Determining the Fetal Inflammatory Response in an Experimental Model of Intrauterine Inflammation in Rats

MICHAEL J. BELL, JOHN M. HALLENBECK, AND VITTORIO GALLO

Center for Neuroscience Research, Children's Research Institute [M.J.B., V.G.], Children's National Medical Center, George Washington University School of Medicine, Washington, D.C., 20010, and Stroke Branch [M.J.B., J.M.H.], National Institute of Neurological Diseases and Stroke, Bethesda, Maryland, 20892

ABSTRACT

Intrauterine infection is a risk factor for developmental brain injuries in childhood. A variety of cytokines known to be toxic to developing brain cells have been isolated from mothers or children at risk for developmental disabilities, and these cytokines have been proposed as mediators of these injuries. We have developed a model of intrauterine inflammation that damages the developing white matter and we now hypothesize that selected cytokines are increased after our experimental inflammatory stimulus. Timed-pregnant Fischer 344 and Lewis rats were injected with 0.1 mg/kg of lipopolysaccharide (LPS) into the cervix at E15. Tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), IL-6, and IL-10 were measured in homogenates of fetal brain and placenta at serial time periods within the first 24 h after the inflammatory stimulus. TNF- α was increased 20-fold in the placenta and more than 5-fold in the fetal brain after the stimulus. IFN- γ was only increased within the fetal brain (20-fold) and IL-6 was only increased in the placenta (10-fold). IL-10 was mildly increased in the placenta and was decreased slightly in the fetal brain. Our observations show that an intrauterine inflam-

matory stimulus can cause large increases in Th1 cytokines within the fetal brain. The placenta can produce selected cytokines but fails to produce IFN- γ , suggesting that the fetal immune system produces this cytokine in response to our stimulus. By studying placental and brain cytokine responses in models such as ours, the mechanisms responsible for the damage to developing white matter can be determined. (*Pediatr Res* 56: 541–546, 2004)

Abbreviations

CNP, 2', 3'-cyclic nucleotide phosphodiesterase
CP, cerebral palsy
IFN- γ , interferon gamma
LPS, lipopolysaccharide
PLP, proteolipid protein
PVL, periventricular leukomalacia
TLR4, toll-like receptor 4
TNF- α , tumor necrosis factor alpha

Inflammation has become a more widely recognized risk factor for the development of brain injuries in children (1–4). Over the past several years, epidemiologic evidence has accumulated implicating inflammation and infection with CP and PVL. Determining the mechanisms responsible for this association is pivotal in developing strategies to ameliorate the injuries in an early stage or preventing them altogether.

Cytokines are soluble mediators of the immune system that are produced in response to infections and also play a role in

normal brain development (5). Th1 cytokines promote the immune response to infection, whereas Th2 cytokines quell the inflammatory response. TNF- α is a Th1 cytokine that can be detected in developing rodent astrocytes during fetal life (6) and plays a role in parturition initiation in the placenta (7,8). In response to inflammatory stimuli such as LPS, TNF- α is rapidly released by microglia as well as other cells within the CNS (9,10). TNF- α is implicated in many forms of degenerative brain injuries and can initiate programmed cell death signaling cascades. IFN- γ is a Th1 cytokine that is not normally present within the CNS during development. IFN- γ is released from Th1 lymphocytes and natural killer cells and it can induce the release of TNF- α from a variety of cell types (11,12). After an inflammatory stimulus, IFN- γ activates microglia, converts astrocytes into antigen presenting cells, and up-regulates major histocompatibility type II antigens on neurons, astrocytes, and oligodendrocytes (13–17). IL-6 is another Th1 cytokine that is produced by macrophages and monocytes

Received December 2, 2003; accepted April 4, 2004.

Correspondence: Michael J. Bell, M.D., Center for Neuroscience Research, Children's Research Institute, Department of Critical Care Medicine, Children's National Medical Center, 111 Michigan Ave., NW, Washington, DC 20010; e-mail: mbell@cnmc.org

Supported by an Intergovernmental Personnel Agreement between the National Institute of Neurological Disorders and Stroke (NINDS) and George Washington University School of Medicine, K12 HD01399, and K08 HD044716 with National Institute of Child Health and Human Development (NICHD).

