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**THE EFFECT OF TOLL-LIKE RECEPTOR AGONISTS ON
SURVIVAL, DIFFERENTIATION AND MYELIN FORMATION BY
OLIGODENDROCYTES**

MANUSCRIPT IN PREPARATION

ABSTRACT.

Toll-like receptors (TLRs) play a key role in controlling innate immune responses to a wide variety of pathogen-associated molecules and endogenous proteins. In this study we investigated expression of TLRs in primary cultures of rat oligodendrocyte progenitor cells (OPCs) at different development stages as well as in mature oligodendrocytes. OPCs and mature oligodendrocytes were found to express high levels of mRNA encoding TLR2 and, to lesser extent, TLR3 and TLR4. In addition, we also investigated the effects of treatment with different TLR agonists on differentiation, maturation, survival and myelin formation by oligodendrocytes. In highly purified oligodendrocytes cultures zymosan and LPS promoted survival, differentiation and myelin sheet formation. Poly I:C, in contrast, was a potent inducer of oligodendrocyte death and inhibited myelin formation.

Together, these data strongly suggest that TLRs in the brain play an important role in differentiation and myelination of oligodendrocytes, and not only in immune responses and injury.

INTRODUCTION

Toll-like receptors (TLRs) play a fundamental role in innate immune responses against invading pathogens. The different human TLRs are expressed in a wide variety of cell types and tissues and they are generally activated by conserved pathogen-associated molecules such as LPS, peptidoglycans, lipoproteins and by bacterial and viral nucleic acids¹⁻³. TLR-mediated signaling is characterized by activation of the NF- κ B signaling pathway resulting in production of pro-inflammatory mediator such as TNF- α , IL-1 β and IL-6⁴⁻⁶. Yet, some response variation has been observed between different TLR family members and between different types of cells. In the human central nervous system astrocytes, microglia and oligodendrocytes express different TLRs⁷. Recently, we have reported that activation through TLR3 but not TLR4 mediates a comprehensive astrocyte response including enhanced production of several neuroprotective, angiogenic and anti-inflammatory mediators rather than a polarized pro-inflammatory response. In organotypic human brain slice cultures the collective activity of mediators which are released by astrocytes upon TLR3 activation leads to significantly enhanced survival of neurons. These findings suggest that at least when expressed on the surface of astrocytes TLR3 activates neuroprotective and tissue repair responses rather than pro-inflammatory host defense responses⁸.

Oligodendrocytes, the myelinating cells of the central nervous system (CNS), wrap their cell membrane around axons to support rapid nerve impulse conduction⁹. They develop from bipotential oligodendrocyte-type-2-astrocyte progenitor (O2A), which can differentiate *in vitro* into either oligodendrocytes or astrocytes¹⁰⁻¹². OPCs persist in human adult CNS and can respond to injury by proliferating and migrating. Previous work has revealed that intracerebral injection of LPS, a TLR4 ligand, induced macrophage activation followed by recruitment and mobilization of oligodendrocyte progenitor cells. In contrast, however, injection of LPS into the brain led to oligodendrocyte death and demyelination^{13,14}. Similarly, marked demyelination has been observed in LPS-induced lesions in the dorsal spinal cord¹⁵. In addition, TLR2 agonist, zymosan, evoked total oligodendrocyte and axonal loss without regeneration. The same study also demonstrated that LPS caused acute loss of oligodendrocytes followed by a dramatic rise in oligodendrocyte numbers¹⁶. To the best of our knowledge, no information has been published so far on the effects of TLR activation on oligodendrocytes *in vitro*.

The goal of the present study was to examine the expression of TLRs in rat oligodendrocytes, and *in vitro* effects of TLR agonists on rat oligodendrocyte survival, differentiation and myelination of these cells. For this reason we compared the effects of zymosan, poly I:C and LPS, agonists for TLR2, TLR3 and TLR4, respectively, on oligodendrocytes at different developmental stages; from progenitor cells to mature oligodendrocytes. We found that rat oligodendrocytes express high levels of TLR2 and, to a

lesser extent, TLR3 and TLR4. In addition, in highly purified oligodendrocytes cultures zymosan and LPS promoted survival, differentiation and myelin sheet formation. Poly I:C, in contrast, was a potent inducer of oligodendrocyte death and inhibited myelin formation.

