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A Novel Mouse Model of *Ureaplasma*-Induced Perinatal Inflammation: Effects on Lung and Brain Injury

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ABSTRACT: Chorioamnionitis is associated with increased lung and brain injury in premature infants. *Ureaplasma* is the microorganisms most frequently associated with preterm birth. Whether *Ureaplasma*-induced antenatal inflammation worsens lung and brain injury is unknown. We developed a mouse model combining antenatal *Ureaplasma* infection and postnatal oxygen exposure. Intraamniotic *Ureaplasma Parvum* (*UP*) increased proinflammatory cytokines in placenta and fetal lungs. Antenatal exposure to *UP* or broth caused mild postnatal inflammation and worsened oxygen-induced lung injury. Antenatal *UP* exposure induced central microgliosis and disrupted brain development as detected by decreased number of calbindin-positive and calretinin-positive neurons in the neocortex. Postnatal oxygen decreased calretinin-positive neurons in the neocortex but combined with antenatal *UP* exposure did not worsen brain injury. Antenatal inflammation exacerbates the deleterious effects of oxygen on lung development, but the broth effects prohibit concluding that *UP* by itself is a compounding risk factor for bronchopulmonary dysplasia. In contrast, antenatal *UP*-induced inflammation alone is sufficient to disturb brain development. This model may be helpful in exploring the pathophysiology of perinatal lung and brain injury to develop new protective strategies. **(*Pediatr Res* 65: 430–436, 2009)**

Preterm births represent 12% of all live births in the United States and account for >80% of all perinatal complications and deaths (1). Very premature newborns have a high risk for developing long-term injury to lung and brain (1). Chronic lung disease of prematurity or bronchopulmonary dysplasia (BPD) remains one of the most frequent sequelae in very premature infants. BPD is characterized by an arrest in lung development, resulting in larger and fewer alveoli (2). Similar structural abnormalities have been demonstrated in newborn rodents exposed to hyperoxia at birth (3). Recent evidence suggests that BPD is an independent risk factor for adverse neurodevelopmental outcome, but the reasons are poorly understood (4). Currently, there is neither prevention nor treatment for BPD. Further advances in our understanding of the mechanisms underlying lung and brain injury are required to increase survival free of morbidity.

Although multifactorial in origin, inflammation plays a major role in the pathogenesis of BPD (5) as indicated by elevated tracheal levels of proinflammatory cytokines in ventilated preterm baboons and human infants developing BPD (6). Antenatal inflammation because of chorioamnionitis is a major risk factor for extreme prematurity. Chorioamnionitis initiates a fetal inflammatory response syndrome (7) that may prime the fetal lung and make it more vulnerable to subsequent postnatal stress (such as mechanical ventilation and oxygen), thus increasing the incidence and/or severity of BPD (6).

Ureaplasma is the microorganism most frequently associated with chorioamnionitis, preterm birth, and pulmonary morbidity (8,9). Despite a strong correlation between the presence of *Ureaplasma* and inflammation, a causal relationship between *Ureaplasma* and BPD has not been formally established (9).

Analogous to the pathogenesis of BPD, fetal inflammatory response syndrome has been proposed to be one link between intrauterine inflammation and subsequent impaired neurodevelopment in preterm infants (10). Thus, the same events leading to BPD may cause brain injury.

We developed a mouse model combining antenatal *Ureaplasma* infection mimicking human chorioamnionitis and postnatal oxygen exposure, mimicking the histopathology of BPD, to explore the hypothesis that adverse antenatal events worsen postnatal lung and brain injury.

METHODS

All animal procedures were approved by the Health Sciences Animal Policy and Welfare Committee of the University of Alberta.

Ureaplasma parvum-*induced chorioamnionitis.* Frozen aliquot of *Ureaplasma parvum* (*UP*) strain DFK3 (previously labeled *Ureaplasma urealyticum* serotype 3) was thawed and cultured in 1/10 serial dilution of modified B-broth (11). The B-broth used for injections in this project did not include yeast extract to minimize potential inflammatory reactions. Preliminary *in vitro* experiments determined that B-broth with and without yeast extract

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Abbreviations: BPD, bronchopulmonary dysplasia; **CaBP,** calbindin protein; **e,** embryonic day; **GFAP,** glial fibrillary acidic protein; **MBP,** myelin basic protein; **MCP,** monocyte chemotactic protein; **MIP,** macrophage inhibiting protein; **MPO,** myeloperoxidase; **p,** postnatal day; **TGF-β_1,** transforming growth factor beta 1; ***UP,*** *Ureaplasma Parvum*

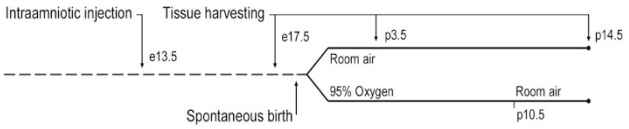

Figure 1. Study flow chart. Injections at embryonic day (e) 13.5 were *UP*, broth, or saline. One additional study group was left without antenatal intervention. Newborn pups were exposed to either room air or hyperoxia. Tissues were collected when indicated. p, postnatal day.

generated the same yield of *UP*. To further reduce the risk of inflammatory reaction of the vehicle, the aliquot indicating maximal *UP* growth was diluted with nine parts of saline immediately before injection.

