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Perinatal inflammation results in decreased oligodendrocyte numbers in adulthood

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ABSTRACT

Aims: Maternal inflammation is a risk factor for preterm birth, and premature infants are often exposed to supplemental oxygen as a life-sustaining therapy. While more immature neonates are surviving, rates of neurodevelopmental impairment are not improving. We developed a novel mouse model with clinically relevant exposures to test the hypothesis that systemic maternal inflammation with transient neonatal hyperoxia exposure will induce a phenotype similar to diffuse periventricular leukomalacia (PVL) like that observed in premature human infants.

Main methods: Timed-pregnant C3H/HeN mice received intraperitoneal injections of lipopolysaccharide (LPS) or saline on embryonic day 16. Newborn pups were placed in room air (RA) or 85% oxygen (O_2) for 14 days, followed by 14 days in RA recovery. Oligodendroglial and microglial populations were evaluated at 14 and 28 days.

Key findings: Brain weight to body weight ratios were lower in mice exposed to LPS. Oligodendrocyte numbers were decreased significantly in the cerebral cortex and hippocampus in groups exposed to LPS or LPS/O_2 at 14 days, and persisted in the cerebral cortex at 28 days for LPS/O_2 mice. At day 14, cleaved caspase 3 was increased and numbers of microglia were elevated in the cerebral cortex and hippocampus of LPS/O_2 animals.

Significance: These data indicate that combining systemic maternal LPS and neonatal hyperoxic exposure impairs myelination, and suggests that this novel mouse model may represent a subtle, diffuse form of periventricular white matter injury that could provide a clinically relevant platform for further study of perinatal brain injury.

Introduction

Advances in neonatal care have resulted in smaller and more immature babies surviving the perinatal period. However, adverse neurologic outcomes remain a significant consequence of premature birth, with as many as 25–50% of very low birth weight (VLBW) babies, born weighing less than 1500 g, experiencing some degree of neurodevelopmental impairment (Robertson et al., 2007; Volpe, 2009a; Allen, 2008). Historically, cerebral palsy was a major defining factor of adverse neurologic outcomes from the perinatal period. However, it is increasingly recognized that long term impairments related to brain injury in preterm neonates represent a spectrum of type and severity, involving mechanisms outside of classic intraventricular hemorrhage or cystic periventricular leukomalacia (PVL) (Back, 2006; Volpe, 2009b). This shift is encapsulated by descriptions of non-cystic PVL, characterized by diffuse periventricular white matter injury in the cerebrum involving a constellation of damage to vulnerable premyelinating oligodendrocytes, microglial activation, astrogliosis, and neuronal injury (Back, 2006; Volpe et al., 2011; Volpe, 2011).

Translational science including investigation of animal models is necessary to understand the mechanisms and develop preventive and treatment strategies for perinatal brain injury in preterm infants (Kinney and Volpe, 2012). Such animal models need to show brain pathology consistent with that seen in human neonates and should be induced by clinically relevant stimuli. While existing animal models have studied the impact of neonatal hypoxia–ischemia, chorioamnionitis, neonatal infection, and neonatal hyperoxia on brain development and injury (Burd et al., 2012; Boksa, 2010; Back et al., 2002; Hagberg et al., 2002), the effects of combined exposures have been reported only recently (Brehmer et al., 2012).

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Maternal inflammation and infection are risk factors for both preterm birth and subsequent cerebral palsy (Burd et al., 2012; Horvath et al., 2012; Soraisham et al., 2013). Systemic maternal inflammation in the absence of chorioamnionitis or other infection is commonly seen in mothers with chronic disease, obesity, preeclampsia, and diabetes (Schmatz et al., 2010). Animal studies have demonstrated that multiple infectious and inflammatory stimuli may induce a range of effects on the developing brain (Rousset et al., 2006, 2008; Debillon et al., 2000; Normann et al., 2009). Premature neonates commonly require ventilator support and supplemental oxygen therapy at a time when decreased antioxidant capacities make them most vulnerable to oxidative stress. Injurious effects of hyperoxic conditions on the developing brain have been reported in existing animal studies (Gerstner et al., 2008; Yis et al., 2008; Ramani et al., 2013; Zaghloul et al., 2012; Sifringer et al., 2012).

In this study, we investigated a novel mouse model of maternal systemic lipopolysaccharide (LPS) administration followed by transient neonatal hyperoxia exposure. Prior work in our laboratory indicates that this model is well suited to examining the effects of neonatal inflammation on developing systems. We have observed an inflammatory lung phenotype similar to severe bronchopulmonary dysplasia (Velten et al., 2010, 2012; Rogers et al., 2009) as well as significant functional and structural alterations in cardiac development that persist into adulthood (Velten et al., 2011).

The combination of systemic maternal inflammation and neonatal hyperoxia has overwhelming potential for injury in the neonatal brain during a critical developmental period. However, the neuropathology after these combined exposures has not been elucidated, even though it is one of the most common courses experienced by VLBW infants in Neonatal Intensive Care Units worldwide. Our hypothesis was that the combination of systemic maternal inflammation and transient neonatal hyperoxia exposure would result in patterns of injury consistent with pathologic changes observed in preterm infants.