MATERIALS AND METHODS

Primary oligodendrocyte cultures

Rat oligodendrocyte progenitor cells (OPCs) were isolated and cultured as previously described¹⁷. Briefly, forebrains were collected from 1-3 day old Wistar rats and after papain digestion the cell suspension was gently triturated and washed with DMEM medium (Life Technologies, Paisley, Scotland) containing 10% FCS (Bodinco, Alkmaar, The Netherlands) and antibiotic supplements (Life Technologies, Paisley, Scotland). Cells were grown in PLL-coated flasks for 10-14 days. OPCs, growing on top of astrocytes were isolated by a shake-off procedure as previously described¹⁸ and cultured for 2 days on PLL-coated cell culture dishes in a defined SATO medium¹⁹ containing 10 ng/mL of the growth factors PDGF-AA and FGF (PeproTech, Rocky Hill, NJ). At the start of the experiments typical cultures contained less than 1 % microglia, 1-3 % astrocytes, and less than 1% neurons as assessed by immunocytochemistry using ED1 (a kind gift of Dr. Christien Dijkstra, VUmc, Amsterdam, the Netherlands), anti-GFAP antibodies (Millipore, Temecula, CA) and TuJ1 (a kind gift of Dr. A. Frankfurter, Charlottesville, VA), respectively. Differentiation was induced by growth factor withdrawal and culturing the cells for 10 days (mature myelinating stage) in SATO medium containing 0.5% FCS with medium changes twice a week. Cells from different growth stages (Fig. 1) were treated with different TLR agonists including zymosan (Invivogen, San Diego, CA), poly I:C (Pharmacia Biotech, Buckinghamshire, UK) and ultra pure LPS (Invivogen, San Diego, CA).

RT-PCR analysis of TLR expression

Total RNA from oligodendrocytes was isolated using the RNeasy Mini kit (Qiagen, Hilden, Germany). The yield and purity was quantified spectrophotometrically by measuring A_{260} and A_{280} optical densities. Total RNA (1 μ g) was reversed transcribed in the presence of oligo(dT)12-18 and dNTPs (Gibco Invitrogen, Paisley, UK) with superscript II reverse transcriptase (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. PCR amplifications were performed on copy DNA using primers specific for rat TLRs and the housekeeping gene GAPDH (Table 1).

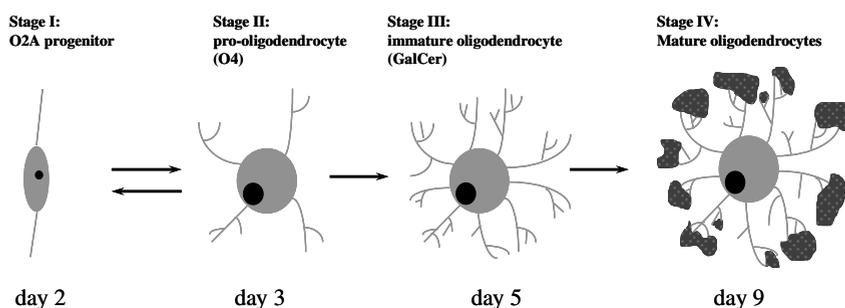


Figure 1: A model for oligodendrocyte development. *In vitro* differentiation of oligodendrocyte progenitor cells (OPCs) in a four-stage process resulting in mature myelinating oligodendrocytes

Table 1: Primer sequences used during RT-PCR.

Gene	Sense	Anti-sense	Length (bp)
GAPDH	CAGTGCCAGCCTCTGCTCAT	ATACTCAGCACCAGCATCAT	303
TLR2	GTCCATGTCTCTGGTTGACTGG	GATACCACAGCCCATGGAAAT	199
TLR3	TAGATGCACAGGGTGAGCAG	AGGCGT TTA TCC GTT CCT TT	426
TLR4	GAGCCGGAAAGTTATTGTGG	AGCAAGGACTTCTCCACTTTCT	150

MTT and LDH assay

Mitochondrial dehydrogenase activity was measured by using the MTT assay. OPCs were seeded into a PLL-coated 24-wells plate in 500 μ l of SATO medium at 1.0×10^6 cells/mL. The cells were treated with 10 μ g/ml zymosan, 1 μ g/mL poly I:C and 200 ng/ml LPS, added at different developmental stages, unless otherwise indicated. TLR agonists were continuously present for the duration of the experiment. Staurosporin-treated cells and untreated cells served as controls. After 10 days, the cells were incubated for 2 h with MTT (0.5 mg/ml, Sigma) in SATO medium. The medium was removed, the MTT-formazan crystals were collected in 15% isopropanol in 100 mM Tris (pH 10) and the absorption at 560 nm was determined. In parallel, a commercially available LDH assay kit (Roche, Indianapolis, IN) was used to quantify LDH release into the medium from the same cells as used for the MTT assay. The medium was centrifuged for 7 min at 7,000 rpm, and LDH release was assayed according

to the manufacturer's instructions. . To compare different experiments, for both assays, the values obtained for the treated cells were correlated to the value of untreated control cells, which was set to 100.

