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Prenatal Infection and Risk for Schizophrenia: IL-1β, IL-6, and TNFα Inhibit Cortical Neuron Dendrite Development

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Prenatal exposure to infection increases risk for schizophrenia, and we have hypothesized that inflammatory cytokines, generated in response to maternal infection, alter neuron development and increase risk for schizophrenia. We sought to study the effect of cytokines generated in response to infection—interleukin-1β (IL-1β), tumor necrosis factor-α (TNFα), and interleukin-6 (IL-6)—on the dendritic development of cortical neurons. Primary mixed neuronal cultures were obtained from E18 rats and exposed to 0, 100, or 1000 units (U)/ml of IL-1β, TNFα, IL-6, or IL-1β + TNFα for 44 h. MAP-2-positive neurons were randomly identified for each condition and the number of primary dendrites, nodes, and total dendrite length was determined. We found that 100 U of TNFα significantly reduced the number of nodes (27%, $p = 0.02$) and total dendritic length (14%, $p = 0.04$), but did not affect overall neuron survival. A measure of 100 U IL-1β + TNFα significantly reduced the number of primary dendrites (17%, $p = 0.006$), nodes (32%, $p = 0.001$), and total dendritic length (30%, $p < 0.0001$), although it did not affect overall neuron survival. At 1000 U, each cytokine significantly reduced the number of primary dendrites (14–24%), nodes (28–37%), as well as total dendritic length (25–30%); neuron survival was reduced by 14–21%. These results indicate that inflammatory cytokines can significantly reduce dendrite development and complexity of developing cortical neurons, consistent with the neuropathology of schizophrenia. These findings also support the hypothesis that cytokines play a key mechanistic role in the link between prenatal exposure to infection and risk for schizophrenia.
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INTRODUCTION

Maternal infection during pregnancy increases the risk of the offspring developing schizophrenia and other neurodevelopmental disorders. The weight of the evidence indicates that maternal influenza infection during pregnancy is associated with a higher incidence of schizophrenia in offspring (reviewed in McGrath and Murray, 2003; Bagalkote *et al*, 2001). Other types of maternal infections have also been implicated, including pneumonia and diphtheria (Watson *et al*, 1984; O'Callaghan *et al*, 1994), rubella (Brown *et al*, 2000b), measles, varicella-zoster, and polio (Torrey *et al*, 1988; Suvisaari *et al*, 1999). Recent studies have moved beyond epidemiologic association with studies that link infections in individual mothers with schizophrenia in their adult children. Respiratory infections in the second trimester increase risk for schizophrenia (Brown *et al*, 2000a). In this same cohort, serologic evidence of maternal exposure to influenza also increased the risk of schizophrenia in offspring (Brown *et al*, 2004a). Finally, the offspring of mothers with elevated IgG and IgM levels, and antibodies to herpes simplex virus type 2 during pregnancy, have an increased risk for schizophrenia (Buka *et al*, 2001a).

The pathological mechanisms responsible for increased risk of schizophrenia in offspring after maternal infection remain largely unstudied. Hypotheses about the role of infection in the etiology of schizophrenia have focused on direct infection of the developing fetus (Yolken and Torrey, 1995) or the generation of antibodies that crossreact with neuronal antigens (Wright *et al*, 1993). As a variety of infections are associated with increased risk of schizophrenia, a feature common to all infections would be a likely candidate mechanism. We have hypothesized that cytokines generated in response to maternal infection alter early brain development and increase risk for schizophrenia (Gilmore and Jarskog, 1997). Cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNFα), have a variety of effects on the central nervous system (CNS) and are expressed by glial and neuronal elements within the CNS (Bartfai and Schultzberg, 1993; Hopkins and Rothwell, 1995; Rothwell and Hopkins, 1995). Cytokines, including IL-1β, IL-6, and TNFα, regulate

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normal brain development and have been implicated in abnormal brain development (Merrill, 1992; Mehler and Kessler, 1994; Mehler and Kessler, 1997). Expression of cytokine mRNA in the CNS is developmentally regulated in mouse, rat, sheep, and human brain (Burns *et al*, 1993; Gadient and Otten, 1994; Pousset, 1994; Mousa *et al*, 1999; Dziegielewska *et al*, 2000), an indication of the important role that cytokines play in neurodevelopment.