At e13.5, pregnant CD-1 mice (Charles River, Saint Constant, QC, Canada) were allocated to either of four study groups: 1—control (no injection), 2—intraamniotic saline injection, 3—intraamniotic broth (*UP* vehicle) injection, and 4—intraamniotic *UP* injection (5000 CFU in each sac). Mice were anesthetized with isoflurane, and the uterus was externalized through a 12 mm abdominal incision (12). The uterus was soaked with prewarmed saline and 10 μL of study substance was injected into each amniotic sac. The abdominal wall was then closed in two layers. After recovery, mice were given water and food *ad libitum*. Remaining *UP* and broth after injection were controlled for contaminating growth. The *UP* was also cultured in a 1/10 serial dilution of regular B-broth to verify viability and to calculate the number of bacteria that had been injected. Fetal tissues were obtained by C-section at e17.5. For each experimental endpoint, 2–3 litters were used.

Oxygen-induced lung injury. In separate experiments, mice were allowed to deliver spontaneously and pups were then exposed to either room air or 95% O_2 from birth to postnatal age (p) 10.5 in sealed Plexiglas chambers (BioSpherix, Redfield, NY) with continuous O_2 monitoring. Exposure of neonatal rodents to 95% oxygen is a well-established model mimicking histopathological features of BPD (3). Dams were switched every 48 h between the hyperoxic and normoxic chambers to prevent damage to their lungs and provide equal nutrition to each litter. Litter size was adjusted to 10 pups to control for effects of litter size on nutrition and growth. Tissues were collected for analyses at different time points as illustrated in Figure 1.

UP *culture.* Tissues were stored in B-broth containing glycerol at −80°C. After thawing, the tissue was ground and cultured in serial 1/10-dilutions of regular B-broth (11). Growth of *UP* was confirmed by urease-test and visualization of *UP* colonies on agar (13). Bacterial contamination was excluded by cultures on sheep blood agar plates and by visual inspection for cloudiness in the *UP* culture vials. Bacterial contaminants were absent throughout the study.

Cytokine analysis. Tissues were immediately frozen in liquid nitrogen and then stored at −80°C. Samples were homogenized in 1 mL of 0.1% Igepal/phosphate-buffered saline and centrifuged. Interleukin (IL)-1α, IL-1β, IL-6, macrophage inflammatory protein 2 (MIP-2), monocyte chemotactic protein 1 (MCP-1), and transforming growth factor beta 1 (TGF-β_1) were analyzed by ELISA (R&D Systems, Minneapolis, MN).

Lung myeloperoxidase assay. Mouse myeloperoxidase (MPO) was measured in P3.5 lung homogenates using an ELISA kit (Hycult Biotechnology b.v. Uden, The Netherlands, catalog number HK210) according to the manufacturer's instructions.

Lung histology. At p14.5, lungs were fixed with 4% formaldehyde through the trachea at a constant pressure of 20 cm H_2O. The trachea was then ligated and lungs immersed in fixative overnight at 4°C. Lungs were processed and embedded in paraffin. Serial step sections, 4 μm in thickness, were taken along the longitudinal axis of the lobe. The fixed distance between the sections was calculated so as to allow systematic sampling of 10 sections across the whole lobe. Lungs were stained with hematoxylin and eosin. Alveolar structures were quantified on a motorized microscope stage using the mean linear intercept method (14).