DOI: 10.1203/01.PDR.0000139407.89883.6B

in the systemic circulation and it promotes immunoglobulin secretion and T cell activation (18). Its role in normal CNS development is unclear, but IL-6 increases rapidly in response to LPS and TNF- α (19). IL-6 receptors are present on both neurons and glia (20) and it has been implicated in both protective (neurotrophin, cellular survival) as well as degenerative processes within the CNS (21). IL-10 is a Th2 cytokine that acts to limit the inflammatory response after stimulus. Its role in normal development is controversial, but IL-10 diminishes the inflammatory actions of Th1 cytokines through a wide variety of actions (22).

Th1 cytokines have been implicated in inflammation-induced developmental brain injuries, but Th2 cytokines have not. TNF- α and IL-6 have been isolated from the placenta, amniotic fluid, and umbilical venous blood, and IFN- γ has been isolated from fetal blood in children that develop CP or PVL (2,23–26). TNF- α immunoreactivity has been demonstrated in PVL lesions at autopsy (27). *In vitro* systems have demonstrated that TNF- α decreases oligodendrocyte progenitor number, affects cell morphology, and inhibits maturation, whereas IFN- γ induces apoptosis [as measured by deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) and annexin V binding] and decreases oligodendrocyte proliferation (28–30). These observations have led to hypotheses that these cytokines produced in response to intrauterine infection and inflammation cause damage to the developing fetal brain. Conversely, although Th2 cytokines can be produced by placental tissues (31) and have been associated with protection from certain brain injuries (32,33), there is no evidence to date that they play a role in inflammation-induced developmental brain injuries (34).

We have developed a model of intrauterine inflammation in timed-pregnant rats to study this process (35). Our model shows i) damage to the developing white matter as demonstrated by decreased oligodendrocyte-specific staining (CNP and PLP) and ii) an increase in apoptotic cell death in the periventricular region as manifested by an increased number of TUNEL-stained nuclei in the periventricular region. We concluded that these observations could be the result of a decreased number of oligodendrocytes, a decreased amount of myelin protein produced by the oligodendrocytes, or a decreased number of cellular processes.

To further assess the clinical applicability of our model, we measured cytokines that are putative mediators of these injuries in children with PVL and CP. We hypothesized that our model of intrauterine inflammation would cause an inflammatory response within the fetal brain, manifested by an increase in TNF- α , IFN- γ , and IL-6. We hypothesized that the inflammatory response to our stimulus would be limited to Th1 cytokines and, therefore, the Th2 cytokine IL-10 would not be affected. Lastly, we hypothesized that the placenta would be a major source of cytokines released in response to the stimulus and, therefore, cytokines would be increased earlier in the placenta than the brain.

MATERIALS AND METHODS

All experiments were performed with the approval of the Institutional Animal Care and Use Committee of Children's

Research Institute of Children's National Medical Center. Timed-pregnant Fischer 344 and Lewis rats were obtained from Charles River Laboratories (Worcester, MA) at E12 and allowed to acclimate to our animal facility for 3 d before experimentation. LPS (*Escherichia coli* serotype 0111:B4) was obtained from Sigma Chemical Co. (St. Louis, MO). OptEIA ELISA kits for rat IFN- γ , TNF- α , IL-6, and IL-10 were obtained from BD Pharmingen (San Diego, CA).

Experimental protocol. Pregnant rats were studied at E15 using a protocol we have previously described (35). Briefly, rats were anesthetized with 4% isoflurane, the cervix was visualized, and 0.1 mg/kg of LPS was administered in a mixture of 0.1 mL of saline. In pilot experiments to develop our model, we found that the cervical wall could be easily visualized using a surgical microscope and a small speculum. We found that we could reliably inject 0.1 mL of dye (Evans blue) within the cervical muscle with high reliability and without leakage into the surrounding tissue. Once injected with LPS, rats recovered from anesthesia and the procedure within 2 min of induction. We have previously established that this dose of LPS is associated with minimal fetal mortality (4%) when animals are allowed to survive to delivery.

