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IL-1 Receptor Antagonist Protects against Placental and Neurodevelopmental Defects Induced by Maternal Inflammation

Sylvie Girard,* Luc Tremblay,†,‡ Martin Lepage,†,‡ and Guillaume Sébire*

The precise role of maternal bacterial infection and inflammation occurring at the end of gestation is a controversial matter. Although it is recognized as an independent risk factor for neurodevelopmental diseases such as cerebral palsy, mental deficiency, and autism, it remains unclear whether it is causal or simply associated with the diseases. In this study, we demonstrate that IL-1 plays a key role in mediating severe placental damage and neurodevelopmental anomalies in offspring. Our results show that end of gestation exposure of pregnant rats to systemic microbial product (LPS) triggers placental inflammation and massive cell death, fetal mortality, and both forebrain white matter and motor behavioral alterations in the offspring. All these effects are alleviated by the coadministration of IL-1 receptor antagonist with LPS, suggesting a possible protective treatment against human placental and fetal brain damage. The Journal of Immunology, 2010, 184: 3997–4005.

Perinatal brain damage and subsequent neurodevelopmental disorders, such as cerebral palsy (CP) (1), mental deficiency, and autism, are major health problems for which few effective preventive or therapeutic interventions are available (1, 2). Paradoxically, improved obstetrical and neonatal care has fallen short of the hope of reducing the incidence of perinatal neurologic handicaps. Although perinatal mortality has decreased over the past 20 y, the incidence of CP has not (1). For instance, a recent population-based prospective study showed that a large proportion of premature newborns under 33 wk of gestation suffer neurologic disabilities, including CP and/or mental deficiency, at 5 y of age (3). Maternal infection/inflammation is one of the main independent risk factors for perinatal brain lesions, both in premature and term newborns (4–6), and also increases the risk of fetal death and premature delivery. Antenatal infection/inflammation was also associated with an enhanced susceptibility to later occurring diseases and is likely to inflict noxious fetal imprints that program the development of some severe neuropsychiatric illnesses, such as schizophrenia, in young adults (7, 8).

Systemic and placental maternal infections (e.g., urinary tract infections or chorioamnionitis) occurring at the end of gestation are recognized as triggering perinatal inflammation. Such maternal infections are mainly due to bacterial microorganisms, Escherichia coli being one of the most prevalent (1). Whereas white matter damage is the predominant form of brain injury associated with perinatal infection (9–11), most neonates with white matter damage show no detectable intracerebral bacterial infection. Therefore, as first hypothesized by Leviton (12), soluble inflammatory mediators arising from maternal infection remote from the fetal brain might be key agents linking maternal infection, fetal inflammatory response, and fetal brain damage (13–15). However, whether cause or effect, the precise role of inflammatory mediators in the pathophysiology of neurodevelopmental disturbances remains unclear. Experimental animal studies of maternal immune activation have further validated the association between activation of various inflammatory mediators (e.g., IL-1, TNF-α, and IL-6) in the placenta and in the perinatal brain and the occurrence of brain lesions (14, 16–20). Increased expression of proinflammatory cytokines, including the β form of IL-1 (IL-1β), has also been detected in the human placenta, amniotic fluid (21), newborn blood (22), and the neonatal brain (23–25) in close temporal association with the development of forebrain periventricular white matter damage. Other observations suggest a possible toxic role of the IL-1 system (26). For instance, IL-1R antagonist (Ra) gene polymorphism homozgyocity (IL-1RN*2 allele) resulting in a proinflammatory orientation of the IL-1β/IL-1Ra ratio was associated with an increase in late intrauterine human fetal death (27).

We hypothesized that IL-1 plays a key role in inducing placental damage and subsequent fetal mortality or neurodevelopmental defects. We used an established animal model of perinatal brain damage and CP (18, 28–30) to determine the role of IL-1 on the placenta, fetal viability, and the developing brain in the context of LPS-induced maternal inflammatory response.