Brain histology and immunohistochemistry. Formalin-fixed brains were embedded in paraffin. Serial sagittal histologic sections were used for cresyl violet staining and immunohistochemistry. Antibodies used for immunostaining were directed against calbindin protein (CaBP, a marker of GABAergic interneurons) (1/2000, rabbit polyclonal; SWant, Bellinzona, Switzerland), calretinin (a marker of a subpopulation of GABAergic interneurons, 1/2000, rabbit polyclonal; SWant), glial fibrillary acidic protein (GFAP, a marker of astrocytes) (1/500, rabbit polyclonal; Dako, Glostrup, Denmark), myelin basic protein (MBP, a marker of myelin) (1/500, mouse monoclonal; Chemicon, Temecula, CA), tomatolectin (a marker of microglia-macrophages) (1/500, biotinylated lectin; Vector, Burlingame, CA), and cleaved caspase-3 (a marker of apoptosis) (1/200, rabbit polyclonal; Cell Signaling, Beverly, MA). For immunohistochemistry, deparaffinized sections were microwaved before overnight incubation with the primary antibodies. Antibodies were detected using an avidin-biotin-horseradish peroxidase kit (Vector), as instructed by the manufacturer. Diaminobenzidine was used as a chromogen. For each marker, at least three sections of each brain were stained in two successive batches. To avoid regional and experimental variations in labeling intensity, for each marker, sections from the different experimental groups were treated simultaneously. All markers were analyzed qualitatively at the levels of the parietal cortex, the underlying white matter, the hippocampus, and the cerebellum. Furthermore, cells or nuclei labeled with CaBP, calretinin, GFAP, tomatolectin, and cleaved caspase-3 were quantified at the level of the parietal cortex, hippocampus, and cerebellum. Two fields were analyzed in each experimental group for each animal (five/group) and for each marker by a blinded observer.

Table 1. *The numbers of* UP *recovered from tissues collected at different time points after injection of 5000* UP *at e13.5*

Tissue, time point—postnatal exposure	No. *UP* recovered
Placentas, e17.5	10^5, 10^6, 10^6
Lungs, e17.5	0, 10^2, 10^4
Lungs, p3.5—room air	0,0, 0, 1, 1, 10
Lungs, p3.5—hyperoxia	0, 0, 10

The part of tissue used for culture was 1/3–1/4 of the whole organ. Results obtained from separate specimen are presented by their exponential magnitude.
e, embryonic day; p, postnatal day.

Statistics. Individual values are presented with the median of the group when appropriate. Mann-Whitney two-sample statistic was used to compare differences between medians of two groups. Two-way analysis of variance was used to test for interactions between pre- and postnatal exposures. Data were analyzed with STATA/SE 8.2 for Windows (StataCorp, College Station, TX) or with GraphPad[4] Prism version for Windows (GraphPad Software, San Diego, CA). The level of confidence was 95%.

RESULTS

UP *infection.* *UP* did not cause preterm delivery, maternal loss and did not affect postnatal survival. Greater numbers of *UP* could be recovered from placenta and fetal lung specimens than originally injected (Table 1). The number of *UP* recovered from the corresponding fetal lungs varied between individual animals and ranged from none to 10^4. *UP* was low or undetectable in p3.5 lungs. Cultures performed on tissues from the three control groups were negative for *UP*.

UP-*induced perinatal inflammation.* Four days after intraamniotic *UP* injection, inflammatory cells were detected in the fetal membranes (Fig. 2*A*). The proinflammatory cytokines IL-1β and MIP-2 were significantly higher in *UP* infected placentas compared with controls (Fig. 2*B–E*).

Similarly, fetal lungs exposed to *UP* from the same set of experiments had significantly increased IL-1α, IL-1β, IL-6, MIP-2, and MCP-1 levels (Fig. 3*A–E*). TGF-β_1 was increased in fetal lungs of all groups that underwent intraamniotic injections (Fig. 3*F*).

Effects of* UP-*induced perinatal inflammation and postnatal oxygen on lung inflammation. In separate experiments, animals were allowed to deliver spontaneously and pups were exposed to room air or hyperoxia. At p3.5, pups exposed antenatally to *UP* had significantly higher MPO levels (Fig. 4*A*). *UP* + O_2 were also increased but not significantly different when compared with the study groups (Fig. 4*A*).

Pups exposed antenatally to *UP* or broth had increased lung IL-1α levels (Fig. 4*B*). O_2 alone significantly increased IL-1α (Fig. 4*B*). Combined *UP* + O_2 exposure further increased IL-1α levels.

Only *UP*-exposed pups had increased levels of IL-1β at p3.5 (Fig. 4*C*). O_2 alone did not increase IL-1β.