At designated times after injection (baseline, 1 h, 2 h, 6 h, and 24 h), rats were again anesthetized with 4% isoflurane. A laparotomy was performed and the uterus exposed. Fetal sacs were isolated, fetal brain and placenta were dissected and placed in 600 μ L of iced lysis buffer (RIPA buffer containing 50 mM Tris-HCl, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, and 1 mM EDTA; recipe from Upstate, Charlottesville VA). Tissue was dissociated, sonicated, and centrifuged at 14,000 rpm at 4°C. Supernatants were collected and stored at -70°C until analysis.

Protein determination. Protein concentration of each supernatant was determined using reagents from BCA following the directions from the manufacturer (Pierce Chemical, Rockford, IL). In brief, 2 μ L of supernatant was mixed with 48 μ L of deionized water and 1 mL of the BCA protein assay mixture. Standards were prepared similarly using BSA as a protein source. Samples were heated to 60°C in a water bath for 30 min and protein concentrations were determined using a spectrophotometer (Beckman DU Series 500, Beckman Coulter, Inc., Fullerton, CA) at 562 nm.

Cytokine determination. TNF- α , IL-1 β , IL-6, and IL-10 concentrations were determined by commercially available ELISA kits (BD Pharmingen) following the directions provided by the manufacturer. Samples (100 μ L) or known standard were added to wells coated with an appropriate capture antibody. After the wells were washed, a biotinylated antibody and an avidin-horseradish peroxidase conjugate were serially applied. Tetramethylbenzidine was used as a chromogen and the reaction was stopped after 30 min with the application of 1 M H₃PO₄. Absorbance was measured at 450 nm by a microplate reader (Thermomax Microplate Reader, Molecular Devices, Sunnyvale, CA), and data were analyzed with Soft Max Pro software (Molecular Devices). Standard curves were determined from known standards and sample cytokine concentrations were calculated. Samples were run in duplicate, and the mean concentration of each sample per milligram of protein

was calculated. For each cytokine, median concentrations of all the samples of a given time point were determined and reported as median (25–75%).

Statistical analysis. Statistical analysis was performed using SPSS version 2.0 (SPSS Inc., Chicago, IL). Because we were interested in studying the acute effects of our LPS injection on baseline cytokine concentrations and not on saline controls, we compared concentrations at times after injection to baseline with the Kruskal-Wallis one-way ANOVA on ranks with Dunn's method. Correlations between cytokines were determined using Pearson correlations ($p < 0.05$ was considered significant).

RESULTS

Four mothers per time period were studied (two Fischer 344 and two Lewis), producing a total of 80 placentas and fetal brains for analysis ($n = 12, 11, 24, 16,$ and 17 at baseline, 1 h, 2 h, 6 h, and 24 h, respectively). Preliminary analysis revealed no differences between Fischer 344 and Lewis rats for any cytokine at any time period studied in either the brain or placenta. This finding is consistent with our previous work demonstrating that each strain is equally affected by our inflammatory stimulus (35). Therefore, data from brains and placentas from both strains were combined for analysis at each time point.

Placental concentrations of the cytokines are summarized in Figure 1. TNF- α significantly increased at 1 h and 2 h after the inflammatory stimulus, returned to baseline at 6 h and was

maximal (20-fold increase) at 24 h [baseline, 4.1 pg/mg (2.9–5.7); 1 h, 17.2 (15.3–19.8); 2 h, 29.6 (16.6–88.4); 6 h, 4.5 (3.0–13.3); 24 h, 79.1 (22.5–94.5); $p < 0.05$ for baseline versus 1 h, 2 h, and 24 h]. Conversely, placental concentrations of IFN- γ remained unchanged during the initial time periods and decreased below baseline by 6 and 24 h [baseline, 13.6 pg/mg (11.2–18.9); 1 h, 16.5 (14.2–19.3); 2 h, 15.3 (7.9–26.7); 6 h, 6.1 (0.9–8.0); 24 h, 5.1 (1.9–8.1); $p < 0.05$ for baseline versus 6 h and 24 h]. Placental IL-6 concentrations were dramatically increased at 2 h and 6 h [baseline, 19.4 pg/mg (13.8–48.0); 1 h, 21.1 (13.8–41.4); 2 h, 170.6 (96.1–282.5); 6 h, 192.1 (61.1–269.4); 24 h, 23.2 (12.7–57.9); $p < 0.05$ for baseline versus 2 h and 6 h]. Placental IL-10 was mildly increased at 6 h and this increase persisted at 24 h [baseline, 2.5 pg/mg (1.7–4.3); 1 h, 6.4 (3.0–8.3); 2 h, 5.4 (1.6–9.2); 6 h, 10.3 (5.8–13.2); 24 h, 6.0 (4.2–8.1); $p < 0.05$ for baseline versus 6 h and 24 h].