Materials and Methods

Animals

Timed pregnant Lewis rats were obtained from Charles River Laboratories (Saint-Constant, Quebec, Canada) at gestational day (G) 16. They were allowed to acclimatize to our animal facility prior to experimental
Critical Role of IL-1 in LPS-Induced Placental Damage

Quebec, Canada) as previously described (31).

A homemade ELISA was used (generously provided by Dr. Giamal Luheshi, McGill University, Douglas Mental Health University Institute, Montreal, NY). T2-weighted respiration-gated images were acquired using a fast spin-echo pulse sequence (repetition time/echo time: 2000/12 ms, eight echoes; field of view: 6 × 6 cm²; matrix: (256)²; number of averages: 8; 20 slices of 1.5 mm). Sets of T1-weighted images were acquired repeatedly to enable simultaneous and continuous monitoring of the placental perfusion (repetition time/echo time: 197/2.5 ms; field of view: 6 × 6 cm²; matrix: (128)²; α: 30°; number of averages: 4; 20 slices of 1.5 mm). Five minutes after the initial set of T1-weighted images, a bolus of contrast agent (gadolinium-diethylene-triaminopentaacetic acid, 0.143 mmol) was injected i.v. in the tail vein, and its distribution was monitored for a period of 45 min. Following the MRI procedure, the pregnant rat was injected i.p. from G18, every 12 h, with either LPS (n = 6; 200 µg/kg in 100 µl pyrogen-free saline from E. coli, 0127:B8; Sigma-Aldrich, Ontario, Canada) or saline solution (controls, n = 6; 100 µl) until G20, when the MRI procedure was repeated. No deleterious effect (i.e., no mortality; behavioral changes, differences in gestational length, litter size, or pups weight at birth) was observed in dams exposed to the MRI scan.

Placental cytokines quantification

In another set of experiments, pregnant rats were injected i.p. from G18, every 12 h, with either LPS (n = 3) or saline solution (controls, n = 3) until G20. Placentas were removed by cesarean section and then either quickly frozen and kept at −80°C until extraction (three to six placentas randomly selected per dam) or fixed in a buffered solution of 4% paraformaldehyde containing 0.1% glutaraldehyde for 48 h before being embedded in paraffin (three to six placentas randomly selected per dam). Proteins were extracted as previously described (29). Cytokines were quantified using a rat ELISA kit for IL-1β, TNF-α, and IL-6 (R&D Systems, Minneapolis, MN) following the manufacturer's instructions, except for IL-1Ra, for which a homemade ELISA was used (generously provided by Dr. Giamal Luheshi, McGill University, Douglas Mental Health University Institute, Montreal, Quebec, Canada) as previously described (31).

Histological analysis of brains and placentas

Rat pups (three pups per litter from three different litters in each experimental condition; n = 9) were euthanized by decapitation at postnatal day (P) 9. Brains were removed, fixed, and paraffin-embedded, and 5 µm-thick coronal sections (∼3.2 to ∼5.6 mm from the bregma) were used to study microglial proliferation by immunofluorescence (IF) as previously described (28). Sections were mounted on silanized slides (VWR, Mississauga, Ontario, Canada) and stained with H&E. Placental sections were also stained for calcium deposits using the Von Kossa staining method (Polysciences, Warrington, PA) following the manufacturer's instructions. IF and immunohistochemistry (IHC) were performed as previously described (28). The Abs used are detailed in Supplementary Table I. Slides were mounted using a DAPI-containing medium (Invitrogen, Ontario, Canada) for IF. Negative controls consisted of an additional set of sections treated in a similar way but without the primary Ab. For IF, counting of labeled cells was performed using Image J analysis software (National Institutes of Health Image, http://rsweb.nih.gov/nih-image/) at ×200 magnification. For HIC, counting of labeled cells was performed in three areas of the placenta at ×400 magnification in triplicate. Mean value for percentage of positive cells from three readings was tabulated and used for data analysis.