Materials and methods

Animal model

Animal study protocols were approved by the Institutional Animal Care and Use Committee at the Research Institute at Nationwide Children's Hospital, Columbus, OH. Adult C3H/HeN mice (8–10 weeks old) were purchased from Harlan Sprague Dawley (Indianapolis, IN). Mice were housed in our facility for at least 7 days prior to breeding. Male and females were paired, and the presence of a vaginal plug was designated as embryonic day 1 (E1). On E16, dams received intraperitoneal injections of LPS (serotype 0111:B4, catalog no. 437627; Calbiochem, Gibbstown, NJ) in saline (approximately 0.1 mL), or saline only. The LPS dose (80 μg/kg) was chosen based on preliminary studies that consistently resulted in a viable litter (Velten et al., 2010). Newborn mice from saline- or LPS-injected dams were pooled and redistributed randomly (yielding mixed litters of 6 to 7 pups) to the two dams in separate cages within 24 h of birth. One litter of pups was exposed to 85% oxygen (O_2) for 14 days in a Plexiglas chamber which was regularly calibrated (chamber calibrated every other day with an Oxygen Analyzer, Hudson RCI, model 5577) while the litter of pups from the corresponding maternal E16 treatment was maintained in room air (RA) for the same period. Day 1 was defined as the first 24 h of O_2 or RA exposure. Litters were either sacrificed at day 14 (after hyperoxia exposure) or maintained in RA for 14 days of recovery and sacrificed at day 28. In this fashion, four treatment groups were created: saline/RA, saline/O_2, LPS/RA, and LPS/O_2. To avoid oxygen toxicity in the dams and reduce confounding maternal effects between groups, the nursing dams from the same E16 treatment groups were rotated between their RA and O_2 litters every 24 h.

Histology processing

At days 14 and 28, two animals from each litter were anesthetized with intraperitoneal administration of ketamine/xylazine (150 mg/kg:15 mg/kg, respectively). Whole-body perfusion was performed with ice cold saline followed by freshly prepared ice cold neutral buffered 4% paraformaldehyde. Brains were removed immediately and immersed in fresh fixative for 24 h, then washed 3× in phosphate buffered saline (PBS) and transferred into PBS until processed and embedded into paraffin blocks. Coronal sections cut serially at 4 μm were placed on positively charged slides and then either stained with hematoxylin and eosin to evaluate general tissue architecture or labeled by indirect immunohistochemistry to examine the distribution of selected cell populations (Table 1). All immunohistochemical methods were visualized with a polymer detection system and the chromogen 3,3′-diaminobenzidene (DAB; kit DS9800; Leica Microsystems, Buffalo Grove, IL). Sections were counterstained with hematoxylin.

Glial cell counts

For both days 14 and 28, oligodendrocyte and microglial counts were performed on selected brain regions (cerebral cortex, hippocampus) of the middle cerebrum at the level of the internal capsule and thalamus. Intracortical oligodendrocytes, defined by their immunoreactivity with 2′, 3′ cyclic nucleotide 3′ phosphodiesterase (CNPase), and microglia, identified by their immunoreactivity with ionizing calcium-binding adaptor molecule 1 (Iba1), were counted between the cingulum and rhinal fissure in four non-overlapping high-power fields (i.e., 100×). Additionally, tissue counts were performed on hippocampal neurons within the dentate gyrus in two non-overlapping high-power fields. Microglial cell bodies were also quantified in four non-overlapping high-power fields in the cerebral cortex and hippocampus. All counts were performed by a single investigator (oligodendrocytes by KMH, microglia by AEG) for consistency.

Caspase 3 expression

Whole brains were extracted from randomly selected pups (2 per litter) at postnatal days 3, 7, and 14. Whole brains were selected as the sample due to the small size of the brains at the early time points,

Table 1
Antibodies for immunohistochemistry analyses.

Antibody	Clone	Dilution	Antigen retrieval	Catalog #	Supplier
CNPase (oligodendrocytes)	11-5B mouse anti-human	1:800	Citrate	ab6319	Abcam, Cambridge, MA
MBP (oligodendrocytes, myelin)	7H11 mouse anti-human	1:200	Citrate	NCL-MBP	Leica Microsystems, Buffalo Grove IL
Iba-1 (microglia, macrophages)	Rabbit anti-human polyclonal	1:1000	EDTA	PP290-AA	Biocare Medical, Concord, CA
GFAP (reactive astrocytes)	Rabbit anti-guinea pig polyclonal	Predilute	None	RB-087-R7	Thermo LabVision, Kalamazoo MI

All antibodies were diluted with Antibody Diluent, catalog# S3022, Dako, Carpentaria CA. Abbreviations: CNPase—2′, 3′ cyclic nucleotide 3′ phosphodiesterase; MBP—myelin basic protein; Iba-1—ionizing calcium-binding adaptor molecule 1; GFAP—glial fibrillary acidic protein.