TUNEL assay

Cells were cultured in PLL-coated 8 well chamber slides at a density of 15,000 cells per well . OPCs were allowed to differentiate for 3 days, after which they were treated for 16 hrs with poly I:C at the indicated concentrations. To identify oligodendrocytes, immunocytochemistry on live cells was performed using the R-mAb (hybridoma was a kind gift of Dr. Guus Wolswijk, Amsterdam, The Netherlands, Ranscht et al., 1982) which recognizes the myelin-typical glycosphingolipids, galactosylceramide and sulfatide. Apoptosis was detected using TUNEL according to the manufacturer's instructions (Apoptag Red, Millipore). Apoptosis was examined by the percentage of TUNEL-positive, R-mAb-positive cells in a given condition. Nuclei were stained with DAPI. In each experiment at least 250 R-mAb positive cells were scored.

Immunocytochemistry

Cells were cultured in PLL-coated 8 well chamber slides (Nalgene Nunc, Naperville, IL) at a density of 15,000 cells per well. Antibody staining of the cell surface components, GalCer and sulfatide, was performed on live cells at 4°C or on fixed, non-permeabilised cells. For live staining, aspecific binding was blocked with 4% BSA in PBS for 10 min, after which the oligodendrocytes were incubated with R-mAb (1:10) for 30 min, washed three times with ice-cold PBS, and incubated for 25 min with the appropriate TRITC-conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). After washing with PBS cells were fixed with 4% paraformaldehyde (PFA). For staining of internal antigens, cells were gently fixed with 2% PFA for 15 min at room temperature (RT), followed by 4% PFA for 15 min at RT, after which they were either permeabilized with ice-cold methanol (MBP) or 0.1% Triton X-100 (PLP) for 10 min. After a 30-min block with 4% BSA, cells were incubated for 60 min with the primary anti-MBP antibody (1:25 in 4% BSA, Serotec, Oxford, UK) or anti-PLP antibody (4C2, 1:10, kind gift of Dr. V. Kuchroo, Harvard Medical School, Boston, MA) at RT. Next, the slides were rinsed with PBS, and incubated for 25 min with the appropriate TRITC-conjugated antibody. For actin filament staining, cells were fixed, after which they were permeabilized and blocked with 0.1% Triton X-100 and 4% BSA, respectively. Nuclei were stained with DAPI and mounting medium (anti-fading) was added to prevent image fading. The cells were analyzed with a conventional fluorescence microscope (Olympus ProVis AX70) equipped with analysis software. Oligodendrocytes were characterized upon morphology, and in each experiment at least 500 cells were scored as MBP-positive or negative. In addition, positive cells bearing MBP-positive membranous structures spread

between the cellular processes were identified as membrane sheet-forming, irrespective of the extent of sheet formation. To compare different independent experiments, the data are expressed as % of control, i.e., untreated cells, which were set to 100.

Western blot analysis

Cells were cultured in 10 cm PLL-coated 10 cm dishes at a density of 1.0×10^6 cells/dish. Cells were washed three times with PBS, harvested by scraping, and centrifuged at 10,000 rpm at RT. Pellets were lysed in TNE-lysis buffer (50 mM Tris-HCl, 5 mM EDTA, 150 mM NaCl, 1% Triton X-100, and protease inhibitor cocktail (Complete Mini, Roche Diagnostics GmbH, Mannheim, Germany), pH 7.4). Protein concentrations were determined by a BioRad DC protein assay (Bio-Rad Laboratories, Hercules, CA) using BSA as standard. Equal amounts of proteins were diluted with reducing sample buffer, heated for 5 min at 98°C and loaded onto 10% SDS-polyacrylamide gels. Proteins were transferred to PVDF membranes using a semi-dry blotting system and a glycine-Tris-methanol buffer. The membranes were rinsed with PBS and incubated for 1 h in blocking solution (Li-Cor Biosciences, Lincoln, NE). After washing with PBS containing 0.1% Tween-20, the membranes were incubated overnight with primary antibodies (PLP, 1:50, CNPase 1:250 (Sigma, St. Louis, MO)) diluted in blocking solution diluted 2x in PBS containing 0.1% Tween-20. After washing with PBS containing 0.1% Tween-20, the membranes were incubated for 1 h with appropriate IRDye-conjugated antibodies (Li-Cor Biosciences, IRDye) and washed three times with PBS containing 0.1% Tween-20. The signals were detected using the Odessey Infrared Imaging System (Li-Cor Biosciences).