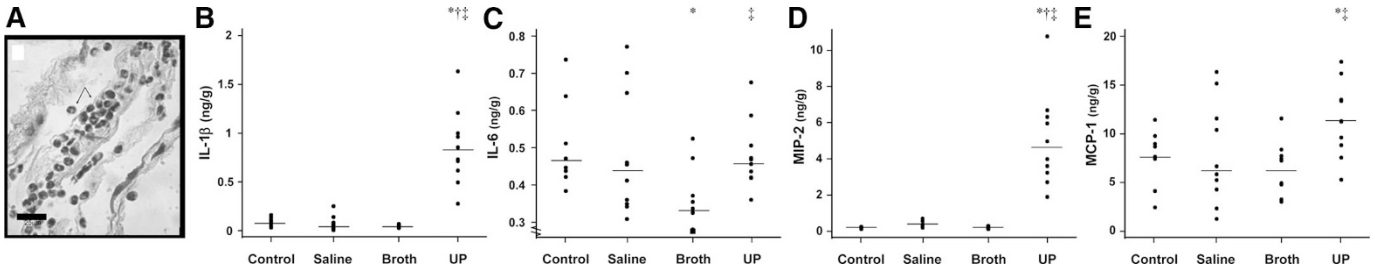

Figure 2. Placental analyses at embryonic day 17.5. Inflammatory cells (*arrows*) detected in the fetal membrane of *UP* infected mice, hematoxylin and eosin staining, magnification ×100 (*A*). The individual levels of IL-1β (*B*), IL-6 (*C*), MIP-2 (*D*), and MCP-1 (*E*) in placentas from the four study groups are presented. The lines represent the median of the group. $n = 9–10$ for each group. Note that *y* axis does not include zero in (*C*). Statistically significant differences: * = *vs* control; † = *vs* saline; ‡ = *vs* broth.

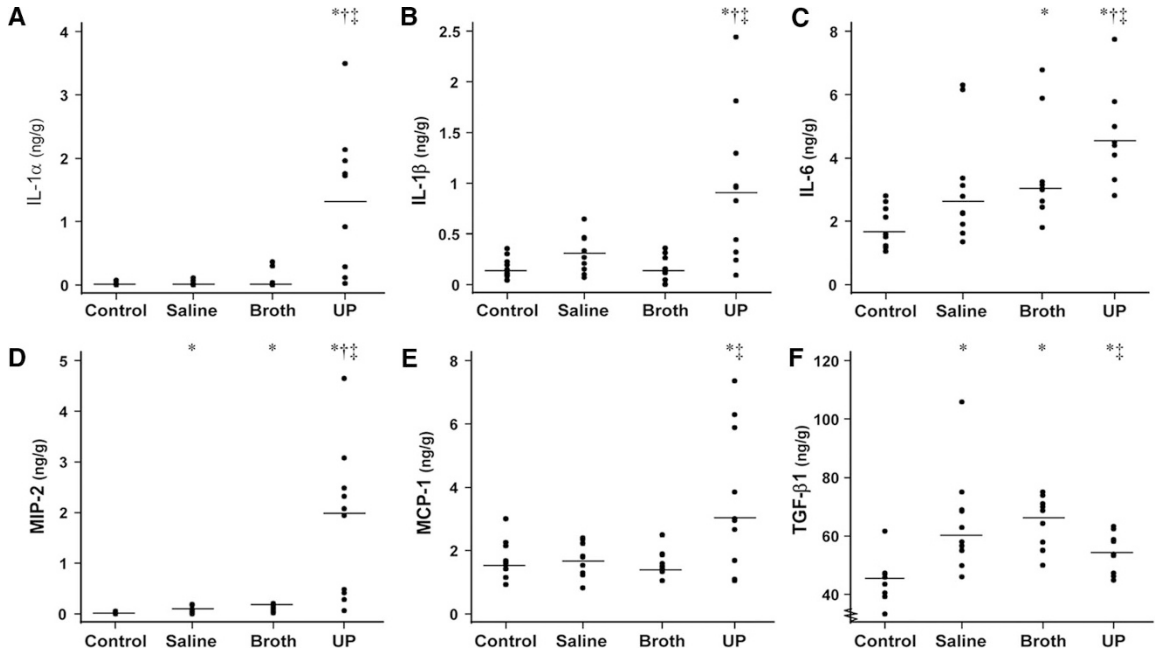

Figure 3. Cytokine levels in fetal lung at embryonic day 17.5. The individual levels of IL-1α (*A*), IL-1β (*B*), IL-6 (*C*), MIP-2 (*D*), MCP-1 (*E*), and TGF-β_1 (*F*) in fetal lungs from the four study groups are presented. The lines represent the median of the group ($n = 9–10$ for each group). Note that *y* axis does not include zero in (*F*). Statistically significant differences: * = *vs* control; † = *vs* saline; ‡ = *vs* broth.

Pups exposed to saline, broth, and *UP* had elevated levels of IL-6 (Fig. 4*D*). O_2 did not increase IL-6 significantly.