Treatment with IL-1Ra

Recombinant human (rh) IL-1Ra at doses of 2, 10, or 20 mg/kg (Kineret, Biovitrum, Sweden) was injected i.p. 30 min before each LPS injection performed as described above. IL-1Ra was used successfully in several animal studies to protect various tissues, including the brain, against inflammatory aggressions (32–34). The range of rhIL-1Ra doses used range from 10 µg to 200 mg/kg (when administered peripherally) depending on the model and the species; classically, 1.5 mg/kg/day rhIL-1Ra was used in humans for treatment of gout. Our goal was to administer rhIL-1Ra peripherally (to dams) and to minimize the potential impact of high doses on physiological function. Based on the above considerations, doses ranging from 2–20 mg/kg were selected. We detected no adverse effect of rhIL-1Ra administration on animal behavior, perfusion of organs (as determined by MRI on placental inflaction and muscle), and placental inflammatory response and no change in gestational length, litter size, or pups weight at birth. At G20, animals either underwent MRI (four to five dams for each dose) or cesarean section for placenta removal (three to four dams for each dose; three to six placentas randomly selected per dams). In another set of experiments, dams gave birth naturally. Histological experiments on pup brains were performed after LPS administration (three to six pups per litter from three litters; n = 9–15 for each experimental condition). Motor behavioral experiments were performed at P30, P35, and P40 using the Rotarod test as previously described (all pups from three to five litters; n = 10–25 for each experimental condition) (28).

MRI analysis

Magnetic resonance images were analyzed using Matlab (The MathWorks, Natick, MA). One slice in the middle of each placenta was selected on T2-weighted images where the umbilical cord attachment site was frequently observed. A region of interest (ROI) was manually drawn to delineate the placenta. The ROI for each placenta was applied to corresponding T2-weighted images to analyze the signal enhancement throughout the acquisition period. Each placenta was visible on at least three contiguous images (slice thickness 1.5 mm) and typically visible on five to six contiguous images. Each placenta was manually delineated by drawing an ROI on each image. To avoid any bias from partial volume artifacts expected on the first and the last images where a placenta is visible, we report the surface of the placenta determined from the central image for each placenta. The surface may depend on the orientation of a placenta relative to the image plane, but this variability is expected to be similar for all study groups. Each placenta was considered individually, with 8–10 placentas for each condition. Given that LPS did not lead to placental or fetal sorption, the mean number of placentas was the same for dams from LPS and control groups. To account for possible changes in signal intensity between different MRI sessions, the signal detected on MRI was normalized to the intensity of the maternal dorsal muscle. All contrast agent perfusion curves were offset to get the same intensity values prior to injection of the agent. Dynamic curves were fitted with a two-exponential function (pulse function, MicroCal Origin 5.0, Microcal, Piscataway, NJ), enabling the determination of the perfusion rate (inverse of the time constant for a rising exponential), the time to reach maximum signal, and the clearance rate (inverse of the time constant for a decreasing exponential).

Data analysis

Data are presented as means ± SEM. Comparisons were performed using ANOVA with the Newman-Keuls post test or the unpaired t test with Welch correction. The significance level was set at p < 0.05.

Results

LPS-induced placental damage

Using MRI, we assessed in vivo the effect of maternal systemic administration of LPS on the placenta. LPS exposure significantly decreased placental signal intensity on T2-weighted images as compared with control placenta at G20 (Fig. 1A–D, Supplemental Fig. 1A). The deleterious effect of LPS on placental perfusion was assessed by dynamic contrast-enhanced T1-weighted imaging. The increased intensity of the MRI signal, produced within a given tissue by contrast agent injection, is correlated to the level of blood perfusion of this tissue. In control dams, the contrast agent was distributed almost uniformly throughout the placenta at G17 (Fig. 1E). Contrast enhancement increased with gestational age (p < 0.05, between G17 and G20; Fig. 1E–G, Supplemental Video 1). LPS exposure led to a decreased and heterogeneous placental accumulation of contrast agent at G20 (Fig. 1E–G, Supplemental Fig. 1B, Supplemental Video 2). In utero inflammation also induced a delay in placental contrast agent accumulation: 1.71 ±
0.19 min in controls at G20 versus 4.55 ± 0.51 in LPS-treated dams (p < 0.001). This is depicted by a decrease in perfusion rate (Fig. 1H) and also by a longer elimination time, as shown by the decreased clearance rate (Fig. 1I). Decrease in contrast agent accumulation varied slightly from one placenta to the next, but a decrease of at least 10% in contrast agent accumulation was observed in 80% of placentas in LPS-exposed dams as compared with controls at G20 (Fig. 1G, Supplemental Table II). These observations were not due to a diffuse drop in maternal blood pressure because the MRI measure of perfusion of maternal muscular tissue (paravertebral muscles) was identical in LPS-exposed and control dams (Supplemental Fig. 2). Thus, our MRI results document, for the first time, several noninvasive imaging markers associated with placental exposure to inflammation. Moreover, our results also support the inclusion of an MRI approach in therapeutic clinical trials aiming to protect the placenta against inflammation.

