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IL-1 Receptor Blockade Prevents Fetal Cortical Brain Injury But Not Preterm Birth in a Mouse Model of Inflammation-Induced Preterm Birth and Perinatal Brain Injury

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Abstract

Problem—Exposure to intrauterine inflammation, associated with preterm birth, has been linked to a devastating spectrum of neurobehavioral disorders. Mechanisms of this injury are unknown. Using a mouse model of intrauterine inflammation, we have observed a disruption of fetal neuronal morphology along with a marked elevation of Interleukin (IL)-1 β in the fetal brain and placenta. In the current study, we hypothesized that IL-1 plays a key role in perinatal brain injury.

Method of Study—Utilizing a mouse model of inflammation-induced preterm birth, we investigated the role of IL-1 in fetal cortical injury as well as preterm birth. In these studies, dams received systemic treatment with IL-1 receptor antagonist prior to administration of intrauterine inflammation.

Results—Systemic maternal antagonism of IL-1 improved fetal cortical neuronal injury associated with the exposure to intrauterine inflammation, without affecting the phenotype of preterm birth. IL-1 receptor antagonist blocked activation of neuronal nitric oxide synthase in perinatal cortex, a key enzyme implicated in neurotoxicity.

Conclusion—Our data suggest that fetal cortical brain injury and preterm birth may occur by divergent mechanisms. Furthermore, our studies indicate maternal administration of IL-1 receptor antagonist (IL-1RA) blocked neuronal nitric oxide synthase activation observed in the brain cortex and, we speculate, that this alteration in activation leads to demonstrated decreased neurotoxicity.

Keywords

Intrauterine inflammation; mouse model; IL-1 receptor antagonist

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INTRODUCTION

There are approximately 500,000 infants born preterm in the United States annually, they account for just over 12 percent of all births.^{1,2} Preterm birth results in not only an increase in acute neonatal morbidity and mortality but also has many long-term implications for survivors. Epidemiological studies have identified an association between intrauterine infection or inflammation and preterm birth,³ and as such to the development of cerebral palsy and other neuropsychiatric conditions such as cognitive dysfunction, schizophrenia and even autism.^{4,5}

Animal models of intrauterine inflammation have been used to recapitulate behavioral, and movement disorders similar to those that occur in exposed children.⁶ The observed adverse neurobehavioral outcomes are now thought to occur not only due to a heightened vulnerability of premature white matter to inflammation but also directly through fetal cortical neuronal injury.^{7,8} This injury has been defined as both abnormal cellular morphology and dendritic arborization as well as delayed neurotoxicity^{9,10} and is proposed to occur via a pro-inflammatory pathway that is triggered in response to intrauterine inflammation.^{11,12} Cytokine response reported by our group and others^{10,13–15} has shown in an acute elevation of IL-6, TNF- α and significant increase in IL-1 β in the fetal brain and placenta.¹⁰

This remarkable increase in IL-1 β stimulated an investigation into the possibility of this cytokine being a key player in the observed fetal neuronal injury. Girard et al in 2010¹⁶ showed that antagonism of IL-1 receptor in a rodent model of systemic inflammation decreased placental injury and improved motor performance in offspring; however, the effect on preterm birth and cortical effects were not studied. We, therefore, sought to use our model of inflammation-induced preterm birth (II-PTB) to investigate the effects on the fetal cortex as well as on the phenotype of preterm birth.

We hypothesized that IL-1 is a key mediator of fetal cortical brain injury and that pre-treatment with an IL-1 receptor antagonist (IL-1RA) could prevent the observed fetal brain injury. Furthermore, we investigated whether maternally administered IL-1RA has the ability to block preterm birth.