UP or broth exposed pups had increased levels of MIP-2 (Fig. 4*E*). O_2 alone did not increase MIP-2 but combined *UP* + O_2 increased MIP-2 significantly.

UP and broth increased the levels of MCP-1 (Fig. 4*F*). O_2 alone increased MCP-1. Combined broth + O_2 further increased the level of MCP-1. There was no significant increase of MCP-1 by combined *UP* + O_2.

Broth increased the level of TGF-β_1 (Fig. 4*G*). O_2 alone did not increase TGF-β_1 at p3.5.

Effects of* UP*-induced perinatal inflammation and postnatal oxygen on lung injury. Antenatal *UP* or broth alone did not adversely affect lung morphometry at p14.5 (Fig. 5). Hyperoxia impaired alveolarization, characterized by larger and fewer alveoli as quantified by the increased mean linear intercept. Oxygen-induced impaired alveolarization was exacerbated by antenatal exposure to *UP* and broth.

Effects of* UP*-induced perinatal inflammation and postnatal oxygen on brain injury. In the neocortex, there were no differences between groups on the density of GFAP-positive cells (Fig. 6*A*1). *UP* alone induced a significant increase in the density of tomatolectin-positive cells (Fig. 6*A*2) and a significant decrease in the density of CaBP-positive cells (Fig. 6*A*3) compared with controls or vehicle groups. Oxygen alone had no effect on these parameters and did not worsen the effect of *UP*. Oxygen alone induced a moderate but significant decrease in the density of calretinin-positive cells while *UP* and *UP* + O_2 induced a major drop in the density of calretinin-positive cells when compared with control or vehicle groups (Fig. 6*A*4). At p14.5, no cleaved-caspase-3-positive cell was detected in any of the groups (data not shown).

Qualitative analysis of MBP immunostaining showed that O_2 alone induced a moderate decrease in the density of MBP immunostaining, whereas *UP* and *UP* + O_2 induced a major drop in the density of MBP staining when compared with control or vehicle groups (Fig. 6*B*).

Similar results were observed in the hippocampus and cerebellum except for CaBP immunostaining where O_2 alone, when compared with controls, induced a moderate but signif-

