine Nelissen, University of Hasselt, Belgium, for providing the T0901317, Bernd Denecke for help with the GeneChip analysis, and the anonymous reviewers for helpful suggestions.

References

[1] J. K. Huang, A. A. Jarjour, B. N. Oumesmar, C. Kerninon, A. Williams, W. Krezel, H. Kagechika, J. Bauer, C. Zhao, A. Baron-Van Evercooren, P. Chambon, C. ffrench-Constant, and R. J. M. Franklin, Rtinoid X receptor gamma signaling accelerates CNS myelination, *Nature Neuroscience*, **14**, 45–53, (2011).

[2] K. René, M. Stillfried, P. Aperdannier, T. Clarner, C. Beyer, M. Kipp, and J. Mey, Expression of retinoid X receptor beta is induced in astrocytes during corpus callosum demyelination, *Journal of Chemical Neuroanatomy*, **43**, 120–132, (2012).

[3] S. van Neerven, E. Kampmann, and J. Mey, RAR/RXR and PPAR/RXR signaling in neurological and psychiatric diseases, *Progress in Neurobiology*, **85**, 433–451, (2008).

[4] E. Kampmann, S. Johann, S. van Neerven, C. Beyer, and J. Mey, Anti-inflammatory effect of retinoic acid on prostaglandin

synthesis in cultured cortical astrocytes, *Journal of Neurochemistry*, **106**, 320–332, (2008).

[5] S. van Neerven, T. Regen, D. Wolf, A. Nemes, S. Johann, C. Beyer, U. Hanisch -K, and J. Mey, Inflammatory chemokine release of astrocytes in vitro is reduced by all-trans retinoic acid, *Journal of Neurochemistry*, **114**, 1511–1526, (2010).

[6] S. van Neerven, A. Nemes, P. Imholz, T. Regen, B. Denecke, S. Johann, C. Beyer, U. Hanisch -K, and J. Mey, Inflammatory cytokine release of astrocytes in vitro is reduced by all-trans retinoic acid, *Journal of Neuroimmunology*, **229**, 169–179, (2010).

[7] J. Xu, P. D. Storer, J. A. Chavis, M. K. Racke, and P. D. Drew, Agonists for the peroxisome proliferator-activated receptor-alpha and the retinoid X receptor inhibit inflammatory responses of microglia, *Journal of Neuroscience Research*, **81**, 403–411, (2005).

[8] C. X. Zhang-Gandhi and P. D. Drew, Liver X receptor and retinoid X receptor agonists inhibit inflammatory responses of microglia and astrocytes, *Journal of Neuroimmunology*, **183**, 50–59, (2007).

[9] L. Massacesi, A. L. Abbamondi, C. Giorgi, F. Sarlo, F. Lolli, and L. Amaducci, Suppression of experimental allergic encephalomyelitis by retinoic acid, *Journal of the Neurological Sciences*, **80**, 55–64, (1987).

[10] M. K. Racke, D. Burnett, S. H. Pak, P. S. Albert, B. Cannella, C. S. Raine, D. E. McFarlin, and D. E. Scott, Retinoid treatment of experimental allergic encephalomyelitis. IL-4 production correlates with improved disease course, *The Journal of Immunology*, **154**, 450–458, (1995).

[11] C. Klemann, B. J. Raveney, A. K. Klemann, T. Ozawa, H. S. von, K. Shudo, S. Oki, and T. Yamamura, Synthetic retinoid AM80 inhibits Th17 cells and ameliorates experimental autoimmune encephalomyelitis, *The American Journal of Pathology*, **174**, 2234–2245, (2009).

[12] D. L. Feinstein, E. Galea, V. Gavrilyuk, C. F. Brosman, C. C. Whitacre, L. Dumitrescu-Ozimek, G. E. Landreth, H. A. Pershadsingh, G. Weinberg, and M. T. Heneka, Peroxisome proliferator-activated receptor-g agonists prevent experimental autoimmune encephalomyelitis, *Annals of Neurology*, **51**, 694–702, (2002).

[13] J. Xu, M. K. Racke, and P. D. Drew, Peroxisome proliferator-activated receptor-alpha agonist fenofibrate regulates IL-12 family cytokine expression in the CNS: relevance to multiple sclerosis, *Journal of Neurochemistry*, **103-**, 1801–1810, (2007).

[14] O. Torkildsen, L. A. Brunborg, A. M. Milde, S. J. Mork, K. M. Myhr, and L. Bo, A salmon based diet protects mice from behavioural changes in the cuprizone model for demyelination, *Clinical Nutrition (Edinburgh, Scotland)*, **28**, 83–87, (2009).