**Placental cytokine expression**

Because perinatal inflammation, and particularly the IL-1 system, is associated with an increased risk of placental and perinatal brain damage, we hypothesized that the IL-1 system may play a key role in the cascade through which maternal inflammation presumably induces its deleterious effects. We therefore tested whether maternal LPS administration is associated with changes in IL-1β and other proinflammatory cytokines within the placenta. Dams exposed to LPS presented a sharp increase of placental IL-1β expression at G20; the concentration of IL-1β was 20-fold higher than IL-6 and 50-fold higher than TNF-α (Fig. 3A). Placental IL-1Ra expression was also induced by exposure to LPS, but to a lesser extent than IL-1β (Fig. 3A). ELISA results were reflected by IHC, confirming increased expression of these cytokines in LPS-exposed placentas at G20, particularly for IL-1β, IL-6, and TNF-α in the labyrinth (Fig. 3B, 3C). IL-1Ra was mainly expressed by trophoblastic cells from the junctional zone (Fig. 3C). Expression of IL-1β and TNF-α did colocalize with macrophages (CD68⁺), although IL-1β expression was not restricted to this cellular lineage (Fig. 3D). IL-6 and IL-1Ra immunostaining did...
growth delay was noticed at birth in LPS-exposed compared with control pups, but was no longer apparent at P3 (28). Pups exposed prenatally to LPS developed white matter brain lesions including a significant increase in proliferating (Fig. 3F) and total (Supplemental Fig. 4) microglial cells in the forebrain white matter at P9. These changes in brain development were associated with a decrease in forced motor capacities, as previously described in our model, in the offspring of LPS-exposed dams (28).

**Effects of maternally administered IL-1Ra**

Based on 1) the massive upregulation of IL-1 in LPS-exposed placental tissues as compared with other proinflammatory cytokines; and 2) the detection of the same IL-1 system activation in perinatal brain injury, as previously described in this model (29), we tested whether maternally administered IL-1Ra protects the placenta, and rat pups, against LPS-induced damage. We chose a range of rhIL-1Ra doses shown to be efficient and well tolerated in rat models and human inflammatory diseases (35). Our results showed that the concomitant administration of IL-1Ra with LPS indeed prevented the decrease in placental signal intensity observed in T2-weighted images in dams exposed to LPS alone (Fig. 4A, 4B). The dose-dependent effect of IL-1Ra on placental perfusion was shown by contrast enhanced T1-weighted images (Fig. 4C–F). Treatment with 10 or 20 mg/kg of IL-1Ra resulted in an increased placental accumulation of contrast agent (Fig. 4C, 4D, Supplemental Table II), whereas both perfusion and clearance rates were maintained (Fig. 4C, 4E, 4F). IL-1Ra administration by itself (10 mg/kg) did not change these parameters (Supplemental Fig. 5). Histologically, placentas exposed to a combination of LPS plus IL-1Ra presented significantly less cellular loss and cavitations (Fig. 5A) and displayed no calcification and no staining for apoptotic marker activated caspase-3 (data not shown) as compared with LPS-exposed placentas. Decreased LPS-induced macrophage infiltration within the placenta, as seen by the drop in CD68+ cells, was noted with all doses of IL-1Ra, including the lowest dose (2 mg/kg). The proportion of CD68+ cells was lowest at 10 mg/kg of IL-1Ra, which kept the placental density of macrophage identical to that of control placentas (Fig. 5B). IL-1Ra treatment also drastically downregulated LPS-induced cytokine expression in the placenta. All doses of IL-1Ra tested abrogated placental expression of TNF-α and also led to a dose-dependent significant decrease in IL-1β and IL-6 expression (Fig. 5C–E). Maternally injected rhIL-1Ra was detected by ELISA in placentas (data not shown).