IL-1β, IL-6, and TNFα are elevated in the maternal–fetal unit after maternal infection in human pregnancies (Hillier *et al*, 1993; Fortunato *et al*, 1996; Yoon *et al*, 2003) and in animal models (Fidel *et al*, 1994; Urakubo *et al*, 2001). Maternally generated cytokines cross the placenta and regulate cell growth and development in the fetus (Medlock *et al*, 1993; Letterio *et al*, 1994; Stallmach and Karolyi, 1994; Li *et al*, 1995; McDuffie *et al*, 2001). An additional source of cytokines in prenatal infection may be the placenta, as the human placenta synthesizes IL-1β, IL-6, and TNFα in response to infection (Fortunato *et al*, 1996; Taniguchi *et al*, 1991; Menon *et al*, 1995). Finally, the fetus itself can mount an inflammatory response, especially of IL-6, in the face of maternal infection (Gomez *et al*, 1998; Yoon *et al*, 2003). IL-1β, IL-6, and TNFα cross the blood–brain barrier in mature rodents (Banks *et al*, 1991; Guiterrez *et al*, 1993; Banks *et al*, 1994). Finally, the blood–brain barrier is incomplete in the fetus (Adinolfi, 1985), making it very likely that systemically generated cytokines gain entry into the fetal brain.

Several recent studies support the hypothesis that cytokines play a key role in the association between maternal infection, altered brain development, and risk for schizophrenia. Maternal blood levels of TNFα (Buka *et al*, 2001b) and IL-8 (Brown *et al*, 2004b) are elevated in pregnancies in which the offspring goes on to develop schizophrenia. Three animal models of maternal infection have recently been advanced as models of schizophrenia that also support our hypothesis. Maternal infection with human influenza virus in mice causes abnormalities in prepulse inhibition (Shi *et al*, 2003), and maternal exposure to *Escherichia coli* cell wall endotoxin, lipopolysaccharide (LPS), disrupts sensorimotor gating in the offspring (Borrell *et al*, 2002). Finally, maternal exposure to poly-I:C, a synthetic double-stranded RNA that stimulates a cytokine response, causes prepulse inhibition abnormalities (Shi *et al*, 2003) and disrupted latent inhibition (Zuckerman and Weiner, 2003; Zuckerman *et al*, 2003). In the influenza model, no virus is detected in the fetal brain (Shi *et al*, 2003), suggesting that the immune responses, especially cytokines, are the likely mediators of the abnormal brain development that leads to long-term behavioral changes. In the poly-I:C and LPS models, it is likely that the immune response to the challenge plays a major role in the mechanism of action, as no infectious agent is present. We have shown that maternal LPS exposure increases cytokine expression in the placenta and amniotic fluid of rats (Urakubo *et al*, 2001).

Cytokines can be neurotoxic to developing neurons, as they decrease survival of serotonergic and dopaminergic neurons (Jarskog *et al*, 1997), hippocampal neurons (Araujo and Cotman, 1995), and cortical neurons (Gelbard *et al*, 1993; Jeohn *et al*, 1998; Marx *et al*, 2001). The neuropathology of cerebral cortex in schizophrenia is subtle and does not appear to involve neuron loss, but rather loss of neuropil (Selemon *et al*, 2003), dendrites and spines (Glantz and Lewis, 2000; Broadbelt *et al*, 2002), and synaptic markers (Glantz and Lewis, 1997). The actions of cytokines on developmental processes that may give rise to schizophrenia, such as dendrite development, are less well known. This study was conducted to determine the effect of cytokines on cortical neuron dendrite development. We hypothesized that the inflammatory cytokines IL-1β, IL-6, and TNFα, which are generated in response to maternal infection, would decrease the development of dendrites on embryonic cortical neurons *in vitro*.

MATERIALS AND METHODS

Cell Culture

Primary cortical neuronal cells were isolated as described previously (Marx *et al*, 2001). Briefly, frontal cerebral cortex was dissected from whole brains of embryonic day 18 Sprague–Dawley rats (Charles River, Raleigh, NC) and the meninges removed. The trimmed tissues were dissociated in 0.125% trypsin and 0.5 mM EDTA for 20 min at 37°C. The reaction was terminated by adding an equal volume of Dulbecco's modified Eagle's medium + 10% fetal bovine serum + 5 units (U)/ml penicillin/streptomycin. The cells were further dissociated by mechanical trituration, followed by filtering through the nylon mesh and centrifugation. The cells (5×10^4/ml/well) were plated on 18-mm glass coverslips coated with poly-D-lysine (100 μg/ml) in 12-well culture plates. Cells were cultured in Neurobasal medium supplemented with B27 (Invitrogen, Carlsbad, CA), 0.5 mM L-glutamine, 25 μM L-glutamate and 5 U/ml penicillin/streptomycin. After 4 h, media was exchanged and cultures were treated with TNFα, IL-6 (R&D Systems, Minneapolis, MN), IL-1β (Boehringer Mannheim, Ridgefield, CT), or TNFα + IL-1β for 44 h at concentrations of 0, 100, or 1000 U/ml, three wells per condition. Specific activity per manufacturer: TNFα, 60 U/ng; IL-6, 12.5 U/ng; and IL-1β, 50 U/ng. Three cultures from three different litters were studied for a total $n = 9$ per treatment condition.