Statistics

Data represents means of at least three independent experiments \pm SD, and statistical analysis was performed using the Student's *t*-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

RESULTS

Expression of TLR-encoding mRNA in primary cultures of rat glia cells

As a first evaluation of TLR expression in rat oligodendrocytes, the presence of TLR-encoding mRNA was examined by RT-PCR in primary cultures of oligodendrocyte progenitor cells at different development stages as described in Fig. 1. We focused our investigation on TLRs, 2, 3 and 4 since our previous studies⁷ suggested that these are the only detectable TLR family members in human oligodendrocytes. The results as represented in Fig. 2 revealed high

expression of TLR2 in oligodendrocytes at stage I and gradually decreasing levels at stage II, III and IV. TLR3 and TLR4 mRNA expression was also detected at different stages but at somewhat lower mRNA levels. Rat astrocytes express high levels of TLR3 and to some extent also TLR4 and TLR2.

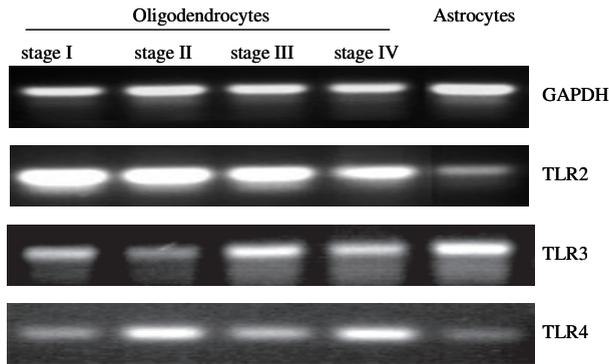


Figure 2: Expression of TLRs in primary rat oligodendrocytes. Oligodendrocyte progenitor cells (OPCs) were obtained from 1-3 days old Wistar rats and mRNA was isolated from at different development stages. TLR expression was examined by RT-PCR. Amounts of cDNA used for the amplification reactions were standardized to produce identical amounts of amplicons for GAPDH.

Effects of TLR agonists on survival of differentiated primary oligodendrocytes

To investigate the effects of TLR agonists on the survival of oligodendrocytes at different development stages, progenitor cells were isolated, plated in 24-well culture plates, and treated with poly I:C, zymosan and LPS at different stages. Treatment started at stage I, stage III or stage IV and was continued until day 11 when cells reached the MBP⁺ stage (mature oligodendrocytes). Cell viability was monitored using an MTT assay. As shown in fig. 3 A, treatment of oligodendrocytes with zymosan induced 150% and 100% MTT activity starting at stage I and stage III, respectively, indicating that zymosan had a positive effect on proliferation and survival of oligodendrocytes. Approximately 30-40% reduction of MTT activity was observed after poly I:C and LPS treatment at different stages as compared to control cultures, except for LPS treatment starting at stage I. Under those conditions LPS did not reduce the MTT levels in cultures of oligodendrocyte progenitor cells (Fig. 3A). To further evaluate the effects of the TLR agonists on maturing oligodendrocytes, the release of LDH, a marker of increased membrane permeability and lethal cell injury, was measured. The data in Fig. 3B indicate that poly I:C, zymosan or LPS treatment had an only marginal effect on the release of LDH whereas the control compound staurosporine induced marked cell death and led to a two-fold increase in LDH release at all stages.