Maternally administered IL-1Ra markedly improved crucial developmental parameters otherwise altered by LPS exposure: 1) by preventing LPS-induced fetal death (Fig. 6A); all IL-1Ra doses tested increased the number of surviving pups to a number similar to control litters; 2) by maintaining the density of microglial cells and the percentage of proliferating microglial cells detected in offspring’s forebrain white matter close to control levels (Fig. 6B–D); and 3) by protecting against LPS-induced motor dysfunctions at P30, P35, and P40: IL-1Ra coadministered with LPS significantly increased performance in the Rotarod test (evaluation of forced motor capacities) as compared with administration of LPS alone. LPS plus IL-1Ra–treated rats reached a motor performance similar to that of control animals with the administration of 10 or 20 mg/kg of IL-1Ra (Fig. 6E).

**Discussion**

Our results show that LPS-triggered maternal inflammatory response at the end of gestation induces massive placental damage. Most importantly, our results clearly link IL-1 activation and both placental and perinatal brain damage through the demonstration of powerful protective effects of prenatal coadministration of...
IL-1Ra together with LPS, as compared with LPS alone, on placental perfusion, fetal death, and offspring’s brain white matter microgliosis and motor functions. IL-1Ra administration also spectacularly shut down the massive placental proinflammatory cytokine hyperexpression induced by systemic maternal LPS exposure and maintained the histological integrity of the placenta. These findings are consistent with previous observations that LPS administered at midgestation in mice induces a high rate of fetal death (36) associated with maternal proinflammatory cytokine induction, including IL-1 (37). Neonatal brain damage induced by postnatal intracerebral LPS injection in rats was also shown to be attenuated by intracerebral administration of IL-1Ra, but not by the TNF-α Ab (38). In contrast, Wang et al. (39) found that IL-1α/β gene knockout did not reduce fetal loss in a mouse model of LPS-induced inflammation. Recently described natural IL-1R agonists, other than IL-1α or IL-1β, may explain the apparent discrepancy with our results, given that IL-1Ra also competitively blocks other IL-1R agonists (35). In addition, the complete absence of IL-1β in this knockout model may produce unfavorable placental effects, as we observed on some of our endpoints, such as the level of placental macrophage infiltration, for which protective effect was relatively modest even at the highest IL-1Ra doses of 20 mg/kg as compared with 10 mg/kg. Finally, compensatory mechanisms may also come into play when the IL-1 gene is congenitally disrupted.

Interestingly, Leviton et al. (40) recently showed that the robust association between chorioamnionitis and perinatal brain lesions was not always accompanied by the detection of bacterial microorganisms within the placenta, leading to the conclusion that “organism recovery does not appear to be needed for placenta inflammation to predict diparetic CP.” This important observation suggests that in humans, as in our animal model, inflammation in the absence of direct placental infection is associated with brain damage in the offspring.