which precluded regional microdissection. Specimens were frozen in liquid nitrogen and maintained at −80 °C until analyses. Frozen whole brains were homogenized, and protein concentrations were determined by Bradford assay. For western blots, proteins were separated on SDS-PAGE gels and transferred to PVDF membranes, which were then probed with rabbit monoclonal anti-cleaved caspase 3 primary antibody (catalog# 9664; Cell Signaling, Boston, MA) at a 1:500 dilution. Antibody binding was detected using anti-rabbit secondary antibody (catalog #170-6515, Bio-Rad Laboratories, Hercules, CA) at 1:12,000 and then developed using enhanced chemiluminescence (ECL Prime Western Blotting Detection, GE Healthcare, UK). Expression levels were quantified using Image Quant Software, Version 5.0 (Molecular Dynamics, Sunnyvale, CA). The density of the band for the protein was normalized to the density of β-actin protein (catalog# ab6276; Abcam, Cambridge, MA).

Statistics

Statistical analyses were performed by two-way analysis of variance (ANOVA) followed by least significant difference (LSD) or Tukey's tests (corrected for multiple analyses). All data are presented as means ± standard error of the mean (SEM), and the results of the two-way ANOVA are indicated. Significance was defined as $p < 0.05$. All analyses were performed with GraphPad PRISM 5 (La Jolla, CA).

Results

Brain and body weight growth patterns differ after LPS or O_2 exposures

At 14 days, hyperoxia exposure caused a modest decrease in body weight with preservation of brain weight (Table 2) leading to an effect of hyperoxia on the brain:body weight ratios (Table 2). At day 28, after 14 days in RA recovery, there was "catch-up" growth of the body weight in the saline/O_2-exposed group and even greater gain in the LPS/O_2 group. There was evidence of excessive weight gain in the mice exposed to LPS which resulted in brain to body weight ratios that were lower in the LPS-treated groups than in the saline-treated groups at day 28 (Table 2).

Gross pathological changes were not observed across treatment groups

Hematoxylin and eosin stained brain sections were evaluated at days 14 and 28. These sections were examined for gross pathological changes including infarction, focal areas of necrosis, cyst formation, significant hemorrhage, or parenchymal abnormalities visible at low magnification. No overt neuropathological abnormalities were observed in any of the treatment groups at either time point (data not shown).

Cortical early myelinating oligodendrocyte cell number persistently decreased after LPS and O_2

Numbers of CNPase-positive cortical oligodendrocytes were decreased by both LPS and O_2 exposures. At 14 days, cortical oligodendrocytes were fewer in LPS/RA and saline/O_2 and LPS/O_2 compared to saline/RA (Fig. 1A and C). The lower numbers of cortical oligodendrocytes persisted at 28 days in the LPS/O_2 group compared to LPS/RA and saline/RA (Fig. 1B and D). Qualitatively, staining with myelin basic protein (MBP, a marker of mature myelinating oligodendrocytes) at 28 days was reflective of the quantitative results from CNPase (Fig. 1E). The intensity of staining and progression of myelination from the corpus callosum through the neocortex was decreased in LPS- and O_2-treated groups at 28 days, and was most pronounced in the hyperoxia groups.

Hippocampal early oligodendrocytes decreased at 14 days

Numbers of CNPase-positive hippocampal oligodendrocytes in the dentate gyrus were significantly decreased by treatment with LPS and O_2. At 14 days, hippocampal oligodendrocytes were decreased in the LPS-treated groups compared to the saline-treated groups, with the lowest numbers in the LPS/O_2-treated group (Fig. 2A and C). Following the 14-day RA recovery, at day 28 there was no significant difference in hippocampal oligodendrocyte numbers (Fig. 2B and D).

Combined LPS/O_2 exposure increased cleaved caspase 3 levels

Cleaved caspase 3 levels measured by western blot in whole brain homogenates were similar across treatment groups at day 3 and day 7 (data not shown). At day 14, increased levels of cleaved caspase 3 were observed in the LPS/O_2 treatment group (Fig. 3).

Microgliosis, but not astrocytosis, observed at 14 days after combined LPS/O_2 exposure

Pups exposed to LPS and O_2 had increased numbers of Iba1-positive microglia in both the cerebral cortex (Fig. 4 A and C) and the hippocampus at day 14 (Fig. 4 B and D) compared to all other groups. However, numbers of Iba1-positive cells were no longer elevated in either region by day 28 (data not shown). Qualitative analysis of sections stained for GFAP-positive astrocytes showed no differences among treatment groups at either day 14 or 28 (data not shown).

Discussion

The complex interaction between the maternal environment and fetal development directly influences the spectrum of neurodevelopmental deficits in VLBW infants (Kinney et al., 2012). Those neonates who

Table 2
Brain weights and brain to body weight ratios.