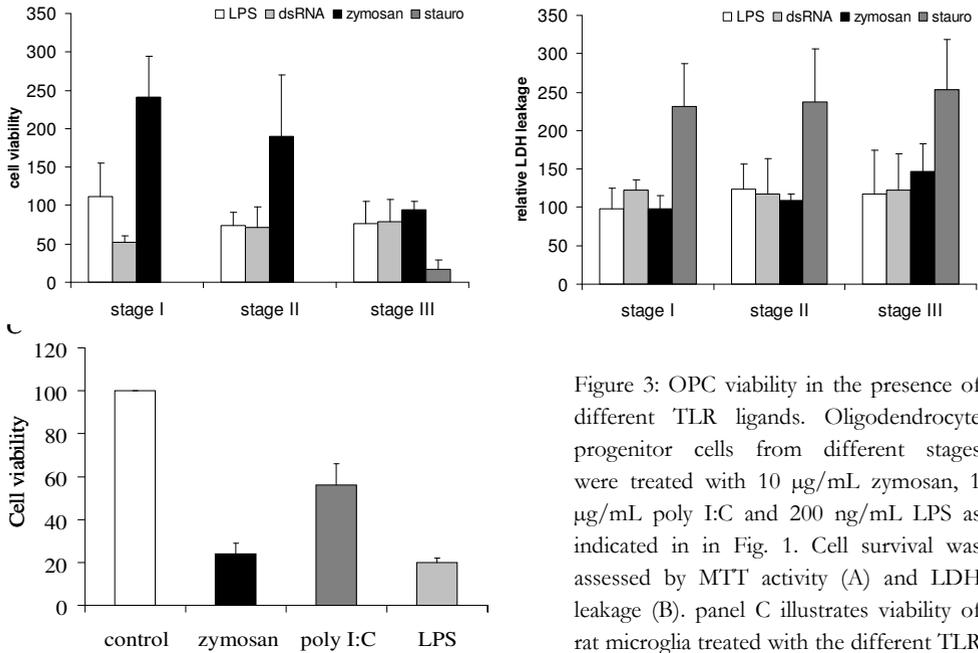


Figure 3: OPC viability in the presence of different TLR ligands. Oligodendrocyte progenitor cells from different stages were treated with 10 $\mu\text{g}/\text{mL}$ zymosan, 1 $\mu\text{g}/\text{mL}$ poly I:C and 200 ng/mL LPS as indicated in in Fig. 1. Cell survival was assessed by MTT activity (A) and LDH leakage (B). panel C illustrates viability of rat microglia treated with the different TLR ligands.

As a control, we evaluated the effect of zymosan, LPS and poly I:C on MTT activity on pure microglia and astrocyte cultures. As shown in Fig. 3 C, approximately 76, 80, 54% reduction of MTT activity was observed on microglia after zymosan, LPS and poly I:C treatment, respectively. The same was found for astrocyte cultures (data not shown). This suggests that the different TLR ligands have a negative effect on the proliferation of microglia and astrocytes, indicating that the effect on oligodendrocytes observed are indeed direct. In addition, incubation of cells with increasing concentrations of poly I:C induced dose-dependent apoptosis. Nuclei of apoptotic OPC were fragmented (fig.4), cells were coagulated and MBP staining was weak even at low dose of poly I:C (1 $\mu\text{g}/\text{mL}$) (data not shown).

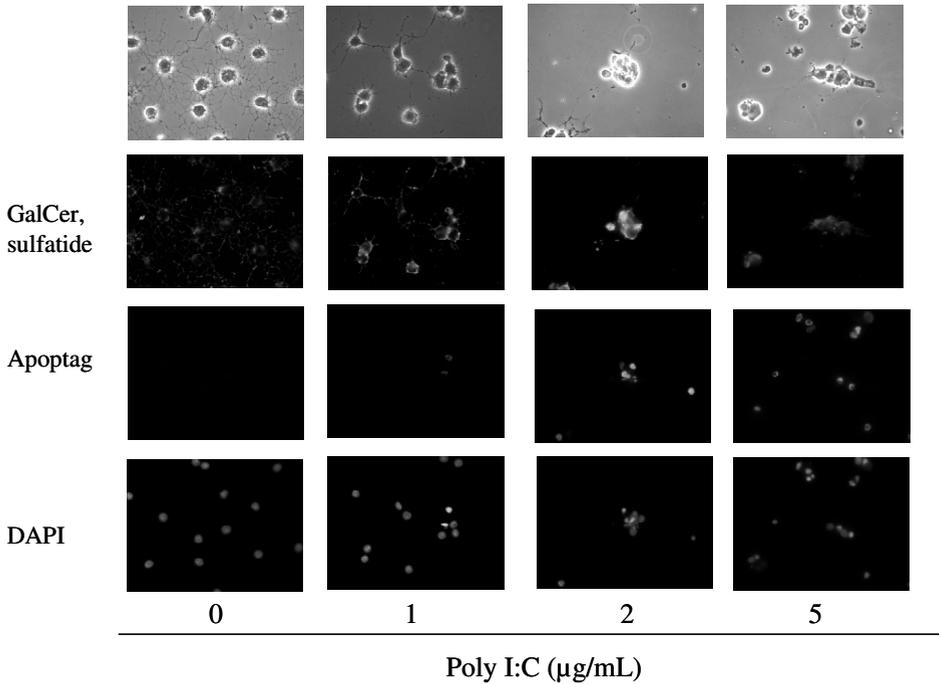


Figure 4: The effect of poly I:C on oligodendrocytes. Oligodendrocytes were treated with different concentration of poly I:C en then stained with GalCer, Sulfatide immunofluorescence, Apoptag and DAPI. See page 202 for a full-color representation of the figure.