The full preventive effects of maternally administered IL-1Ra against fetal death, forebrain white matter microgliosis, and CP-like motor dysfunction is associated with a strong protective effect against placental damage. Thus, the deleterious impact of maternal IL-1 may first act upon the placenta. Ensuing placental insufficiency could then contribute to fetal mortality and morbidity. Our data show that a small minority of LPS-exposed placentas presents a complete lack of perfusion as compared with control placentas. In addition, most fetuses were alive at the time of cesarean section (G20), and no sign of apparent resorption was detected in the others. Thus, it seems unlikely that such a fetal resorption process participates in the severe placental damage, including cavitary lesions, detected at G20. Although LPS does not appear to cross the placenta (41), maternal IL-1 may exert its noxious effects on fetal organs by passing directly through the
placenta and/or indirectly via molecular cascades spanning both maternal and fetal sides of the placenta and then diffusing via the blood stream to fetal organs including the brain. There are conflicting reports with respect to the ability of maternal cytokines to cross the normal or diseased placental barrier (42–44). To our knowledge, the ability of IL-1Ra to cross the placental barrier has not yet been reported. Under certain conditions, IL-1β has been shown to leak through the blood-brain barrier either by passive

**FIGURE 4.** Protective effect of IL-1Ra monitored by MRI. Concomitant administration of IL-1Ra with LPS prevented the decrease of placental T2-weighted signal, which remained at the level of controls at G20 (A, B). IL-1Ra treatment prevented the decrease of contrast enhancement induced by LPS at G20 in a dose-dependent manner (C). Solid lines in C are fits to the data points. From these fits, it is seen that IL-1Ra also prevented the LPS-induced decreased accumulation of contrast agent (D) and the decrease in both placental perfusion (E) and clearance rates (F), which returned to control levels (G20). Data are from three to five dams (5–10 placentas per dam) in each experimental condition. Error bars represent means ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001 by one-way ANOVA with Newman-Keuls post test.

**FIGURE 5.** Protective effect of IL-1Ra on placental inflammation and histological integrity. Histological analysis of the placentas performed at G20 showed that the IL-1Ra administration concomitantly to LPS preserved the tissue integrity by a decrease in cell loss (A, original magnification ×12.5) as compared with LPS alone (Fig. 2A). IL-1Ra also prevented the CD68+ macrophage infiltration in the placentas, which remained at the control level for a dose of 10 mg/kg of IL-1Ra (G20) (B). IL-1Ra had a major impact on cytokine production at G20, which remained at the control level for IL-1β (C) and TNF-α (D) with all of the IL-1Ra doses tested and with a dose-dependent effect for IL-6 reaching the control level at doses of 10 and 20 mg/kg of IL-1Ra (E) (n = 9 for each experimental condition). Error bars represent means ± SEM. Scale bar, 1 mm.
diffusion or via IL-1R2 binding followed by translocation of ligand-receptor complexes (45). IL-1Ra may interfere with such potential transfer by blocking IL-1/IL-1R2 binding. Propagation of the fetal inflammatory response from blood to brain compartments might be facilitated perinatally by the immature, more permeable blood-brain barrier, particularly under inflammatory conditions. On the other hand, systemic fetal inflammatory response triggered by maternal LPS injection induces IL-1 mRNA and IL-1 synthesis within the brain (16, 29). IL-10 has been used as a prenatal treatment to reduce white matter damage induced by prenatal exposure to E. coli; such IL-10 effect may act via the IL-10 inhibition of IL-1 synthesis (46). Although our own study focuses mainly on cytokine expression within the placenta, it is possible that other inflammatory mediators (e.g., chemokines, PGs) also play a role in fetal outcome. IL-1 is a central cytokine implicated in numerous inflammatory processes that can lead to the induction of those other mediators. Because blockade of the IL-1 pathway using IL-1Ra protects the placenta and prevents deleterious effects of LPS-induced inflammation on fetuses, it is possible that this system may be at the apex of the molecular cascade leading to placental and neurodevelopmental defects. On the other hand, several studies implicate proinflammatory cytokines in the physiopathology of premature birth. There are conflicting reports as to the ability of LPS to trigger premature birth depending on species, strains, and, possibly, disparities in protocols of LPS administration (47). IL-10 appears to prevent LPS-induced preterm delivery and fetal death via the downregulation of uteroplacental IL-1 and TNF-α. However, our experimental protocol did not induce any preterm delivery. Thus, data from our model do not enable a useful discussion on the effect of IL-1Ra, as compared with other molecules, on preterm delivery. In sum, our results are in agreement with those showing that various proinflammatory cytokine blockade strategies and downstream production of PG have protective gestational effects (46).