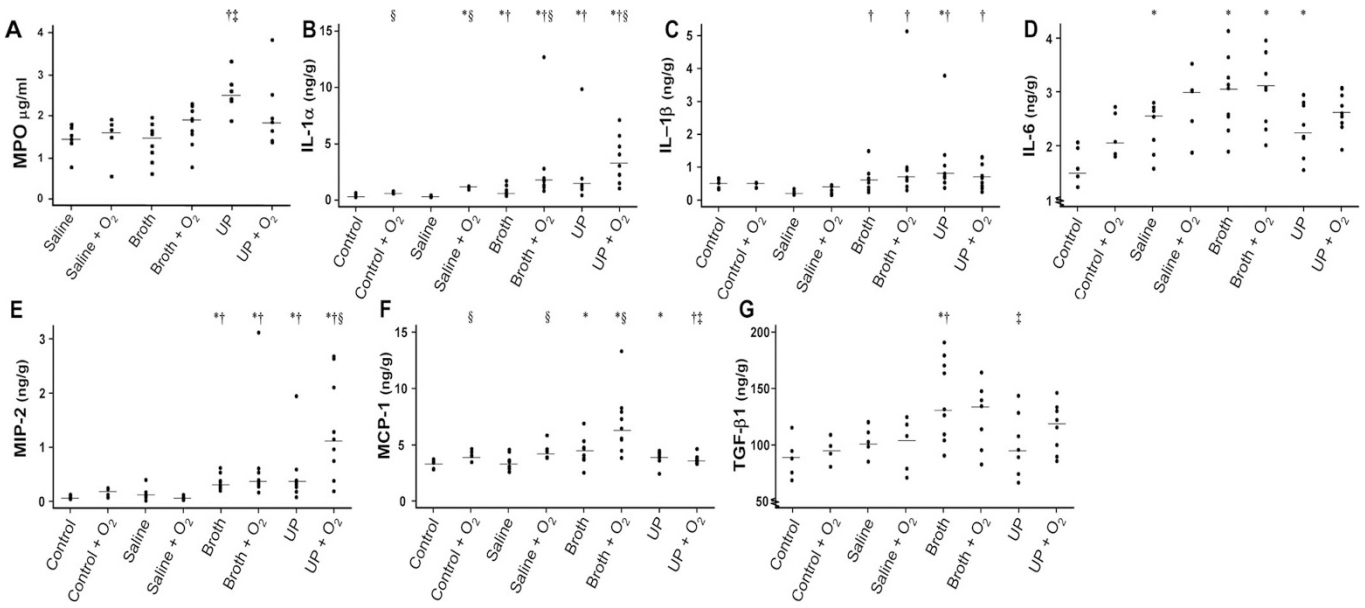

Figure 4. MPO and cytokine levels in 3.5 d postnatal lungs. The individual levels of MPO (*A*), IL-1α (*B*), IL-1β (*C*), IL-6 (*D*), MIP-2 (*E*), MCP-1 (*F*) and TGF-β_1 (*G*) in lungs obtained from the four study groups exposed to either room air or hyperoxia after birth are presented. The lines represent the median of the group. $n = 5$–9 for each group. Note that *y* axis do not include zero in (*C*) and (*F*). Statistically significant differences: * = *vs* control and equal postnatal exposure; † = *vs* saline and equal postnatal exposure; ‡ = *vs* broth and equal postnatal exposure; and § = *vs* same study group exposed to room air after birth. Interaction of pre- and postnatal exposure detected in (*F*), $p < 0.05$.

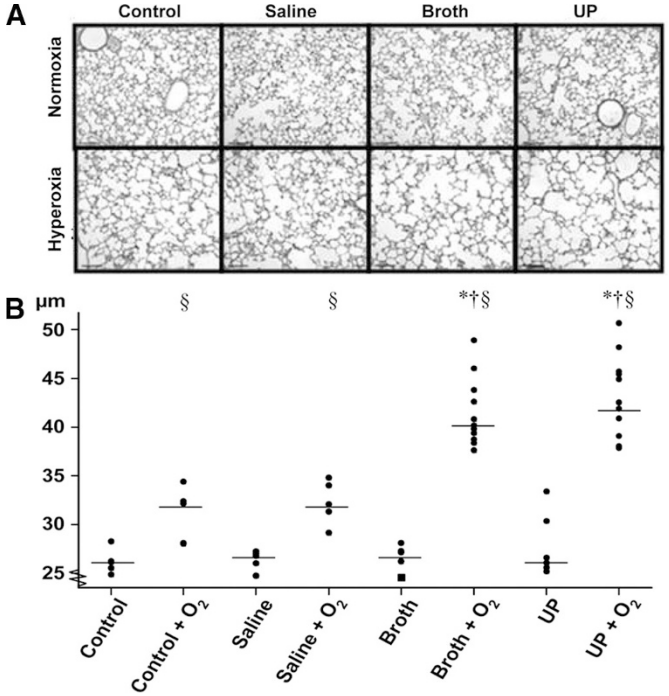

Figure 5. Lung injury at postnatal day 14.5. Hematoxylin and eosin staining of representative lung sections obtained from the four study groups exposed to either room air or hyperoxia after birth, magnification ×20 (*A*). In (*B*) individual results of the mean linear intercept for the four study groups exposed to either room air or hyperoxia after birth are presented. The lines represent the median of the group. O_2 = 95% oxygen from birth to p10.5. $n = 5$ for each group except broth + O_2 and *UP* + O_2 ($n = 15$). Note that *y* axis does not include zero. Statistically significant differences: * = *vs* control and equal postnatal exposure; † = *vs* saline and equal postnatal exposure; § = *vs* same study group exposed to room air after birth. Interaction of pre- and postnatal exposure detected, $p < 0.001$.

icant decrease in the density of CaBP-positive cells in the cerebellum (data not shown).

DISCUSSION

We show that intraamniotic *UP* injection in mice causes antenatal infection, transient lung colonization, initiates a mild fetal lung inflammation, without a significant effect on lung injury. *UP* and broth aggravated postnatal oxygen-induced lung injury. The effect of broth on postnatal lung inflammation and histology is a confounding factor of the study and prohibits us to conclude that *UP* by itself is a compounding risk factor for BPD. In contrast, antenatal *UP* exposure specifically induces central microgliosis, a delay in myelination and disturbs neuronal maturation.

Various animal models using intraamniotic endotoxin or *Ureaplasma* injection suggest that antenatal inflammation primes the fetal lung leading to exacerbated postnatal lung inflammation (15–18). In chronically ventilated preterm baboons, antenatal *Ureaplasma* infection worsens postnatal lung function (19) and causes more extensive fibrosis compared with noninfected ventilated gestational age-matched controls (20). Rodent models have been limited to either antenatal lipopolysaccharide exposure (21) or postnatal *Ureaplasma* pneumonia (22,23). The present study was conducted to explore the putative role of *Ureaplasma*-induced antenatal inflammation on subsequent neonatal lung and brain injury. To accomplish this, a new, clinically relevant animal model was developed. This model was based upon an established BPD-model using newborn mice that are born at the saccular stage of lung development. Newborn mice exposed to hyperoxia develop histologic anomalies seen in human BPD characterized by an arrest in alveolar development (24). For the present work, this model was expanded to include antenatal *UP*