Treatment	N		Day 14[†]			Day 28		
	Litters *d14/d28*	Pups *d14/d28*	Brain weight[†] *g*	Body weight[+,†] *g*	Brain:body weight[a,†]	Brain weight[+] *g*	Body weight[+] *g*	Brain:body weight[a,+]
Saline/RA	*6/5*	*11/10*	0.44 ± 0.01	8.56 ± 0.11	5.01 ± 0.05	0.44 ± 0.01	13.94 ± 0.59	3.16 ± 0.11
Saline/O_2	*6/5*	*11/10*	0.41 ± 0.01[#]	8.05 ± 0.15[*,#]	5.28 ± 0.17[#]	0.43 ± 0.02[#]	13.39 ± 0.61[#]	3.24 ± 0.14[*,#]
LPS/RA	*4/4*	*9/7*	0.46 ± 0.01	9.55 ± 0.20[*]	4.77 ± 0.08	0.47 ± 0.02	16.88 ± 0.82[*]	2.64 ± 0.10[*]
LPS/O_2	*4/4*	*9/7*	0.42 ± 0.02	8.33 ± 0.47[#]	5.15 ± 0.17	0.46 ± 0.01	16.64 ± 0.40[*,^]	2.77 ± 0.08[*,^]

An effect of LPS (+); effect of O_2 (†); and interaction between LPS and O_2 (‡) were indicated by two-way ANOVA, $p < 0.05$. LSD post hoc analyses indicated the following significant findings: *different than saline/RA; #different than LPS/RA; ^different than saline/O_2.
Abbreviations: LPS = lipopolysaccharide, O_2 = hyperoxia, RA = room air.
[a] Data are expressed as g/100 g animal.