Few studies have used MRI to characterize normal and pathological placentas in rodents and humans (48–50). We show that T2-weighted MRI without administration of contrast agent, a safe MRI technique routinely and efficiently applied in second- and third-trimester pregnant women to detect fetal malformations, is a useful diagnostic tool to detect experimentally induced placental inflammation in rodents. In humans, chorioamnionitis is often suspected prenatally based on clinical symptoms but is only proven postnatally through time-consuming pathological examinations, the reliability of which is often limited by sampling biases. Histopathological chorioamnionitis is associated with 50% of premature rupture of membranes and premature deliveries (51). Prenatal detection of human placental inflammation by MRI offers a safe and noninvasive opportunity for early in utero diagnosis of this common and threatening pathology. To our knowledge, a single study, based on a small sample of patients, has correlated
MRI with placental pathologies in humans using T2*, T1*, and diffusion-weighted images. Our results suggest that inflammatory-induced changes in T2*-weighted intensity correlate with placental damage. Thus, we speculate that MRI may noninvasively and usefully detect these changes in humans.

Anti-inflammatory treatments tested in humans in an attempt to protect the fetus against maternal inflammatory response have failed mainly because of severe adverse effects. Thus, antibiotics administered to infected pregnant mothers failed to protect the fetal brain (52). Kenyon et al. (53) recently showed in humans that antibiotic treatment given at the end of preterm pregnancies failed to reduce the burden of neurologie consequences in offspring, but rather doubled the occurrence of CP. This unexpected impact of antibiotics may be due to uncontrolled, and even possible exacerbation, of the inflammatory response triggered by antibiotic-induced release of bacterial components. Alternatively, prenatal administration of corticosteroids initially raised the hope of providing beneficial neuroprotective effects based on findings such as radiological improvement of neonatal brain damage (21). Unfortunately, additional trials and meta-analysis showed that pre- or postnatal corticosteroids eventually do not provide neurobehavioral benefit and could result in adverse effects in certain therapeutic designs (54). Conflicting results and fetal adverse effects such as ductal constriction have been reported in neonates treated prenatally with nonsteroidal anti-inflammatory drugs (55–57), emphasizing the need to better restrict the target of blocking agents to optimally balance beneficial and adverse effects. Maternal IL-1Ra therapy, as we tested, at doses already recommended for human inflammatory diseases (58) may meet this safety/efficacy criterion. However, translation from animals to humans requires the utmost caution because cytokines are involved in many neurodevelopmental mechanisms and other physiological functions (59).

Our findings show that maternal inflammation during pregnancy acts indirectly through IL-1 to severely damage the placenta, leading to fetal death or to abnormal fetal brain development and neurologic disorders in offspring. We also show the remarkable efficiency of maternal IL-1Ra administration in preventing life-threatening brain damage and lifelong neurodevelopmental injuries. With the caveat described above, we suggest that further studies are needed to validate the possible use of IL-1Ra in human clinical trials in the hope of preventing the frequent and heavy burden of prenatal injuries linked to common maternal infectious/imune activation at the end of pregnancy.

Acknowledgments

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Acknowledgments

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Disclosures

The authors have no financial conflicts of interest.
Supplementary material legends:

**Supplementary Table 1.** Antibodies used for IHC and IF.

**Supplementary Table 2.** Comparison of contrast agent accumulation in the placentas of dams exposed to different experimental conditions relative to controls at G20.

**Supplementary Video 1.** Example of sequential contrast-enhanced $T_1$-weighted images of control dam at G20; analysis from these images are graphically represented in Fig 1f.

**Supplementary Video 2.** Example of sequential contrast-enhanced $T_1$-weighted images of LPS-treated dam at G20; analysis from those images are graphically represented in Fig 1f.