MATERIALS AND METHODS

Model of Inflammation-induced Preterm Birth

For the mouse model of intrauterine inflammation, time-pregnant CD-1 out-bred mice (Charles River, Wilmington, MA) were utilized. Animals were handled in accordance with the Institutional Animal Care and Use Committees at the University of Pennsylvania, Philadelphia, PA and Johns Hopkins University, Baltimore, MD. As delivery day in CD-1 mice routinely occurs on 19th day of gestation (E19), survival surgery and intrauterine (IU) injections of lipopolysaccharide (LPS) or normal saline (NS) were performed on E15 of gestation to mimic the phenotype of preterm birth, per previously described protocols.^{8–10,17} Briefly, mice were randomized to receive either LPS (250 μ g μ g/dam in 100 μ L of NS; from *Escherichia coli*, 055:B5, Sigma Chemical Co, St Louis, Missouri) or an equivalent volume

of normal saline (NS; control), and received the intrauterine infusion between 1st and 2nd gestational sac via a mini-laparotomy under general anesthesia with isoflurane. With this model, 95% of dams deliver preterm.^{8-10,17}

Pharmacologic Intervention

For our studies we utilized, IL-1RA, an FDA-approved Class B drug in pregnancy, Kineret (Amgen). Previous investigators have found an at least 75% homology between mouse and human IL-1RA making it feasible to use this pharmacologic agent in mouse and expecting it to have a similarly specific mechanism of action.¹⁸ At E15, 30 minutes prior to receiving intrauterine injections of LPS or NS, dams were injected intraperitoneally (IP) with either IL-1RA at 10mg/kg or normal saline (NS). This dose was selected based on work from Girard et al experiments (2010)¹⁶, demonstrating that at this dose, antagonist protects against placental and neurodevelopmental deficits in a rat model of maternal inflammation. For the experimental design, dams were divided into three groups: NS IP plus NS IU group (10 dams), NS IP plus LPS IU group (13 dams), IL-1RA IP plus LPS IU group (20 dams). Outcomes that were examined were preterm birth phenotype, fetal cortical neuronal development *in vitro* (immunocytochemistry and cell viability assays), and postnatal day (PND) 5 cortex *in vivo* studies (qPCR and Western blot analysis) for markers of excitotoxicity.

Preterm birth phenotype determination

Experimental groups were allowed to delivery spontaneously to determine effect of IL-1RA on preterm birth phenotype. The animals were checked twice a day until delivery, following the intrauterine injections.

Primary Cortical Neuronal Cultures

For the purposes of fetal neuronal cell culture and tissue harvest for qPCR, dams were humanely euthanized 4–6 hours after surgery by utilizing carbon dioxide (CO₂). Using sterile technique, 3–4 fetal cortices per dam were removed from the right uterine horn following euthanasia as described previously.⁸⁻¹⁰ The fetal brains were harvested by incision of the calvaria and placed into cold Ca/Mg free Hanks Balanced Salt Solution (pH 7.4; Invitrogen). The cortices were separated from the meninges, olfactory bulbs, brain stem and cerebellum. They were then mechanically minced using curved forceps in neurobasal medium (NBM; Invitrogen) with 0.03% trypsin and incubated at 37 degrees Celsius and 5% CO₂ for 15 minutes. The tissue was removed from the incubation solution and added to 10% fetal bovine serum (FBS) in NBM and cells were dissociated by trituration. NBM (FBS-free) was supplemented with B-27, L-glutamine and used as neuronal-selective media. Under these conditions >95% of the cells are neurons. Cells were plated at a density of 4×10^4 cells/mL on poly-L-lysine coated glass coverslips and grown in culture at 37° Celsius. For these fetal experiments, 5 dams per experimental group were utilized. Three to four fetal cortices from each dam.