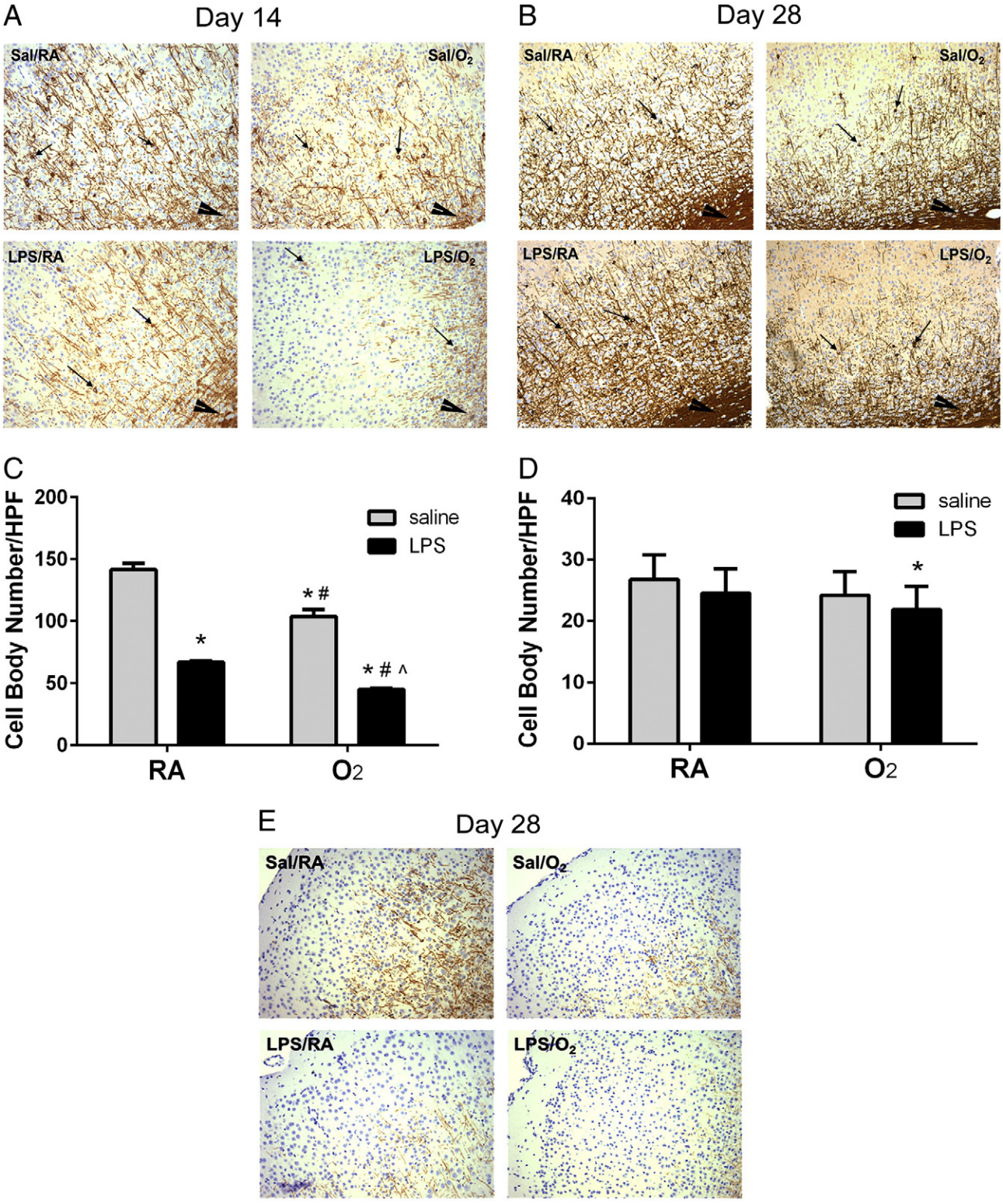

Fig. 1. Oligodendrocyte numbers are decreased in the cerebral cortex by treatment with LPS and/or O_2. A and B, representative images of saline/RA, saline/O_2, LPS/RA and LPS/O_2 treatment groups, labeled with CNPase as a marker for early myelinating oligodendrocytes, are shown (100×). C and D, labeled cortical oligodendrocyte cell bodies were counted per high-power field in the cerebral cortex between the cingulum and rhinal fissure. Significant effect of LPS and an effect of O_2 were indicated by two-way ANOVA at both day 14 and day 28, $p < 0.05$, $n = 2$–4 litters, $n = 1$–2 pups per treatment group. LSD post hoc analyses indicated: *different than saline/RA; #different than LPS/RA; ^different than saline/O_2. Arrowheads provide orientation toward the white matter at the base of the cortex. Small arrows identify representative CNPase-positive cell bodies.

experience seemingly benign hospital courses are still at risk for impaired neurodevelopment, despite normal-appearing routine head ultrasonography (El-Dib et al., 2010). Our findings in this study demonstrate a pattern of cellular injury consistent with aspects of diffuse PVL as described in human neonates. The phenotype is subtle compared to others' previously described, enhancing the potential for clinical relevance of our model.

In examination of the brain:body ratios, there were lower brain weights associated with the 14 days of hyperoxic exposure, thus leading to increased ratios in the O_2-treated groups at day 14 (Table 2).