**Supplementary Figure 1.** Placental and fetal MRI from LPS-exposed dams. $T_2$-weighted MRI showing the decreased definition of fetal organ images induced by LPS exposure compared to control (a). Distinct patterns of placental signal enhancement in LPS-exposed dams, after contrast agent injection (b); the left placenta has a homogeneous enhancement (similar to control) as compared to the right placenta presenting a decreased and heterogeneous perfusion: areas of contrast enhancement (black arrow) contrasting with areas of complete lack of contrast enhancement (white arrow). See related data in Supplementary Table 2.

**Supplementary Figure 2.** MRI of the paravertebral muscle.
There was no difference in the level of blood perfusion between paravertebral muscles of LPS-exposed compared to unexposed dams.

**Supplementary Figure 3.** Histological aspect of placental cavitary areas. Von Kossa staining (a), activated caspase-3 (b), and CD68 (c) staining surrounding placental cavitations (*) from LPS-exposed dams. Scale bars, 25 μm.

**Supplementary Figure 4.** LPS-induced microgliosis at P9.
Pups from LPS-exposed dams presented an increased density of microglial cells (Iba-1+) within the white matter’s forebrain, as compared to control, at P9. Error bars represent means ± SEM, *=p<0.05 by t-test with Welch correction.

**Supplementary Figure 5.** MRI comparison between control and IL-1Ra (10 mg/kg) exposed placenta. IL-1Ra (10 mg/kg) alone did not induce any change, neither in mean signal intensity on T2-weighted images (a), nor in contrast agent enhancement monitored by T1-weighted images (b).
## Supplementary Table 1: Antibodies used for IHC and IF

<table>
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<tr>
<th>Name</th>
<th>Company</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionized calcium Binding adapter molecule 1 (Iba-1)</td>
<td>Wako Chemicals, VA, US</td>
<td>1:500</td>
</tr>
<tr>
<td>Proliferating cell nuclear antigen (PCNA)</td>
<td>Santa Cruz Biotechnology, CA, US</td>
<td>1:500</td>
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<tr>
<td>CD68</td>
<td>Chemicon, ON, Canada</td>
<td>1:250</td>
</tr>
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<td>Active Caspase-3</td>
<td>Chemicon, ON, Canada</td>
<td>1:10</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Serotec, NC, US</td>
<td>1:500</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Chemicon, ON, Canada</td>
<td>1:500</td>
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<tr>
<td>IL-6</td>
<td>R &amp; D Systems, MN, US</td>
<td>1:500</td>
</tr>
<tr>
<td>Anti-mouse Alexa Fluor conjugated</td>
<td>Invitrogen, ON, Canada</td>
<td>1:500</td>
</tr>
<tr>
<td>Anti-rabbit Alexa Fluor conjugated</td>
<td>Invitrogen, ON, Canada</td>
<td>1:500</td>
</tr>
<tr>
<td>Anti-mouse-HRP</td>
<td>Santa Cruz Biotechnology, CA, US</td>
<td>1:100</td>
</tr>
<tr>
<td>Anti-rabbit-HRP</td>
<td>Serotec, NC, US</td>
<td>1:100</td>
</tr>
</tbody>
</table>
**Supplementary Table 2:** Comparison of contrast agent accumulation in the placentas of dams exposed to different experimental conditions relative to controls at G20.

| Level of contrast agent accumulation relative to controls at G20 (%) | Proportion of placentas for different levels of contrast agent accumulation (%) |
|---|---|---|---|---|---|
| | LPS (n=25) | IL-1Ra (2 mg/kg) + LPS (n=26) | IL-1Ra (10 mg/kg) + LPS (n=29) | IL-1Ra (20 mg/kg) + LPS (n=34) | IL-1Ra (10 mg/kg) (n=17) |
| 90 and more | 20 | 44 | 69 | 91 | 41 |
| 75-90 | 32 | 11 | 14 | 0 | 53 |
| 50-75 | 28 | 41 | 14 | 6 | 6 |
| 25-50 | 12 | 4 | 3 | 3 | 0 |
| 0-25 | 8 | 0 | 0 | 0 | 0 |
Percentage of Iba-1+ cells

Control

LPS