Immunocytochemistry

Cells were fixed in 4% paraformaldehyde and stained at days *in-vitro* (DIV3) to assess morphologic characteristics. Cells were permeabilized with 0.5% Triton X and stained using double fluorescence. A mouse monoclonal antibody to microtubule-associated protein 2 (MAP2; Sigma-Aldrich M1406) at a dilution of 1:100 was used to identify dendrites and cell bodies. A rabbit polyclonal antibody to neurofilament 200 (NF200; Sigma-Aldrich N4142) at a dilution of 1:400 was used to label the cell body. Primary antibodies were incubated overnight at 4 degrees Celsius. Secondary antibodies were used as follows: Goat anti-mouse Alexa Fluor 488 (Invitrogen) and Goat anti-rabbit Alexa Fluor 568 (Invitrogen) at a dilution of 1:500 at 37 degrees Celsius for 1 hour. Cells were visualized on a Zeiss microscope at 40X (final magnification of 400X). A total of 150 cells per group (3 slides per dam, 10 neurons per slide, n=5 dams per group) were photographed and analyzed.

Dendritic Process Quantification

Cells stained by immunocytochemistry were photographed. Individual neurons were selected for analysis if they had no clear connections to other neurons. A dendrite was counted as such if it was a single clear projection from the cell body. Two separate observers counted the number of dendrites manually. Counted dendrites were grouped according to treatment group and statistically analyzed by SigmaStat software. The data was non-parametric in its distribution and therefore a Kruskal-Wallis One Way – ANOVA on Ranks with a Dunn's post-test was performed.

Morphologic Characterization

In addition to quantification of dendritic processes the following morphologic characteristics were analyzed: cell body diameter and dendritic area/dendrite (a surrogate for dendritic volume). For each of the analyzed cells, maximum cell body diameter was measured using ImageJ software. For the measurement of dendritic area/dendrite, all dendrites were manually traced and area recorded (ImageJ) and divided by the number of dendrites per cell, cells without any dendrites were excluded from this analysis. This process was done for each neuron after which the average area was divided by the number of dendrites to yield a dendritic area/dendrite measurement. The data for both of these measurements as analyzed with SigmaStat software was non-parametric and therefore a Kruskal-Wallis One Way – ANOVA on Ranks with a Dunn's post-test was performed.

Mitochondrial Activity Assessment

Mitochondrial activity was used as a marker for delayed neurotoxicity. Primary neuronal cultures at DIV14 were assessed for mitochondrial activity using an In Vitro Toxicology Assay Kit, MTT based (Sigma-Aldrich), company supplied protocol was used. Analysis of data was done with SigmaStat, distribution was parametric and therefore One Way – ANOVA was performed with Neuman-Keuls post-test.

Quantitative Polymerase Chain Reaction (qPCR)

PND 5 brains were harvested and processed, using the Trizol method (Invitrogen, Carlsbad, CA), per manufacturer's recommendations. RNA was extracted and cDNA was created as

per protocol. QPCR was performed as previously reported^{9,19–20} for evaluation of NMDA R1, nNOS. Pooled fetal brains were used from each treatment group were utilized for the analysis and the comparison of the mRNA expression.

Western blotting

To determine the protein expression of phospho-SER-847 nNOS, Western blot analysis was performed using tissue homogenates from PND5 brains. Tissues were homogenized and the amount of crude protein present in each sample was determined using the BCA protein assay (Pierce Biotechnology, Inc., Rockford, IL). Forty micrograms of protein were mixed with 2× SDS sample buffer and subjected to SDS-PAGE. The separated proteins were transferred electrophoretically to PVDF membranes. Membranes were blocked in Tris-buffered saline with 5% nonfat dried milk powder for 2 hours. For phospho-SER-847, an antibody that recognizes nNOS phosphorylated at SER-847 (50 kD) and was used in a 1:1000 dilution. An anti-goat secondary antibody was used in a 1:15,000 dilution. Blots were incubated overnight with the primary antibody, incubated for 1 hour with the secondary antibody and then developed with the ECL system (Amersham Pharmacia Biotech, Piscataway, NJ) to detect antibody-antigen complexes. Protein expression was quantified using the National Institutes of Health imaging software (Image J) using three to six samples from each treatment group.

RESULTS

Maternal antagonism of IL-1 did not prevent preterm birth

We sought to investigate whether IL-1RA may prevent the phenotype of preterm birth in the mouse model. Dams following surgery were followed through until delivery. As expected, in the NS+NS group, no litters delivered prematurely; however, in both the LPS+NS and the LPS+IL-1RA group, 80–95% of the litters delivered prematurely at 48 hours after survival surgery ($p>0.05$). There was no difference in regards to length of gestations ($p>0.05$).