Effects of TLR agonists on morphology and differentiation of OPCs

Differentiation of progenitor cells was initiated by withdrawal of growth factors. To asses the effects of the different TLR agonists on the differentiation of OPCs, treatment was commenced on stage I from day 2 onwards and ended at the MBP⁺ stage at day 11. At this stage the morphology and levels of myelin proteins were examined. Cells shown in Fig. 5A were labeled with phalloidin, which reveals the cellular distribution of F-actin and the full extent of very fine processes. In control cultures of oligodendrocytes and zymosan-treated cells actin filaments were predominantly extended to the cell edges whereas poly I:C induced redistribution of actin filament to the cell body. In LPS-treated cells actin filaments were predominantly present in processes.

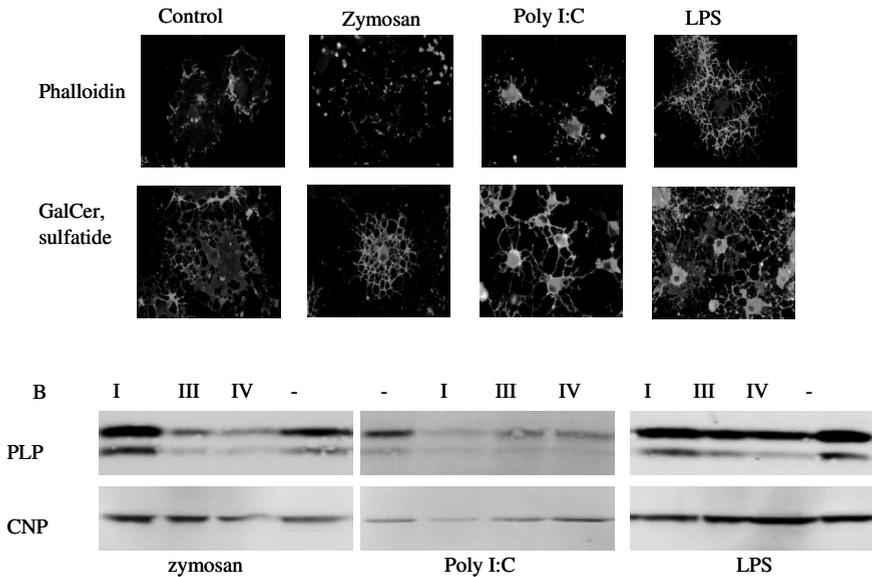


Figure 5: Morphological and western blot evaluation A) Immunofluorescence staining of oligodendrocytes treated with 10 $\mu\text{g}/\text{mL}$ zymosan, 1 $\mu\text{g}/\text{mL}$ poly I:C and 200 ng/mL LPS after 10 days differentiation *in vitro* (from stage I to stage IV). Cells were stained for phalloidin and GalCer/sulfatide. B) Representative western blot for CNPase and PLP in differentiated OPCs after zymosan, poly I:C and LPS treatment. See page 203 for a full-color presentation of this figure.

To further characterize the effects of TLR agonists on the differentiation of oligodendrocyte progenitor cells, we studied the expression and localization of differentiation markers including GalCer, sulfatide, CNPase and PLP in cells treated at different stages. Immunofluorescence staining of control cultures in the MBP⁺-stage revealed that GalCer/sulfatide immunofluorescence staining was found in the processes and cell bodies (Fig. 5A). Addition of zymosan and LPS resulted in a higher degree of differentiation as reflected by increased numbers of cells with extended process branching and extensive membrane networks. In contrast, after poly I:C treatment most cells had a less complex morphology as compared to control cells, with fewer processes and less intense GalCer/sulfatide staining, suggestive a less differentiated phenotype. In cells treated with poly I:C, GalCer/sulfatide staining was mainly found in the cell body. In addition, we determined CNPase and PLP protein expression by Western blot analysis. Whereas CNPase expression,

expressed from stage III on, was not changed during treatment with either zymosan or LPS, poly I:C treatment started from stage I resulted in decreased CNPase expression. In addition, zymosan treatment of oligodendrocytes started from stage I resulted in enhanced PLP expression, which was markedly decreased after treatment with poly I:C but not with LPS (fig. 5B).