Brain concentrations of cytokines are summarized in Figure 2. Brain TNF- α and IFN- γ were both increased at all time periods after 2 h [TNF- α : baseline, 12.6 pg/mg (9.0–14.0); 1 h, 11.9 (9.9–13.5); 2 h, 19.0 (13.5–108.5); 6 h, 111.9 (88.1–129.1); 24 h, 29.5 (19.5–34.8); IFN- γ : baseline, 3.8 pg/mg (1.1–7.6); 1 h, 3.2 (1.6–6.8); 2 h, 38.4 (8.8–72.4); 6 h, 80.8 (65.4–91.7); 24 h, 20.3 (19.9–23.5); $p < 0.05$ for baseline versus 2 h, 6 h, and 24 h for both TNF- α and IFN- γ]. Brain concentrations of both IL-6 and IL-10 showed slight declines during the study period, reaching significance below baseline at 24 h [IL-6: baseline, 142.3 pg/mg (110.7–185.3); 1 h, 164.8

Figure 1. Placental cytokines after an intrauterine inflammatory stimulus. TNF- α increased at 1 h and 2 h after the inflammatory stimulus and then demonstrated a 20-fold increase at 24 h (* $p < 0.05$ for baseline vs 1 h, 2 h, and 24 h). Placental concentrations of IFN- γ remained constant in the initial time periods and decreased below baseline by 6 h and 24 h (** $p < 0.05$ for baseline vs 6 h and 24 h). Placental IL-6 concentrations were dramatically increased at 2 h and 6 h (* $p < 0.05$ for baseline vs 2 h and 6 h). Placental IL-10 was mildly increased at 6 h and this persisted at 24 h (* $p < 0.05$ for baseline vs 6 h and 24 h).

Figure 2. Brain cytokines after an intrauterine inflammatory stimulus. TNF- α and IFN- γ were both increased at all time periods after 2 h (* $p < 0.05$ for baseline vs 2 h, 6 h, and 24 h for both TNF- α and IFN- γ). Brain concentrations of both IL-6 and IL-10 showed slight declines during the study period, reaching significance below baseline at 24 h (** $p < 0.05$ for baseline vs 24 h for both IL-6 and IL-10).

(146.4–221.3); 2 h, 138.3 (105.3–169.7); 6 h, 122.2 (108.2–155.9); 24 h, 49.7 (35.1–76.2); IL-10: baseline, 116.8 pg/mg (83.4–142.9); 1 h, 115.5 (99.0–176.2); 2 h, 54.0 (45.6–127.6); 6 h, 58.6 (45.7–75.3); 24 h, 26.6 (21.8–33.7); $p < 0.05$ for baseline versus 24 h for both IL-6 and IL-10). *In vitro* studies suggest TNF- α and IFN- γ are both required for toxicity of some glial cells, including oligodendrocytes and their precursors. We found a very high correlation between brain TNF- α and IFN- γ ($r^2 = 0.872$, $p < 0.000001$), suggesting both cytokines are increased concurrently.

DISCUSSION

In this study, we hypothesized that our inflammatory stimulus would increase Th1 cytokines, would not significantly change Th2 cytokines, and the placenta would be the main source of the cytokine response. Our results demonstrate a vigorous Th1 cytokine response in both the brain and placenta and minimal change in Th2 cytokine response in both the brain and placenta, thereby supporting the first two hypotheses. With regard to the final hypothesis, placental TNF- α was increased before an observed increase within the brain, suggesting that the placenta may be a fetal source of this cytokine. But, brain IFN- γ increased dramatically without a placental IFN- γ response, suggesting that the placenta is not a source of this cytokine in response to our stimulus.