Pre-treatment with IL-1RA prevented abnormal dendritic arborization in fetal cortical neurons following exposure to LPS

The NS-exposed fetal neurons (negative control) had a median of 4 dendrites per neuron and discrete cytoskeletal morphology at DIV3. As expected based on our previous experiments, LPS-exposed groups had a median of 2 dendrites at DIV3 which was significantly different than NS-exposed neurons ($p<0.001$). Administration of maternal IL-1RA appeared to rescue this difference. IL-1RA neurons had a median of 4 dendrites per neuron, this was similar to the NS-exposed group ($p>0.005$) but remained statistically different from the group of LPS-exposed neurons ($p<0.001$, Dunn's method) (Figure 2).

IL-1RA results in improved dendritic morphology

Although the total number of dendrites per neuron was rescued by IL-1RA, the dendrites appeared visually more diffuse and spread out (Figure 3). To further investigate these observed cytoskeletal morphologic differences, the cell body diameter and dendritic areas were calculated. The largest cell body diameter was calculated for each neuron. As was observed with dendritic count, neurons in the IL-1RA group were similar to NS neurons and

statistically different than LPS-exposed neurons. Cell bodies of LPS-exposed neurons had a significantly larger cell body diameter than the other two groups ($p<0.05$; ANOVA, Dunn's method) (Figure 4). Despite this effect, visually IL-1RA neurons still appeared different from NS neurons, therefore an attempt to quantify dendritic volume was done, see materials and methods for details. In this analysis, all three of the experimental groups were statistically significantly different from each other ($p<0.05$; ANOVA, Dunn's method) (Figure 5). This seems to indicate that although IL-1RA may rescue some aspects of dendritic morphology following LPS administration (such as preserving cell body size) it does not prevent all morphologic abnormalities.

IL-1RA prevents delayed cortical neurotoxicity

We have previously shown that exposure to intrauterine inflammation results in delayed neurotoxicity as measured by a decrease in mitochondrial function at DIV14 (and, hence, a decrease in viability).¹⁰ Administration of IL-1RA, prior to intrauterine inflammation, resulted in a prevention this delayed neurotoxicity and increased neuronal viability ($p>0.05$, One-Way ANOVA; Figure 6).

Decreased excitotoxicity in IL-1RA treated groups

Markers of neurotoxicity through excitotoxic pathways were investigated *in vivo* at PND 5. NMDAR-1 (Subunit 1 of NMDA receptor, implicated in excitotoxicity) and neuronal nitric oxide synthase (nNOS) were chosen as markers of neurotoxicity. qPCR transcripts showed a protective effect of IL-1RA in PND 5 cortex (Figure 7). While there was no difference in total nNOS between LPS and NS-exposed fetal neurons was seen in qPCR signal, nNOS activity is dependent on the post-translational modification of signal with dephosphorylation at SER-847 resulting in increased activity. In our isolates, phosphorylation was increased in IL-1RA treated cells similarly to NS-exposed neurons and in contrast to LPS-exposed cells ($p<0.05$, when compared to housekeeping gene, β actin; Figure 8).

DISCUSSION

In our study, we utilize a well-established mouse model of inflammation- induced preterm birth (II-PTB) to investigate the role of IL-1 in the propagation of perinatal brain injury and discern potential mechanisms of the injury. Our data builds upon the growing body of literature that implicates IL-1 as a key player in modulation of perinatal brain injury following exposure to intrauterine inflammation. We are able to demonstrate for the first time that preterm birth and perinatal brain injury associated with maternal intrauterine inflammation may occur by divergent mechanisms and to offer a possible mechanism of the neuronal injury.