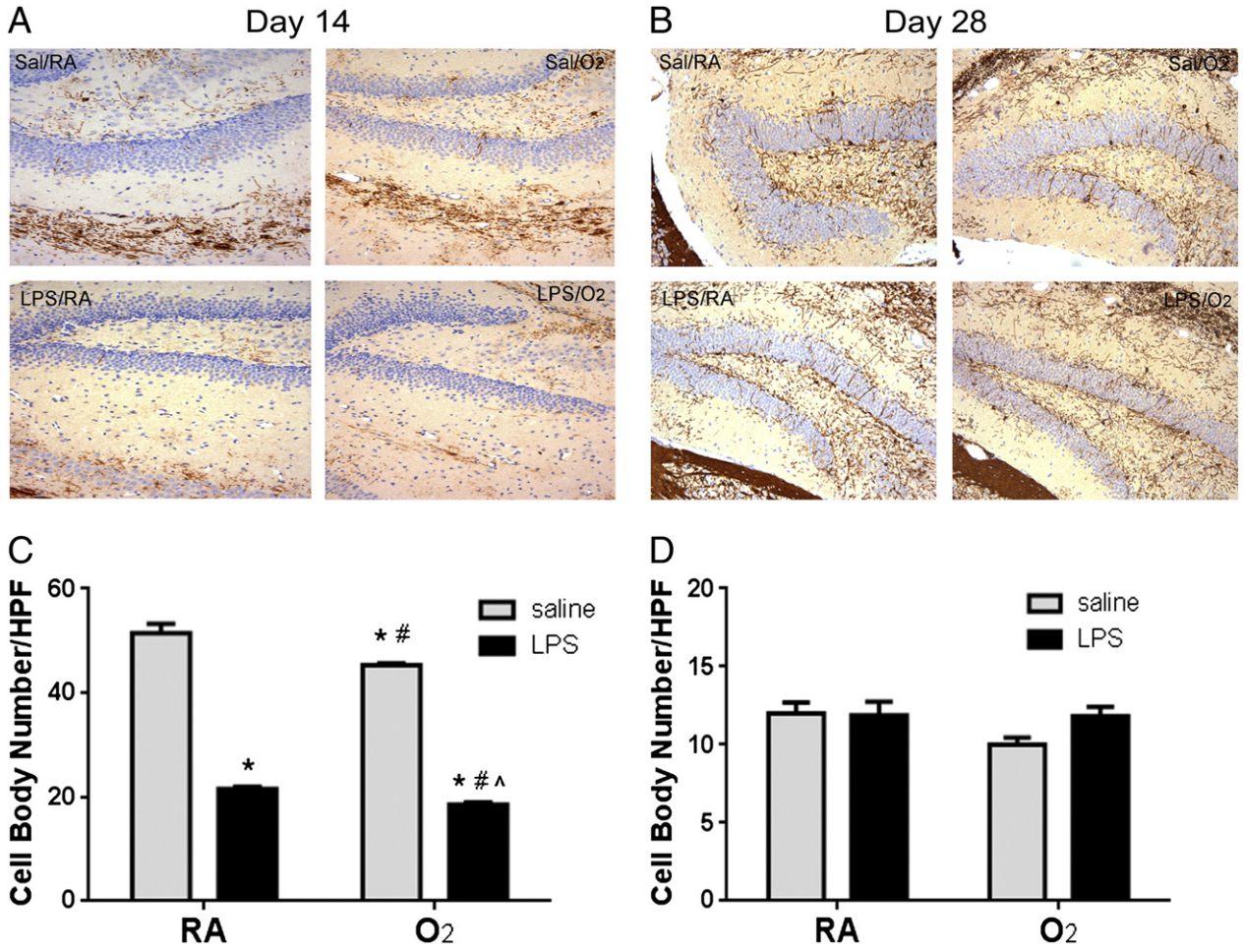

Fig. 2. Oligodendrocyte numbers are decreased in the dentate gyrus of the hippocampus by treatment with LPS and/or O_2. A and B, representative images of saline/RA, saline/O_2, LPS/RA and LPS/O_2 treatment groups, labeled with CNPase as a marker for early myelinating oligodendrocytes, are shown (100×). C and D, labeled hippocampal oligodendrocyte cell bodies were counted per high-power field in the dentate gyrus. Significant effect of LPS and an effect of O_2 were indicated by two-way ANOVA at day 14, $p < 0.05$, $n = 2–4$ litters, $n = 1–2$ pups per treatment group. LSD post hoc analyses indicated: *different than saline/RA; #different than LPS/RA; ^different than saline/O_2. No statistical differences were indicated at day 28.

Unexpectedly at day 28, after 14 days of room air recovery, the brain: body weight ratios were lower as a result of a significant increase in body weight in the LPS-exposed groups, independent of O_2 exposure. These findings suggest a lasting effect of the maternal inflammatory response. Velten et al. reported that birth weights of LPS-exposed pups were decreased compared to those of saline controls. In the same study, body weights of LPS/O_2 exposed animals were significantly lower than those of saline/RA controls at 8 weeks of age, when impaired cardiac function was identified (Velten et al., 2012). Based upon these combined findings, we could speculate that the LPS-exposed pups develop metabolic disturbances related to intrauterine growth restriction associated with maternal inflammation, an aspect of this model that will require further investigation.

The complexity of understanding and preventing brain injury in VLBW infants requires a translational and comprehensive approach (Kinney and Volpe, 2012). As such, several animal models have been developed that vary in insult type, timing, and severity. Current models provide a system for studying one or more of the pathophysiologic components of human PVL or gray matter injury which include loss of oligodendrocyte progenitors, impaired myelination, increased microglial activation, varying degrees of focal necrosis, reactive astrocytosis, neuronal injury, and a range of functional deficits (Kinney and Volpe, 2012; Burd et al., 2012; Boksa, 2010; Hagberg et al., 2002). Similar to other animal models examining LPS or hyperoxia as single interventions (Burd et al., 2012; Boksa, 2010; Gerstner et al., 2008; Wang et al., 2006), our work also found decreased numbers of cortical and hippocampal early myelinating oligodendrocytes with persistently impaired myelination at 28 days (Figs. 1 and 2). In contrast to neuropathologic studies of human PVL and existing animal models, we did not identify significant macro or microscopic foci of necrosis nor astrocytosis or glial scarring, suggesting a more subtle pattern of white matter injury. These findings may be due to a less severe insult in our model, or possibly the timing of the interventions and analysis precluded us from observing additional abnormalities that may be present either prior to the conclusion of the hyperoxic exposure at day 14 or further into adulthood. Understanding the functional implications of exposure to maternal inflammation and neonatal hyperoxia in the setting of a diffuse histopathological injury could further enhance the clinical relevance of this model.

The additive effect of maternal inflammation with hyperoxia exposure on oligodendrocyte cell populations was particularly impressive in the LPS/O_2 treatment group. While apparent recovery of both early CNPase-positive oligodendrocytes and MBP-positive cell numbers were appreciated at 28 days compared to 14 days after maternal LPS exposure alone, this recovery was not seen in the combined LPS/O_2 exposure group. Recovery of oligodendrocyte cell number has been reported in animal models of PVL. Back et al. showed a loss of oligodendrocyte progenitors soon after hypoxic–ischemic injury, followed by