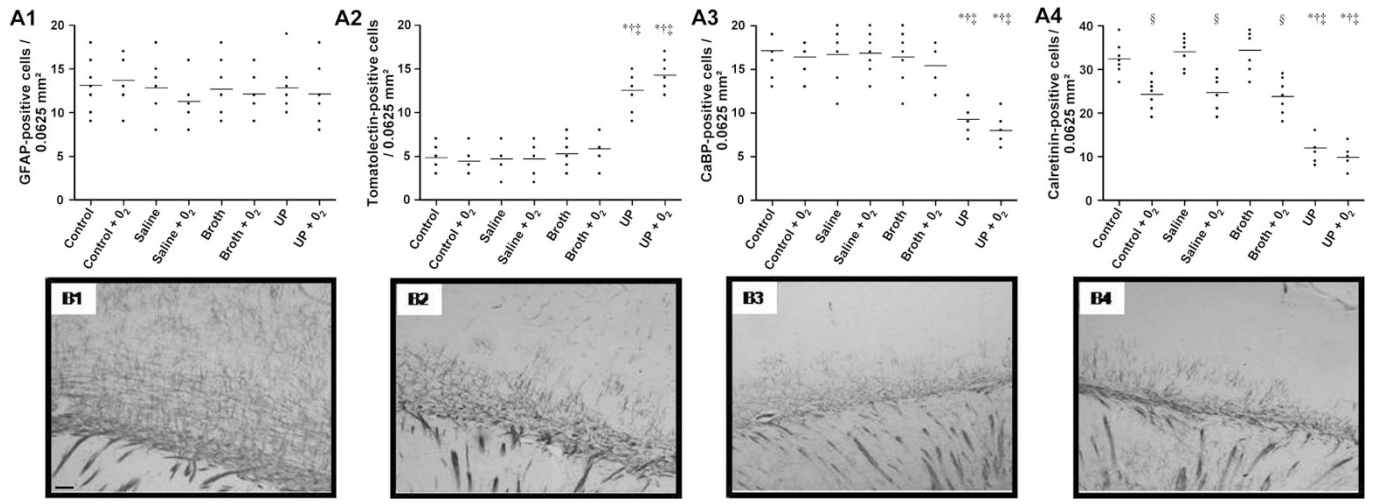

Figure 6. Brain immunohistochemistry at postnatal day 14.5. Immunohistochemistry (*A*1–4): Quantification of neocortical cells labeled with antiglial fibrillary acidic protein (*A*1), tomatolectin (*A*2), anticalbindin protein (*A*3), and anticalretinin (*A*4) in the four study groups. Statistically significant differences: * = *vs* control and equal postnatal exposure; † = *vs* saline and equal postnatal exposure; ‡ = *vs* broth and equal postnatal exposure; § = *vs* same study group exposed to room air after birth. No interaction of pre- and postnatal exposure detected. MBP immunostaining (*B*1–4): Representative immunostaining of neopallial labeling with anti-MBP in control (*B*1), control + O_2 (*B*2), *UP* (*B*3), and *UP* + O_2 (*B*4) p14.5 mice. Bar = 40 μm.

infection, the most common potential pathogen encountered in human chorioamnionitis. In addition, we investigated whether *Ureaplasma*-induced chorioamnionitis affects brain development, because neurologic impairment is often observed in children with BPD (4).

Ureaplasma are fastidious in regard to culture requirements (13). It was, therefore, important to confirm our ability to cause intrauterine infection in mice. The number of *UP* recovered from lungs after birth was low, consistent with findings in antenatal *Ureaplasma*-induced infection in sheep (18). We also confirmed that intrauterine *UP* infection induces mild antenatal inflammation in the placenta and fetal lungs, in agreement with studies showing an association between *Ureaplasma* and elevated cytokine levels in amniotic fluid in sheep (18), baboons (19), and humans (25).

The present study expands the information obtained from earlier investigations by combining an antenatal "hit" (*UP* infection) with a postnatal "hit" (exposure to hyperoxia). Chronic oxygen exposure causes mild lung inflammation and peaks usually at p7.5 in mice (26). In our study, antenatal *UP*-exposure caused mild postnatal lung inflammation as detected at p3.5 by increased lung MPO and IL-1α and MIP-2 levels-the same cytokines shown to be increased in sheep (18); this inflammation was amplified by hyperoxia.