The connection between II-PTB and neurologic damage is well established.²¹ While not all scenarios of preterm birth are a result of activated inflammatory cascades, previous studies by our group have shown that in non-inflammation based model of preterm birth (RU-486),⁹ the observed neurological effects are absent while preterm birth is present. This supports a scenario in which fetal treatment with IL-1RA may be most beneficial for preterm birth in the setting of inflammation as opposed to all types of preterm birth.

Traditionally, IL-1 is considered a mediator of the peripheral immune response; however, recent data has increasingly implicated it as a key mediator of pathogenesis in a number of neurologic disorders.²² There are several reports in the literature that microglial cells respond rapidly to insults including infection or inflammation, and injury by releasing cytokines, including IL-1.²³ A rat model of ischemic brain injury utilized by Yamasake et al reported that intracerebroventricular injection of IL-1RA reduced ischemic brain damage.²⁴ Girard et al used an established rat model of maternal immune activation to show that IL-1 receptor antagonism prevented placental damage, *in utero* fetal death, maintained the density of microglial cells and protected against characteristic motor dysfunction.¹⁶

While cerebral palsy and white matter damage are clearly areas of interest, the damage done to the preterm brain is not limited to these areas. Ex-preterm infants have higher rates of cognitive and neurobehavioral disorders. These disease processes are more linked with cortical neuronal damage.²⁵ Previous work by our group has characterized the link between II-PTB and fetal cortical neuronal injury to include abnormal dendritic arborization and delayed neuronal toxicity and cell death.^{9,10} In this current experimental model we show that maternal antagonism of IL-1 β improves the arborization and decreases the observed delayed neurotoxicity. Damage to the developing neuron as mediated by IL-1 β occurs by a yet unidentified mechanism, however excitotoxicity via glutamatergic mechanisms likely plays a key role.²⁶ These previous findings are supported by our conclusions regarding nNOS in this current study.

Our experiments not only continue to build on the growing body of evidence which implicates IL-1 β in fetal neuronal damage but also provides the possibility for development and repurposing of a pharmacologic agent IL-1RA in the field of improving neonatal neurologic morbidity. Our data show that this effect is at least in part specific to targeting the developing CNS system as it does not affect the overall outcome of II-PTB. Other groups²⁷ have shown correlative work which confirms that pharmacologic agents which target IL-1 β and TNF α are insufficient to block preterm birth. Here, we show that even without preventing the phenotype of PTB, there may be previously undiscovered benefit to blockage of IL-1 β , specifically with respect to the developing CNS. More experiments need to be done to determine where the IL-1 antagonism is working – in the maternal/fetal or placental compartment.

One clear disadvantage of our study was that it was a pre-treatment model and is investigating the mechanistic side of the injury; therefore, making it difficult to extrapolate the data to suggest that IL-1 antagonism may be a future neuroprotective agent. Clinically, preterm birth and intrauterine inflammation may not have any prior risk factor and may present at the time that the inflammatory cascade has already been initiated. However, there are situations in which pretreatment could be useful perhaps used in a similar fashion to prophylactic betamethasone for women who are at high risk for early delivery. The benefit of IL-1RA in the more common scenario following initiation of the inflammatory cascade was, however, clearly not addressed by our current experimental model. An important future experiment would be injection of IL-1RA following injection of LPS to determine whether the long-term neurologic adverse effect is still present and still as strong to IL-1RA. Experiments by Girard et al (2012)²⁸ have already investigated the late administration of

IL-1RA on placental pathology, and are in support of our hypothesis of IL-1RA being beneficial when administered following systemic maternal LPS exposure.

An advantage of our experiments, similar to those done by Girard et al, is that we have used a pharmacologic agent already used in humans, specifically Anakinra (brand name Kineret). This agent is a non-glycosylated form of IL-1RA (receptor antagonist) prepared using *Escherichia coli* and recombinant DNA technology. It is administered subcutaneously and used primarily in the management of rheumatoid arthritis in patients who have not responded well to more traditional disease-modifying anti-rheumatic drugs. Making it a particularly interesting target for our purposes, no adverse effects have been observed in animal models, and it is considered pregnancy class B. Also of interest is the recent approval in January 2013 of the drug for use in neonatal-onset multisystem inflammatory disease (NOMID) which may importantly provide additional support to its safety profile in infants and children.