Immunohistochemistry

After 44 h of cytokine exposure, cultures were rinsed in Hank's balanced salt solution, fixed in 4% paraformaldehyde, and permeabilized with 0.2%. Triton X-100 in phosphate-buffered saline (PBS) and incubated overnight with goat polyclonal antibody MAP-2 (1 : 100). To confirm that neurons in culture expressed the respective cytokine receptors, three cover glasses each were incubated overnight with goat polyclonal antibodies for TNF receptor-1 (1 : 100) and TNF receptor-2 (1 : 100), or rabbit polyclonal IL-1 receptor-1 (1 : 500) or IL-6 receptor-α (1 : 200). MAP-2 and cytokine receptor antibodies were purchased from Santa Cruz (Santa Cruz, CA). To determine if glial cells were present, cultures were incubated overnight with monoclonal mouse anti-GFAP (Boehringer Mannheim, 1 : 500). Cultures were then processed using the avidin–biotin peroxidase complex (ABC) method (Vector). Following immunochemistry and osmium tetraoxide intensification, cultures were rinsed in PBS and counterstained with toluidine blue. A negative control experiment was also performed to rule out

nonspecific staining in the absence of primary antibodies. In this experiment, ABC immunochemistry procedures were followed, but no primary antibody was included.

Cell Counting and Dendrite Morphology

Primary cortical neurons were cultured at low density (5×10^4 cells/ml/well) to allow morphological analysis of single neurons. Neuronal survival was analyzed by counting neurite-bearing MAP-2-immunostained cells with clear neuronal morphology at $\times 200$ magnification using an ocular grid. In all, 12 grid areas were examined per coverslip using a blinded, randomized procedure (0.25 mm^2 per grid area $\times$ 12 per coverslip = total sample area of 3.00 mm^2 per coverslip). Cell counts were converted to percentages of intraexperimental controls.

For morphometry, the first 10 single neurons that had two or more dendrites encountered from the center of the coverslip were digitally captured at $\times 60$. Dendrites of scanned cells were traced using Neurolucida and NeuroExplorer software (Micro Bright Field, Williston, VT). Morphometry data from individual neurons were collected for a number of primary dendrites per neuron, branch points per neuron, and total dendrite length per neuron (Figure 1). Primary dendrites were defined as neurites originating at the neuronal somata. A total of 90 neurons were studied for each treatment condition.

Data Analysis

T-tests and one-way analysis of variance (ANOVA) with *post hoc* Dunnett's multiple comparison tests were performed. Significance was set at $p<0.05$ using a two-tailed test for all analyses.

RESULTS

Cortical neurons in culture expressed receptors for IL-1, IL-6, and TNF (types 1 and 2); Figure 1). There were no GFAP-positive cells present in the cultures. Representative MAP-2-positive neurons and their dendrite tracings are presented in Figure 2. At a concentration of 100 U, TNFα significantly reduced the number of nodes (27%, $p=0.02$) and total dendrite length (14%, $p=0.04$; Table 1) of MAP-2-labeled cortical neurons in culture. IL-1β + TNFα (100 U) significantly reduced the number of primary dendrites (17%, $p=0.006$), nodes (32%, $p=0.001$), and total dendrite length (30%, $p<0.0001$; Table 1). There were no significant effects of IL-1β or IL-6 on the number of primary dendrites, nodes, or total dendrite length at this concentration. At 100 U, there were no significant effects of IL-1β, IL-6, TNFα, or IL-1β + TNFα on neuron survival ($p>0.05$ for each cytokine).

At a concentration of 1000 U, IL-1β significantly reduced the number of primary dendrites (14%, $p<0.05$), nodes (37%, $p<0.01$), as well as total dendrite length (30%, $p<0.01$; Figure 3) on cortical neurons. TNFα also significantly reduced the number of primary dendrites (24%, $p<0.01$), nodes (29%, $p<0.01$), and total dendrite length (27%, $p<0.01$). IL-6 significantly reduced the number of primary dendrites (16%, $p<0.05$), nodes (33%, $p<0.01$), and total dendrite length (25%, $p<0.01$). Finally, IL-1β + TNFα significantly reduced the number of primary dendrites (17%, $p<0.01$), nodes (28%, $p<0.05$), and total dendrite length (29%, $p<0.01$). At 1000 U, there was a small but significant decrease in neuronal survival for IL-1β (14%, $p<0.05$), IL-6 (14%, $p<0.05$), TNFα (17%; $p<0.05$), and IL-1β + TNFα (22%, $p<0.01$; Figure 4).