In determining the utility of an animal model to study a human disease, we feel it is important that i) the cells and structures injured in the human condition are similarly affected

in the animal model, ii) the putative mediators of injury in the human condition are present in the animal model, and iii) these mediators have a plausible rationale for producing the injury observed. Once these conditions are met, mechanisms of injury can then be assessed in the animal model and further comparisons can be made with the human disease.

With the completion of the present study, we believe we have begun to satisfy these criteria for our model. We have previously shown damage to the developing corpus callosum by oligodendrocyte-specific immunostaining (CNP and PLP) and an increased number of apoptotic nuclei after our inflammatory stimulus (35). In this study, we have found that two important Th1 cytokines are increased relatively early and persistently within the fetal brain after our stimulus is applied to the uterus of the mother. Others have found these cytokines are toxic to developing oligodendrocytes in culture (28,29), so studying the mechanism of injury in our model may provide clues to the mechanisms of injury in children.

We are the first group to report on the placental contribution to the cytokine response and we have found that our intrauterine stimulus causes increased placental TNF- α and IL-6, but not IFN- γ . This suggests that although TNF- α produced in the placenta may simply cross the placental-fetal barrier, IFN- γ is produced by the fetal immune system to cause its effects. In contrast, the IL-6 response observed within the placenta is not within the fetal brain. If this is proven true in other models and in humans, pharmacological treatments to mitigate these effects would necessarily need to target both the maternal and fetal immune systems.

Models of inflammation have been developed in attempts to mimic developmental brain injuries. Several groups have instilled live bacteria within pregnant animals to develop an inflammatory response (36,37) but quantification of the cytokine response in these models has not been reported. LPS administration has been used as an inflammatory stimulus in a variety of different methodologies. Duncan and colleagues (38) delivered LPS intravenously on 5 consecutive days. Serum IL-6 increased in the fetuses after the first two injections but then returned to baseline and other cytokines were not reported. This model also showed that LPS delivered in this manner had systemic effects on the developing fetus (decreased blood pressure and decreased oxygen saturation of arterial blood) and the treatment led to damage within the developing white matter. Our model did not estimate these physiologic changes caused by LPS injection due to size constraints in our animals. However, we measured tissue, rather than serum, concentrations of cytokines, which may be a more accurate reflection of the actions of these mediators. We chose to measure TNF- α and IFN- γ due to their association with oligodendrocyte damage as well (28,29). In the future, it would be interesting to determine the placental response to this intravenous method of LPS delivery.

Our findings are in relatively close agreement with models using intraperitoneal LPS injections. Cai and colleagues (39) injected timed-pregnant rats with LPS in two separate paradigms. In the first experiments, a relatively large dose of LPS (4 mg/kg) was administered on a single day (E18) and time-dependent increases in TNF- α and IL-1 β mRNA were observed in fetal brains. The mRNA of both cytokines were detectable at 1 h and maximal at 24 h. In subsequent experiments, the authors injected LPS in a smaller dose (500 μ g/kg) on both E18 and E19 and observed decreases in myelin basic protein staining within white matter structures at P8. Although this study reports mRNA concentrations rather than protein, the time course of TNF- α expression is similar to that observed in our study. Both studies confirm that LPS injection in pregnant animals can initiate a significant inflammatory response within the fetus, including the production of Th1 cytokines within the fetal brain. However, we observe this increase at the same dose of LPS that causes histologic changes later during development, and our model of injury assesses the placental cytokine response. Intraperitoneal injections of LPS bypass the placenta and may not mimic human chorioamnionitis as closely as models such as ours or those that involve bacterial infection of the uterus.