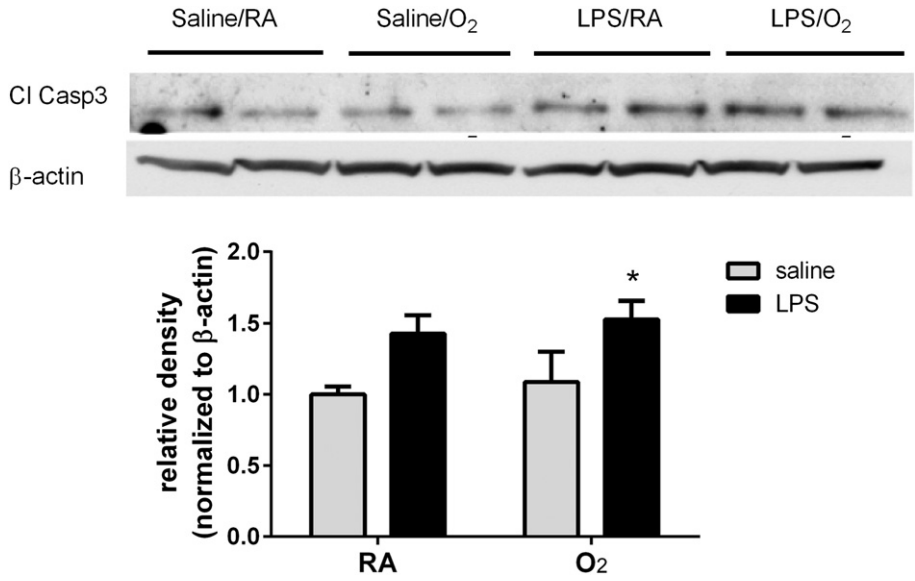

Fig. 3. Cleaved caspase 3 protein levels were elevated in the brains of animals exposed to LPS, with or without O_2. Proteins from whole brain homogenates obtained from saline/RA, saline/O_2, LPS/RA and LPS/O_2 treatment groups at day 14 were analyzed for cleaved caspase 3 by western blot. An effect of O_2 and an interaction between O_2 and LPS were indicated at both days 14 and 28 by two-way ANOVA, $p < 0.05$, $n = 2–4$ litters, $n = 1–2$ pups per treatment group. LSD post hoc analyses indicated: *different than saline/RA; #different than LPS/RA; ^different than saline/O_2.

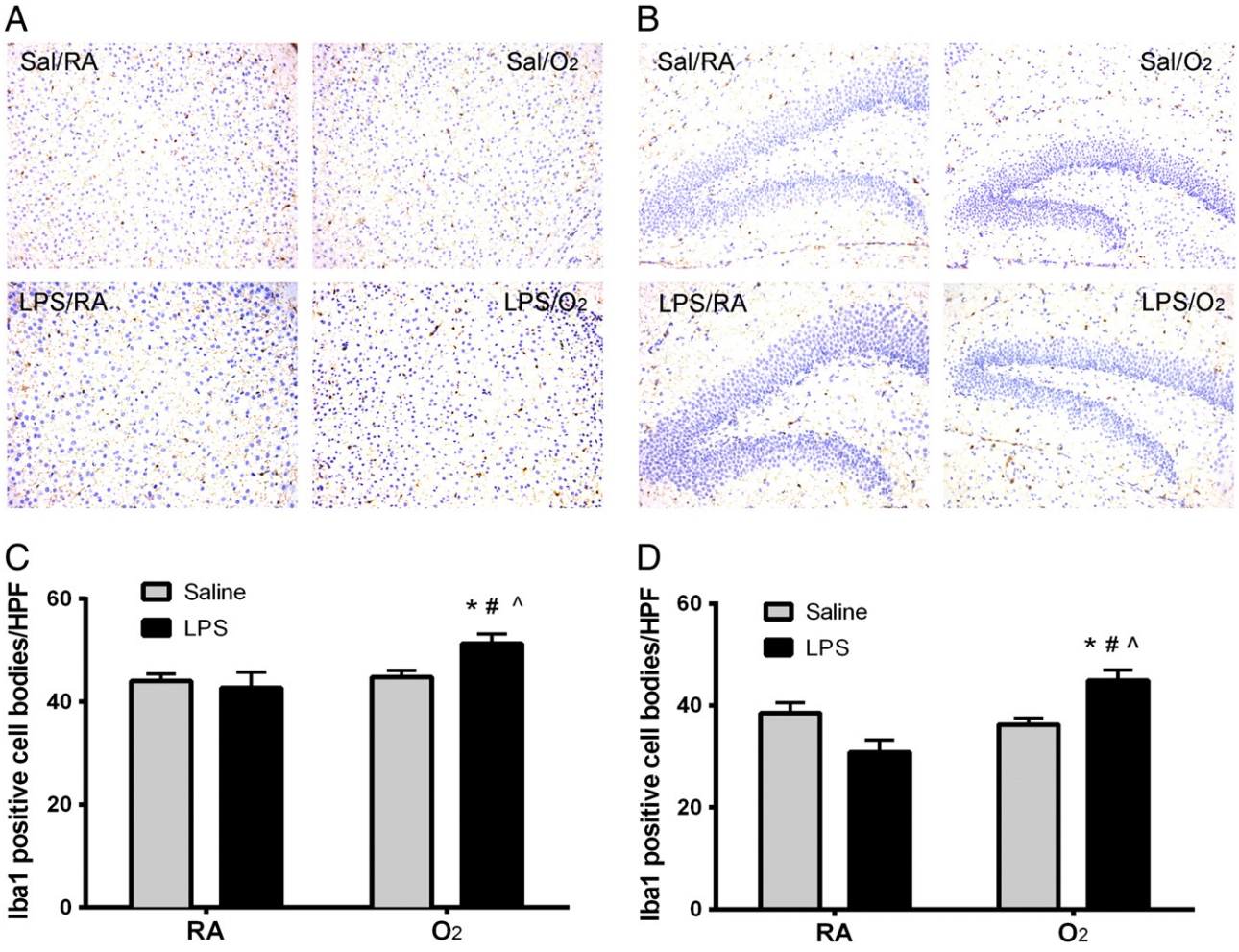

Fig. 4. Immunohistochemical staining and quantification of cortical and hippocampal microglia. A, representative images of saline/RA, saline/O_2, LPS/RA and LPS/O_2, treatment groups of at day 14 are shown (100×). B, cortical and hippocampal microglial cell bodies were counted per high-power field in the cingulum and rhinal fissure and the dentate gyrus, respectively. A significant effect of LPS was observed by two-way ANOVA, $p < 0.05$, $n = 2–4$ litters, $n = 3–4$ pups per treatment group. LSD post hoc analyses indicated: *different than saline/RA.