Hyperoxia impaired postnatal alveolarization in all four study groups; *UP* by itself did not impair lung development. By contrast, alveolarization was most impaired in the broth and *UP* groups, indicating that the substances injected in these two groups primed the lungs to be more vulnerable to hyperoxia, lending additional validity to the "multiple hit hypothesis" and providing the first experimental evidence that an antenatal condition can subsequently aggravate chronic hyperoxia-induced alveolar impairment. The mechanisms involved in this process are speculative but may include alterations of the innate immune system emanating from the first insult (27).

The fact that pups exposed to broth only also exerted arrested alveolar development after hyperoxia is a confounding factor that prohibits us to conclude that *UP* by itself is a compounding risk factor for BPD. Few studies have used multiple control groups including saline and broth to detect the potential effects of the culture vehicle for *Ureaplasma*. None of the studies have explored the long-term effects of broth. The chronically ventilated baboon studies for example, the model closest to the clinical setting, and the only one in which long-term *Ureaplasma* effects have been studied, included untreated controls (19) or historical controls only (20) but no broth group (19,20). In the most recent sheep studies, the broth and saline groups did not differ in terms of inflammation in tissues collected during early pregnancy (18), similar to our own findings. However, when extending the observations over longer term, inflammation by broth became evident only days after birth and the deleterious effect on lung development was apparent only after introducing a second insult, hyperoxia.

Ureaplasma requires strenuous culture requirements difficult to circumvent. In an attempt to minimize potential inflammatory effects of broth although still allowing adequate growth conditions for *UP*, the yeast extract was excluded but horse serum and bovine peptones remained (11). We also diluted the broth with saline before injection to reduce possible adverse effects of these essential *UP* growth components. The finding that broth has biologic activity is important information when validating experimental models to study the role of *Ureaplasma* on perinatal morbidity. Ideally, the *UP* vehicle should be inert to the host animal. The mechanism by which broth exerts the deleterious effect is not clear, but among all other groups, broth groups had the highest levels of lung TGF-β_1, (both ante- and postnatally), a growth factor associated with impaired alveolar development (28,29).

To our knowledge, this is the first report of an animal model of *Ureaplasma*-induced inflammation to include investigations on brain development. We demonstrate that *Ureaplasma*-induced perinatal inflammation is sufficient in itself to disturb brain development in mice, further supporting the role of inflammatory factors in the pathogenesis of perinatal brain damage. Indeed, it has been suggested that cognitive limitations frequently diagnosed in preterm infants might be associated with an exposure to intrauterine infection (30,31).

Neuropathological studies performed in preterm infants have revealed that gray and white matter abnormalities tend to go hand in hand. With regards to white matter injury, recent studies have shown myelin defect without accompanying oligodendrocyte death (32). In the gray matter, various abnormalities have been reported, including loss of GABAergic interneurons (33) and decreased concentrations of the calcium-binding protein parvalbumin (a marker of interneurons) in the cerebral cortex of brains with diffuse white matter damage (34).

The disturbances of brain development observed in the present study could participate in the process leading to impaired cognitive function. The combined decreased density of calbindin protein-positive and calretinin-positive neurons is in favor of a disturbed production, maturation, and/or survival of interneurons, which play a key role in associative and cognitive functions (35). Furthermore, decreased MBP staining most likely reflects abnormal myelination, which is often observed in surviving premature infants and can participate to limited cognitive functions (36).

Interestingly, *UP*-induced central microglial activation, suggesting that systemic inflammation led to central inflammation. This microglial effect seemed specific as astroglia was not affected by *UP*. Microglial activation most likely participated to the observed effects of *UP* exposure on interneurons and myelin.

Exposure to lipopolysaccharide or inflammatory cytokines is well known to sensitize the developing brain to subsequent hypoxic-ischemic or excitotoxic brain damage (37,38). In contrast, a sensitization effect of infection or inflammation to subsequent hyperoxic brain insult has not been previously reported. In the present study, the observed effects of *Ureaplasma* on the developing brain were not exacerbated by combined hyperoxia, suggesting that inflammation *per se* was sufficient to disrupt brain ontogeny. Further studies looking at additional parameters of brain development and maturation will be necessary to address this important question of the potential synergy between inflammation and hyperoxia in the perinatal period.

In conclusion, this novel model, designed to be as clinically relevant as possible, provides evidence for the concept that antenatal events potentiate postnatal events and worsens neonatal outcome. It also demonstrates for the first time that antenatal *UP* infection disturbs brain development. The use of mice provides the potential advantage to study genetically modified animals. We believe that this model will help in identifying new mechanisms underlying perinatal lung and brain injury and new therapeutic strategies to prevent these morbidities.

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