We have shown here that maternally administered antagonism of IL-1 receptor can ameliorate fetal neuronal damage. This provides further evidence to support a mechanism and possible future therapeutic target in the battle against the devastating neurobehavioral outcomes associated with prematurity. Most importantly, we suggest that IL-1 works through an excitotoxic cascade as pretreatment with the antagonist decreased upregulation of NMDA receptor and activation of the key enzyme in the neurotoxic cascade, nNOS.

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References

1. Engle WA, Kominiarek MA. Late preterm infants, early term infants, and timing of elective deliveries. *Clin Perinatol*. 2008 Jun;35(2):325, 41, vi. [PubMed: 18456072]
2. Hamilton, BE.; Martin, JA.; Ventura, SJ. Online: Centers for Disease Control and Prevention. 2013. Births: Preliminary data for 2012. Report No.: 2012
3. Goncalves LF, Chaiworapongsa T, Romero R. Intrauterine infection and prematurity. *Ment Retard Dev Disabil Res Rev*. 2002; 8(1):3–13. [PubMed: 11921380]
4. Yoon BH, Romero R, Park JS, Kim CJ, Kim SH, Choi JH, et al. Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. *Am J Obstet Gynecol*. 2000 Mar; 182(3):675–81. [PubMed: 10739529]
5. Meyer U, Feldon J, Schedlowski M, Yee BK. Immunological stress at the maternal-foetal interface: A link between neurodevelopment and adult psychopathology. *Brain Behav Immun*. 2006 Jul; 20(4):378–88. [PubMed: 16378711]
6. Saadani-Makki F, Kannan S, Lu X, Janisse J, Dawe E, Edwin S, et al. Intrauterine administration of endotoxin leads to motor deficits in a rabbit model: A link between prenatal infection and cerebral palsy. *Am J Obstet Gynecol*. 2008 Dec; 199(6):651 e1–651.e7. [PubMed: 18845289]
7. Leviton A, Gressens P. Neuronal damage accompanies perinatal white-matter damage. *Trends Neurosci*. 2007 Sep; 30(9):473–8. [PubMed: 17765331]
8. Burd I, Chai J, Gonzalez J, Ofori E, Monnerie H, Le Roux PD, et al. Beyond white matter damage: Fetal neuronal injury in a mouse model of preterm birth. *Am J Obstet Gynecol*. 2009 Sep;201(3): 279e1, 279.e8. [PubMed: 19733279]