Effects of TLR agonists on maturation and myelin sheet formation

Next, we studied the effects of TLR agonists on maturation and myelin sheet formation by oligodendrocytes. Purified OPCs were grown for two days in SATO medium containing growth factors (stage I), after which differentiation was initiated upon growth factor withdrawal. At day 4 after differentiation, most cells were differentiated and expressed GalCer and CNPase (stage III). To assess the effects of TLR agonists on myelin sheets, treatment was commenced at day 4. To assess their effect on mature myelin sheets treatment was commenced at day 8 (stage IV) and continued until MBP+ stage (day 11). Morphology and levels of myelin protein expression were examined. Cells shown in Fig. 6 were stained for PLP. At the MBP+ stage zymosan and LPS supplementation from either stage III or stage IV resulted in more prominent PLP staining (Fig. 6) especially in myelin-like membranes and processes and intense MBP staining (data not shown) as compared to non-treated cells. In poly I:C treated cells, PLP (Fig. 6) and MBP staining (data not shown) were very weak and mainly present in the cell body and in primary processes.

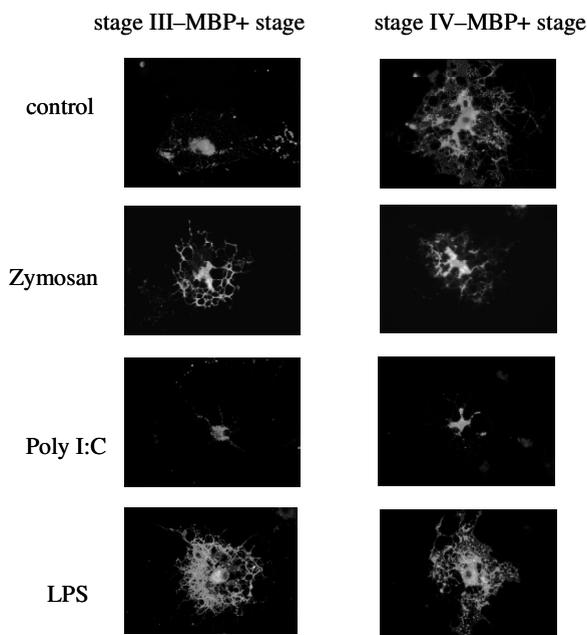


Figure 6: PLP protein expression in oligodendrocytes. Oligodendrocytes were treated from stage III and stage IV onwards with 10 $\mu\text{g}/\text{mL}$ zymosan, 1 $\mu\text{g}/\text{mL}$ poly I:C or 200 ng/mL LPS and were stained at stage IV for PLP. See page 204 for full-color presentation of this figure.

To assess the effects of TLR ligands on differentiation, myelination and demyelination of oligodendrocytes, treatments were started at stage I, stage III and stage IV, respectively. Quantification of the number of MBP-positive cells (differentiation) and of the MBP-positive sheets (myelination) during primary oligodendrocyte differentiation is summarized in Fig. 7. Treatment of OPCs (stage I) with zymosan resulted in a higher degree of maturation and myelination, as reflected by the increased numbers of MBP+ cells and MBP+ sheets, especially when treatment was started at stage IV. LPS treatment had less effects on maturation and myelination of oligodendrocytes. In contrast, poly I:C significantly decreased the percentage of MBP+ cells and MBP+ sheets, which is in line with the changes of PLP expression (Fig. 5). These results suggest that accelerated oligodendrocyte differentiation occurs after treatment with zymosan, but not after treatment with poly I:C. Similarly, myelin sheet formation was also increased after zymosan and LPS supplementation.

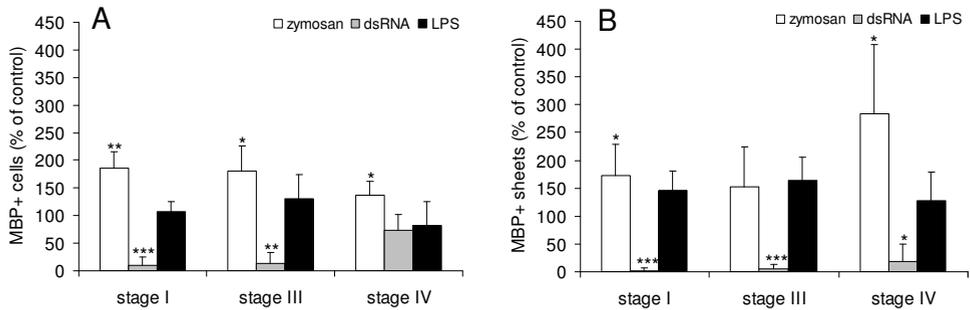


Figure 7: Quantification of MBP-positive cells and MBP-positive sheets. Oligodendrocyte progenitor cells were treated with TLR agonists at different development stages; from stage I onwards, stage III and stage IV. At their final stage cells were stained for MBP and MBP+ cells (A) and MBP+ sheets (B) were quantified by IF. Results presented as percentages of control cells. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$ versus control cells.