Others have directly injected LPS into the developing brain of developing animals to initiate the inflammatory response. Pang and colleagues (40) stereotactically injected LPS into the cerebral ventricles of P5 rats and found that the white matter was selectively damaged. At a number of time periods after the stimulus, brain TNF- α , IL-6, and IL-1 β concentrations were measured. They observed a vigorous TNF- α response within hours that persisted over 24 h, which is similar to our findings. In contrast, they also observed a rapid increase in IL-6, which we did not observe. Recently, this group has shown that IL-1 β may play a pivotal role in this model (41). We chose our cytokines because our preliminary data showed that TNF- α

was present in the brain at the time of delivery whereas IL-1 β was not observed. In the future, it would be interesting to determine whether our model shows a similar IL-1 β response. Lenhardt and colleagues (42) injected LPS into the cerebral ventricles and similarly demonstrated selective injury to the developing white matter. Through a series of experiments, they deduced that LPS binding to TLR4 may be responsible for damaging the developing glia but they did not investigate the cytokine response in their experiments. Direct injection of LPS into the brain causes localized trauma and likely activates a wide variety of immunologic responses that may be additive or even synergistic in nature. Because epidemiologic studies as well as evidence from our model suggest that the placenta plays a role in this process, it is the burden of investigators using intracerebral injection models to prove that their stimulus mimics chorioamnionitis closely enough to make their applicability to humans apparent.

Our study has several limitations. We measured cytokine concentrations in the entire brain and this may not reflect the cytokine milieu within the true region of interest, the periventricular white matter. We considered dissecting the developing white matter from the adjacent structures but were concerned about introducing biases using this approach. Because developing white matter and gray matter have widely variable protein contents during development, inclusion of a small amount of adjoining structures might significantly affect the protein content and tissue homogeneity of the isolated samples. Because we standardized tissue cytokine concentrations to the total protein concentration of the homogenate, minor changes in the efficiency of the white matter dissection could cause significant bias in the results. Also, brain and placental tissue samples in this study included some amount of fetal blood. We strove to eliminate as much fetal blood contamination as possible, but the concentrations of cytokines we observed were necessarily influenced by some fetal blood contamination. This small amount of contamination probably explains why we observed minimal baseline concentrations of IFN- γ in the brain. However, if the observed increases in cytokines were solely due to fetal blood, both placental and brain cytokine concentrations would have increased in parallel. As we demonstrated, IFN- γ concentrations within the placenta did not increase whereas those in the brain increased 20-fold. Lastly, we could not control for changes in protein synthesis caused by our LPS treatment. If the overall protein concentration of the fetal brains or placentas was changed by our treatment, then the cytokine concentrations that we calculated (standardized to overall protein content) would reflect this alteration. This effect would likely take some time to manifest itself and would be expected at the later time periods of the study. For instance, if our LPS treatment significantly increased overall protein synthesis, this would explain why brain IL-6 and IL-10 concentrations are decreased slightly at 24 h after treatment. Further studies to control for protein synthesis would be necessary to fully explain this effect.

In conclusion, we have demonstrated that our experimental model of intrauterine inflammation causes a vigorous and specific Th1 cytokine response in the fetal brain and placenta. Since these cytokines may play a role in the development of

brain injuries in children, investigating the mechanisms of cell damage and death and the effects of our treatment on cells of the oligodendrocyte lineage may help in understanding these processes in children. If our findings of a fetal response to inflammation are proven true in other models and in children, pharmacological agents that can cross the placental barrier will be required for clinical trials to ameliorate the cytotoxic effects of inflammation. We believe that other studies seeking to mimic intrauterine inflammation should demonstrate that purported mediators in human disease are present at critical times during development.