9. Burd I, Bentz AI, Chai J, Gonzalez J, Monnerie H, Le Roux PD, et al. Inflammation-induced preterm birth alters neuronal morphology in the mouse fetal brain. *J Neurosci Res.* 2010 Jul; 88(9): 1872–81. [PubMed: 20155801]
10. Burd I, Brown A, Gonzalez JM, Chai J, Elovitz MA. A mouse model of term chorioamnionitis: Unraveling causes of adverse neurological outcomes. *Reprod Sci.* 2011 Sep; 18(9):900–7. [PubMed: 21421895]
11. Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. *Am J Obstet Gynecol.* 1998 Jul; 179(1):194–202. [PubMed: 9704787]
12. Svigos JM. The fetal inflammatory response syndrome and cerebral palsy: Yet another challenge and dilemma for the obstetrician. *Aust N Z J Obstet Gynaecol.* 2001 May; 41(2):170–6. [PubMed: 11453266]
13. Cai Z, Pan ZL, Pang Y, Evans OB, Rhodes PG. Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. *Pediatr Res.* 2000 Jan; 47(1):64–72. [PubMed: 10625084]
14. Duggan PJ, Maalouf EF, Watts TL, Sullivan MH, Counsell SJ, Allsop J, et al. Intrauterine T-cell activation and increased proinflammatory cytokine concentrations in preterm infants with cerebral lesions. *Lancet.* 2001 Nov 17; 358(9294):1699–700. [PubMed: 11728550]
15. Bell MJ, Hallenbeck JM. Effects of intrauterine inflammation on developing rat brain. *J Neurosci Res.* 2002 Nov 15; 70(4):570–9. [PubMed: 12404511]
16. Girard S, Tremblay L, Lepage M, Sebire G. IL-1 receptor antagonist protects against placental and neurodevelopmental defects induced by maternal inflammation. *J Immunol.* 2010 Apr 1; 184(7): 3997–4005. [PubMed: 20181892]
17. Elovitz MA, Wang Z, Chien EK, Rychlik DF, Phillippe M. A new model for inflammation-induced preterm birth: The role of platelet-activating factor and toll-like receptor-4. *Am J Pathol.* 2003 Nov; 163(5):2103–11. [PubMed: 14578208]
18. Cominelli F, Bortolami M, Pizarro TT, Monsacchi L, Ferretti M, Brewer MT, et al. Rabbit interleukin-1 receptor antagonist. cloning, expression, functional characterization, and regulation during intestinal inflammation. *J Biol Chem.* 1994 Mar 4; 269(9):6962–71. [PubMed: 7509813]
19. Elovitz MA, Mrinalini C. Can medroxyprogesterone acetate alter toll-like receptor expression in a mouse model of intrauterine inflammation? *Am J Obstet Gynecol.* 2005 Sep; 193(3 Pt 2):1149–55. [PubMed: 16157128]
20. Elovitz MA, Mrinalini C, Sammel MD. Elucidating the early signal transduction pathways leading to fetal brain injury in preterm birth. *Pediatr Res.* 2006 Jan; 59(1):50–5. [PubMed: 16327009]
21. Burd I, Balakrishnan B, Kannan S. Models of fetal brain injury, intrauterine inflammation, and preterm birth. *Amer J Repro Imm.* 2012; 67:287–94.
22. Fogal B, Hewett SJ. Interleukin-1beta: A bridge between inflammation and excitotoxicity? *J Neurochem.* 2008 Jul; 106(1):1–23. [PubMed: 18315560]
23. Yao J, Keri JE, Taffs RE, Colton CA. Characterization of interleukin-1 production by microglia in culture. *Brain Res.* 1992 Sep 18; 591(1):88–93. [PubMed: 1446236]
24. Yamasaki Y, Matsuura N, Shozuhara H, Onodera H, Itoyama Y, Kogure K. Interleukin-1 as a pathogenetic mediator of ischemic brain damage in rats. *Stroke.* 1995 Apr; 26(4):676, 80. discussion 681. [PubMed: 7709417]
25. Zikopoulos B, Barbas H. Altered neural connectivity in excitatory and inhibitory cortical circuits in autism. *Front Hum Neurosci.* 2013 Sep 27; 7:609. [PubMed: 24098278]
26. Stroemer RP, Rothwell NJ. Exacerbation of ischemic brain damage by localized striatal injection of interleukin-1beta in the rat. *J Cereb Blood Flow Metab.* 1998 Aug; 18(8):833–9. [PubMed: 9701344]
27. Fidel PL Jr, Romero R, Cutright J, Wolf N, Gomez R, Araneda H, et al. Treatment with the interleukin-1 receptor antagonist and soluble tumor necrosis factor receptor fc fusion protein does not prevent endotoxin-induced preterm parturition in mice. *J Soc Gynecol Investig.* 1997 Jan-Feb; 4(1):22–6.
28. Girard S, Tremblay L, Lepage M, Sebire G. Early detection of placental inflammation by MRI enabling protection by clinically relevant IL-1Ra administration. *Am J Obstet Gynecol.* 2012 Apr; 206(4):358.e1–9. [PubMed: 22304959]

Figure 1. Experimental design

At E15 of gestation, dams were divided into 2 groups: intraperitoneal (IP) pretreatment with IL-1 receptor antagonist (IL-1RA) or sham IP pretreatment with normal saline (NS). Following 30 minutes, sham group was divided into intrauterine (IU) injections with lipopolysaccharide (LPS; positive control) or NS (negative control), and IL-1RA group received IU LPS (treatment group). As a result, there were three groups: NS IP plus NS IU group (10 dams), NS IP plus LPS IU group (13 dams), IL-1RA IP plus LPS IU group (20 dams).