[15] K. Nelissen, M. Mulder, I. Smets, S. Timmermans, K. Smeets, M. Ameloot, and J. J. Hendriks, Liver X receptors regulate cholesterol homeostasis in oligodendrocytes, *Journal of Neuroscience Research*, **90**, 60–71, (2012).

[16] J. F. Bogie, S. Timmermans, V. A. Huynh-Thu, A. Irrthum, H. J. Smeets, J. A. Gustafsson, K. R. Steffensen, M. Mulder, P. Stinissen, N. Hellings, and J. J. Hendriks, Myelin-derived lipids modulate macrophage activity by liver X receptor activation, *PLoS One*, **7**, (2012).

[17] G. Cui, X. Qin, L. Wu, Y. Zhang, X. Sheng, Q. Yu, H. Sheng, B. Xi, J. Z. Zhang, and Y. Q. Zang, Liver X receptor (LXR) mediates negative regulation of mouse and human Th17 differentiation, *Journal of Clinical Investigation*, **121**, 658–670, (2011).

[18] M. Kipp, S. Gingele, F. Pott, T. Clarner, V. van d, B. Denecke, L. Gan, V. Siffrin, F. Zipp, W. Dreher, W. Baumgartner, S. Pfeifenbring, R. Godbout, S. Amor, and C. Beyer, BLBP-expression in astrocytes during experimental demyelination and in human multiple sclerosis lesions, *Brain, Behavior, and Immunity*, **25**, 1554–1568, (2011).

[19] J. Crandall, Y. Sakai, J. Zhang, O. Koul, Y. Mineur, W. E. Crusio, and P. McCaffery, 13-cis-Retinoic acid suppresses hippocampal cell division and hippocampal-dependent learning in mice, *Proceedings of the National Academy of Science of the United States of America*, **101**, 5111–5116, (2004).

[20] S. van Neerven, J. Mey, E. A. Joosten, H. W. Steinbusch, K. M. van, M. A. Marcus, and R. Deumens, Systemic but not local administration of retinoic acid reduces early transcript levels of pro-inflammatory cytokines after experimental spinal cord injury, *Neuroscience Letters*, **485**, 21–25, (2010).

[21] I. Paterniti, T. Genovese, E. Mazzon, C. Crisafulli, R. Di Paola, M. Galuppo, P. Bramanti, and S. Cuzzocrea, Liver X receptor agonist treatment regulates inflammatory response after spinal cord trauma, *Journal of Neurochemistry*, **112**, 611–624, (2010).

[22] G. Praxinos and K. B. J. Franklin, The Mouse Brain in Stereotaxic Coordinates, Academic Press, 2001.

[23] J. Mey, New therapeutic target for CNS injury? The role of retinoic acid signaling after nerve lesions, *Journal of Neurobiology*, **66**, 757–779, (2006).

[24] P. Kastner, M. Mark, M. Leid, A. Gansmuller, W. Chin, J. M. Grondona, D. Decimo, W. Krezel, A. Dierich, and P. Chambon, Abnormal spermatogenesis in RXR beta mutant mice, *Genes & Development*, **10**, 80–92, (1996).

[25] J. Xu and P. D. Drew, 9-cis-Retinoic acid suppresses inflammatory responses of microglia and astrocytes, *Journal of Neuroimmunology*, **171**, 135–144, (2006).

[26] D. Ito, Y. Imai, K. Ohsawa, K. Nakajima, Y. Fukuuchi, and S. Kohsaka, Microglia-specific localisation of a novel calcium binding protein, Iba1, *Molecular Brain Research*, **57**, 1–9, (1998).

[27] S. A. Marshall, J. A. McClain, M. L. Kelso, D. M. Hopkins, J. R. Pauly, and K. Nixon, Microglial activation is not equivalent to neuroinflammation in alcohol-induced neurodegeneration: The importance of microglia phenotype, *Neurobiology of Disease*, **54**, 239–251, (2013).

[28] P. Acs, M. Kipp, A. Norkute, S. Johann, A. Braun, Z. Berente, S. Komoly, and C. Beyer, 17β-estradiol and progesterone prevent cuprizone provoked demyelination of corpus callosum in male mice, *Glia*, **75**, 807–814, (2009).

[29] K. S. Rawji and V. W. Yong, The benefits and detriments of macrophages/microglia in models of multiple sclerosis, *Clinical and Developmental Immunology*, **2013**, 1–13, (2013).