increased oligodendrocyte cell number but persistently impaired myelination, suggesting a maturational or functional defect of remaining oligodendrocytes (Back et al., 2002). This may suggest that prolonged exposure to hyperoxia during a critical period in development induces further cell death rather than allowing for a reactive oligodendrogliosis. A model of systemic neonatal inflammation (LPS on day 3) and 24 hour hyperoxia exposure (day 6) has been reported by Brehmer et al. (2012). In comparison to the model presented here, there was an increase in caspase 3 protein associated with hyperoxia and a decrease in MBP after single LPS or O_2 or combined exposure. In contrast to our findings associated with maternal inflammation and 14 days of neonatal hyperoxic exposure, they observed a "protective effect" of LPS exposure on early oligodendrocyte survival and report an increase in oligodendrocyte apoptosis with O_2 exposure alone. These data suggest that while hyperoxia induces cell death, LPS exposure may impair oligodendrocyte maturation (Brehmer et al., 2012). Both effects would impair myelination as evidenced by the decrease in MBP. The differences between models may be related to both the timing of LPS administration and contribution of maternal inflammatory response, as well as the difference in length of hyperoxic exposure. The finding that hyperoxia is associated with increased oligodendrocyte cell death would also support the lack of recovery seen at 28 days after combined LPS/O_2 exposure.

As mentioned, one possible mechanism for the decrease in oligodendrocytes in LPS/O_2-treated mice is apoptosis. To examine this possibility, western blots for cleaved caspase 3 protein levels were performed on whole brain homogenates at early time points (Fig. 3). The increased level of caspase 3 in the LPS/O_2-exposed mice up to day 14 indicates that a substantial amount of apoptosis was taking place, and that the combined exposures had a more profound effect on apoptosis than either the LPS or O_2 exposures alone. This observation, indicating ongoing cell loss during the hyperoxic period, could explain the persistence of decreased oligodendrocyte numbers in the cerebral cortex even after a recovery period in room air. It is important to note that, because of the small brain size at early time points, caspase 3 protein levels were determined from whole brain homogenates. Analyses of specific brain regions may reveal areas of increased vulnerability and are the subject of ongoing investigation.

Another potential mechanism for the decrease in oligodendrocytes is the increased numbers of microglia present in both the cerebral cortex and hippocampus at day 14 (Fig. 4) in LPS/O_2 exposed pups. Microglia are present in abundance during critical periods in the developing brain (Volpe, 2011) and would be activated by the inflammatory stimuli imposed in this model. Although these cells do not persist after room air recovery, their presence and increased activation during early critical developmental stages may contribute to the decrease in oligodendrocyte numbers either by causing direct injury with release of reactive oxygen and nitrogen species and inflammatory cytokines, or by indirectly altering the neural microenvironment in such a fashion as to impair oligodendrocyte survival and maturation (Kaur and Ling, 2009; Pang et al., 2010; Kaindl et al., 2008).

The decreased numbers of cortical and hippocampal oligodendrocytes and transient microgliosis that we observe in our model align with recent descriptions of 'encephalopathy of prematurity' (Volpe, 2009b). The finding of persistently impaired myelination may be in part related to recent reports in the magnetic resonance imaging literature in which former premature infants continue to exhibit decreased cerebral volumes into early childhood and adulthood (Dyet et al., 2006; Nosarti et al., 2002). Oligodendrocytes are immature and susceptible to injury at 23 to 32 weeks in a human infant (Back, 2006). In the current experiment, the timing of the maternal and neonatal interventions represents a comparable period in mouse brain development (Clancy et al., 2001). Thus, the clinical relevance of our novel "two-hit" model, LPS in utero combined with subsequent postnatal hyperoxia, is compelling in a biological sense and offers a plausible representation of the pathophysiology observed in VLBW human infants. The two stimuli recapitulate a common clinical scenario and are associated with histologic neuropathology that is subtle when compared to existing models. Further study of this novel model may add insight into the complex interactions between the maternal environment, fetal brain development, and inflammatory insults during critical periods. In the future, this model could potentially be used as a platform to investigate novel preventive and therapeutic strategies to improve neurodevelopmental outcomes in VLBW survivors.

Conclusion

Vulnerable premature infants often experience multiple inflammatory insults perinatally, including maternal inflammation or infection, followed by neonatal oxygen exposure as a life-saving medical intervention. In the current animal model, exposure antenatally to maternal inflammation and subsequent neonatal hyperoxia results in significant alterations of glial cell populations in the developing mouse brain. Further interrogation of this model may provide a mechanism to identify preventive and therapeutic targets.

Conflict of interest statement

There are no conflicts of interest, financial or otherwise, to disclose by the authors.

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