Figure 2. Number of processes of cultured neuronal cells at days *in vitro* (DIV) 3 IL-1 receptor antagonist (IL-1RA) prevented abnormal dendritic arborization following exposure to intrauterine inflammation. The normal saline (NS)+lipopolysaccharide (LPS group; positive control) had statistically fewer dendrites ($p < 0.001$; ANOVA, Dunn's method) than either NS+NS (negative control) or IL-1RA+LPS group ($n=5$ dams per group) with a median of 2 dendrites. * $p < 0.05$

Figure 3. Immunocytochemistry for microtubule-associated protein 2 (MAP2) at days *in vitro* (DIV) 3

In **A.1-2** normal saline (NS) +NS (negative control), as expected, there were large numbers of clear and narrow dendrites; **B.1-2**, representative images for positive control cells. **B.1**, NS+lipopolysaccharide (LPS) neuron with 2 dendritic processes. **B.2** NS+LPS neuron without any clear dendrites; **C1-2** IL-1 receptor antagonist (IL-1RA)+LPS neurons.

Figure 4. Effect of IL-1 receptor antagonist (IL-1RA) on cell body diameter

The cell diameter of neurons at days *in vitro* (DIV) 3 was significantly larger in the normal saline (NS)+lipopolysaccharide (LPS) group than either of the other experimental groups ($p < 0.05$; ANOVA on ranks, Dunn's method). * $p < 0.05$

Figure 5. Dendritic area at days *in vitro* (DIV) 3

The area of each dendrite was significantly different between all the groups ($p < 0.05$; ANOVA on ranks, Dunn's method). Normal saline (NS)+NS had the lowest, IL-1 receptor antagonist (IL-1RA)+lipopolysaccharide (LPS) the intermediate and NS+LPS the largest area per dendrite. * $p < 0.05$

Figure 6. IL-1 receptor antagonist (IL-1RA) prevents delayed neurotoxicity as shown by normal mitochondrial function at days *in vitro* (DIV) 14

Normal saline (NS)+ lipopolysaccharide (LPS; positive control) has significantly less mitochondrial activity ($p < 0.05$; One-way ANOVA, Newman Keuls) and, consequently less viable cells, than either NS+NS or IL-1RA+LPS. $*p < 0.05$

Figure 7. Quantitative Polymerase Chain Reaction (qPCR) for NMDA receptor subunit 1 (NMDA R1) in postnatal day 5 brain cortex *in vivo*

An obligatory part of the NMDA receptor, subunit R1, is increased in brain cortex with exposure to inflammation (normal saline (NS)+lipopolysaccharide (LPS)), indicating an increased susceptibility to excitotoxic neuronal injury. Administration of IL-1 receptor antagonist (IL-1RA) in IL-1RA+LPS appears to be neuroprotective as the results are similar to that of negative control (NS+NS; $p < 0.05$, One-Way ANOVA, Newman-Keuls). * $p < 0.05$

Figure 8. Effect of prenatal administration of IL-1 receptor antagonist (IL-1RA) on neuronal nitric oxide synthase (nNOS), an enzyme implicated in neurotoxicity, in postnatal day 5 brain cortex *in vivo*

A, There were no differences messenger RNA levels in nNOS between the groups. **B**, there were activation differences *in vivo* between the groups. As dephosphorylation at serine 847 (Ser847) leads to activation of the enzyme, the results from Western blot, demonstrated a decreased amount of phosphorylated enzyme thus indicating an activation with exposure to inflammation (NS+LPS). – NS – normal saline, LPS lipopolysaccharide; * $p < 0.05$