REFERENCES

- Wu Y, Colford J 2000 Chorioamnionitis as a risk factor for cerebral palsy: a meta-analysis. *JAMA* 284:1417–1424
- Yoon BH, Jun JK, Romero R, Park KH, Gomez R, Choi JH, Kim IO 1997 Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha), neonatal brain white matter lesions, and cerebral palsy. *Am J Obstet Gynecol* 177:19–26
- Grether JK, Nelson KB 1997 Maternal infection and cerebral palsy in infants of normal birth weight. *JAMA* 278:207–211
- Shalak L, Perlman J 2002 Infection markers and early signs of neonatal encephalopathy in the term infant. *Ment Retard Dev Disabil Res Rev* 8:14–19
- Munoz-Fernandez M, Fresno M 1998 The role of tumor necrosis factor, interleukin 6, interferon gamma and inducible nitric oxide synthase in the development and pathology of the nervous system. *Prog Neurobiol* 56:307–340
- Gendron R, Nestel F, Lapp W, Baines M 1991 Expression of tumor necrosis factor alpha in the developing nervous system. *Int J Neurosci* 60:129–136
- Arntzen KJ, Kjollsdal AM, Halgunset J, Vatten L, Austgulen R 1998 TNF, IL-1, IL-6, IL-8 and soluble TNF receptors in relation to chorioamnionitis and premature labor. *J Perinat Med* 26:17–26
- Romero R, Mazor M, Sepulveda W, Avila C, Copeland D, Williams J 1992 Tumor necrosis factor in preterm and term labor. *Am J Obstet Gynecol* 166:1576–1587
- Chung I, Benveniste E 1990 Tumor necrosis factor alpha production by astrocytes: induction by lipopolysaccharide, interferon gamma and interleukin 1 beta. *J Immunol* 144:2999–3007
- Lee S, Liu W, Dickson D, Brosnan C, Berman J 1993 Cytokine production by human fetal microglia and astrocytes: differential induction by lipopolysaccharide and interleukin 1 beta. *J Immunol* 150:2659–2667
- Farrar M, Schreiber R 1993 The molecular cell biology of interferon gamma and its receptor. *Annu Rev Immunol* 11:571–611
- Boehm U, Klamp T, Groot M, Howard J 1997 Cellular responses to interferon gamma. *Annu Rev Immunol* 15:749–795
- Ting J, Shigekawa B, Linthicum D, Weiner L, Frelinger J 1981 Expression and synthesis of murine immune response-associated (Ia) antigens by brain cells. *Proc Natl Acad Sci U S A* 78:3170–3174
- Fierz W, Endler B, Reske K, Wekerle H, Fontana A 1985 Astrocytes as antigen-presenting cells I. Induction of Ia antigen expression on astrocytes by T cells via immune interferon and its effects on antigen. *J Immunol* 134:3785–3793
- Turnley A, Miller J, Barlett P 1991 Regulation of MHC molecules on MBP positive oligodendrocytes in mice by IFN gamma and TNF alpha. *Neurosci Lett* 123:45–48
- Neumann H, Boucraut J, Hahnel C, Miggeld T, Wekerle H 1996 Major histocompatibility complex (MHC) class I gene expression in single neurons of the central nervous system: differential regulation by interferon gamma and tumor necrosis factor alpha. *J Exp Med* 185:305–316
- Torres C, Aranguiz I, Rubio N 1995 Expression of interferon gamma receptors on murine oligodendrocytes and its regulation by cytokines and mitogens. *Immunology* 86:250–255
- Akira S, Taga T, Kishimoto T 1993 Interleukin-6 in biology and medicine. *Adv Immunol* 54:1–78
- Vallieres L, Rivest S 1997 Regulation of the genes encoding interleukin-6, its receptor and gp130 in the rat brain in response to the immune activator lipopolysaccharide and the proinflammatory cytokine interleukin 1 beta. *J Neurochem* 69:1668–1683
- Gadient R, Otten U 1994 Expression of interleukin-6 and interleukin-6 receptor mRNAs in rat brain during postnatal development. *Brain Res* 637:10–14
- Gadient R, Otten U 1997 Interleukin-6—a molecule with both beneficial and destructive potentials. *Prog Neurobiol* 52:379–390
- Vitkovic L, Maeda S, Sternberg E 2001 Anti-inflammatory cytokines: expression and action in the brain. *Neuroimmunomodulation* 9:295–312