DISCUSSION

The aim of this study was to investigate the effect of TLR ligands on the differentiation, maturation and myelination of oligodendrocytes. Our data indicate that rat OPCs and mature oligodendrocytes express TLR2, 3 and 4, which is in line with the expression of these TLRs by human oligodendrocytes⁷. The clear expression of TLR2 in all three types of OPCs is remarkable, especially in the first stage of development, and suggests that TLR2 may play a role in the maturation of OPCs. In addition, all these TLRs appear to be functional. As in the case of astrocytes⁸, engagement of different TLRs appears to induce differential effects, which are only partially influenced by the developmental stage of oligodendrocytes. While engagement of TLR2 and TLR4 induces at least some stimulatory effect on differentiation and myelin sheet formation, activation of oligodendrocytes with poly I:C, a TLR3 agonist, had quite different effects. Some of the apparently confusing functional data on TLR3 activation may in part relate to the fact that TLR3 is not the only receptor for double-stranded RNA or poly I:C, which is often used as a synthetic agonist in experimental systems. Recent evidence has clarified that intracellular RNA helicases such as retinoic acid-inducible gene-I (RIG-I) and the melanoma differentiation-associated gene-5 (mda-5), also known as Helicard, may in fact be more important than TLR3 in mediating responses to viral double-stranded RNA as well as to exogenous poly I:C in some cell types^{20,21}. In addition poly

I:C also activates scavenger receptor A²², suggesting that the effects that we see with poly I:C treatment may not only be mediated by TLR3.

Morphological evaluation revealed that culturing of OPCs in medium supplemented with LPS and zymosan resulted in higher degree of differentiation reflected by an increased number of cells with extended process branching, and by the formation of extensive membrane networks. Poly I:C treatment, on the other hand, induced a less mature phenotype. Poly I:C-treated cells had a less complex morphology as compared to either control cells, LPS- or zymosan-treated cells.

During oligodendrocyte differentiation and maturation different myelin proteins are expressed, CNPase first, followed by MBP and PLP. At later stages, zymosan and LPS supplementation altered oligodendrocyte differentiation as revealed by increased numbers of MBP- and PLP-positive cells. Poly I:C treatment decreased numbers of MBP- and PLP-positive cells. This suggests that the activation of TLR2 and TLR4 on oligodendrocytes promotes cell differentiation and maturation, whereas, TLR3 reduces maturation and induces apoptosis. These data appear conflicting with our previous data suggesting that TLR3 may have a protective role in the brain. Our data also indicate that zymosan and LPS alter oligodendrocytes myelination as revealed by increasing numbers of MBP-positive sheets. At the MBP+ stage poly I:C significantly reduced the number of MBP+ sheets suggesting that poly I:C inhibits myelin formation.

Several *in vivo* studies, however, have shown that injection of LPS or zymosan into the brain results in oligodendrocyte death and demyelination^{14,16} and that exposure of mixed glial cultures to LPS induced death of oligodendrocytes²³. In addition, the apparent anti-inflammatory and clinically beneficial effects of poly I:C in experimental models of CNS are somewhat confusing²⁴. Together with our results, it can be assumed that the activation of TLR4 or TLR2 on microglia or astrocytes and not on oligodendrocytes induces OPC and oligodendrocyte toxicity by the release of mediators such as TNF- α , IL-1 β and NO, which are known to be toxic for oligodendrocytes.

In summary, we demonstrate here that primary oligodendrocytes express different TLRs and that the activation especially of TLR2 and TLR4 promotes cell differentiation, maturation and myelin formation by oligodendrocytes. Our studies warrant future *in vivo* and *in vitro* studies to more precisely establish the effects of TLRs on differentiation and myelination by human oligodendrocytes. Overall, our results indicate that TLRs are not only involved in the induction of injury of oligodendrocytes but that the activation of TLRs on oligodendrocytes themselves plays an important role in differentiation and maturation.

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