- Yoon B-H, Romero R, Tang SH, Jun JK, Kim I-O, Choi J-H, Syn HC 1996 Interleukin-6 concentrations in umbilical cord plasma are elevated in neonates with white matter lesions associated with periventricular leukomalacia. *Am J Obstet Gynecol* 174:1433–1440
- Grether J, Nelson K, Dambrosia J, Phillips T 1999 Interferons and cerebral palsy. *J Pediatr* 134:324–332
- Kashlan F, Smulian J, Shen-Schwarz S, Anwar M, Hiatt M, Hegyi T 2000 Umbilical vein interleukin 6 and tumor necrosis factor alpha plasma concentrations in the very preterm infant. *Pediatr Infect Dis J* 19:238–243
- Zhang W, Wang L, Zhao Y, Kang J 2000 Changes in cytokine (IL-8, IL-6 and TNF-alpha) levels in the amniotic fluid and maternal serum in patients with premature rupture of the membranes. *Zhonghua Yi Xue Za Zhi (Taipei)* 63:311–315
- Iida K, Takashima S, Ueda K 1995 Immunohistochemical study of myelination and oligodendrocytes in infants with periventricular leukomalacia. *Pediatr Neurol* 13:296–304
- Cammer W 2000 Effects of TNF alpha on immature and mature oligodendrocytes and their progenitors *in vitro*. *Brain Res* 864:213–219
- Selmaj K, Raine C, Farooq M, Norton W, Brosnan C 1991 Cytokine cytotoxicity against oligodendrocytes. *J Immunol* 147:1522–1529
- Molina-Holgado E, Vela J, Arevalo-Martin A, Guaza C 2001 LPS/IFN-gamma cytotoxicity in oligodendroglial cells: role of nitric oxide and protection by the antiinflammatory cytokine IL-10. *Eur J Neurosci* 13:493–502
- Trautman MS, Collmer D, Edwin SS, White W, Mitchell MD, Dudley DJ 1997 Expression of interleukin-10 in human gestational tissues. *J Soc Gynecol Investig* 4:247–253
- Kline AE, Bolinger BD, Kochanek PM, Carlos TM, Yan HQ, Jenkins LW, Marion DW, Dixon CE 2002 Acute systemic administration of interleukin-10 suppresses the beneficial effects of moderate hypothermia following traumatic brain injury in rats. *Brain Res* 937:22–31
- Spera PA, Ellison JA, Feuerstein GZ, Barone FC 1998 IL-10 reduces rat brain injury following focal stroke. *Neurosci Lett* 251:189–192
- Dudley DJ, Hunter C, Mitchell MD, Varner MW 1997 Amniotic fluid interleukin-10 concentrations during pregnancy and with labor. *J Reprod Immunol* 33:147–156
- Bell M, Hallenbeck J 2002 Effects of intrauterine inflammation on developing rat brain. *J Neurosci Res* 70:570–579
- Yoon BH, Kim CJ, Romero R, Jun KJ, Park KH, Choi ST, Chi JG 1997 Experimentally induced intrauterine infection causes fetal brain white matter lesions in rabbits. *Am J Obstet Gynecol* 177:797–802
- Debillon T, Gras-Leguen C, Veriell V, Winer N, Caillon J, Roze J, Gressens P 2000 Intrauterine infection induces programmed cell death in rabbit periventricular white matter. *Pediatr Res* 47:736–742
- Duncan J, Cock M, Scheerlinck J-P, Westcott K, McLean C, Harding R, Rees S 2002 White matter injury after repeated endotoxin exposure in the preterm ovine fetus. *Pediatr Res* 52:941–949
- Cai Z, Pan Z-L, Pang Y, Evans O, Rhodes P 2000 Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. *Pediatr Res* 47:64–72
- Pang Y, Cai Z, Rhodes P 2003 Disturbance of oligodendrocyte development, hypomyelination and white matter injury in the neonatal rat brain after intracerebral injection of lipopolysaccharide. *Brain Res Dev Brain Res* 140:205–214
- Cai Z, Pang Y, Lin S, Rhodes P 2003 Differential roles of tumor necrosis factor alpha and interleukin 1-beta in lipopolysaccharide-induced brain injury in the neonatal rat. *Brain Res* 975:37–47
- Lenhardt S, Lachance C, Patrizi S, Lefebvre S, Follett P, Jensen F, Rosenberg P, Volpe J, Vartanian T 2002 The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. *J Neurosci* 22:2478–2486