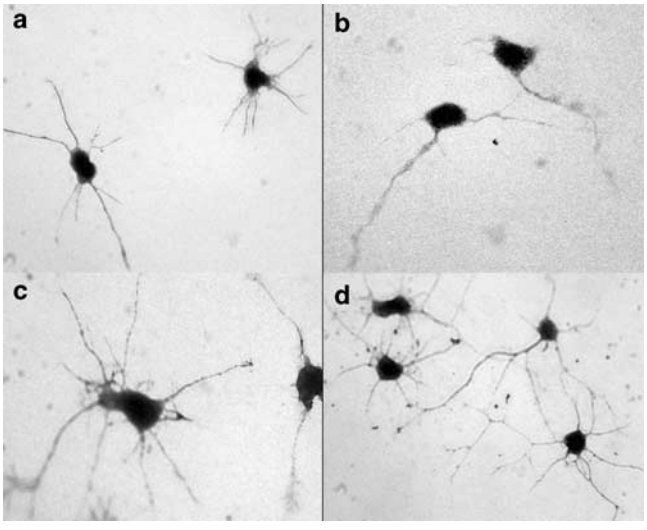

Figure 1 Immunodetection of receptors for IL-1β, IL-6, and TNFα on embryonic cortical neurons *in vitro* using antibodies to (a) IL-1r, (b) IL-6r, (c) TNFr1, and (d) TNFr2 ($\times$ 60 magnification).

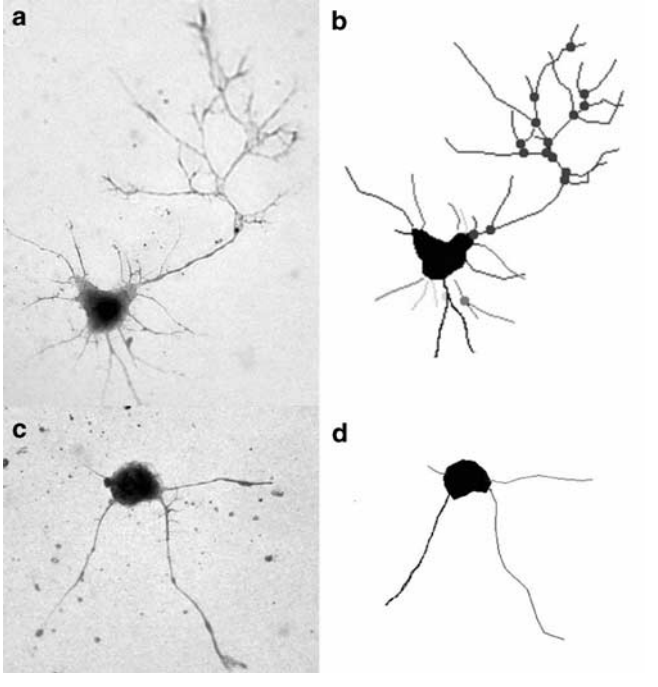

Figure 2 Representative MAP-2-labeled neurons and their dendrite tracings from neurolucida: (a and b) control; (c and d) 100 U of IL-1β + TNFα ($\times$ 60 magnification).

Table 1 Morphometric analysis of cortical neurons in primary culture treated with 100 U of IL-1β, IL-6, TNFα, or IL-1β+TNFα for 44 h

		Primary dendrites	Nodes	Total dendritic length
TNFα	Control	8.7 (4.0)	6.7 (6.5)	331.8 (183.9)
	TNFα	8.0 (3.9)	4.9 (4.0)	282.1 (139.5)
	P-value	>0.05	**0.024**	**0.043**
IL-6	Control	8.4 (3.5)	6.3 (4.6)	322.0 (139.6)
	IL-6	8.9 (3.7)	5.6 (4.1)	328.1 (155.8)
	P-value	>0.05	>0.05	>0.05
IL-1β	Control	8.6 (3.6)	5.4 (4.3)	317.9 (143.8)
	IL-1β	7.8 (3.3)	5.5 (5.0)	307.6 (167.4)
	P-value	>0.05	>0.05	>0.05
IL-1β/TNFα	Control	8.9 (3.3)	5.1 (3.9)	337.3 (134.0)
	IL-1β/TNFα	7.3 (3.2)	3.4 (3.4)	235.3 (128.6)
	P-value	**0.0006**	**0.0012**	**<0.0001**

TNFα and IL-1β+TNFα significantly reduced dendrite morphology. *T*-tests were performed to compare treatment to control.
Bold numerals represent *P* values.

DISCUSSION

This study demonstrates that the inflammatory cytokines IL-1β, IL-6, and TNFα, which are typically generated in response to infection, can significantly inhibit the development of dendrites in embryonic cortical neurons. TNFα (100 U) decreases the number of nodes and total dendrite length; the combination of IL-1β and TNFα at 100 U causes a robust decrease in the number of primary dendrites, nodes, and total dendrite length, suggesting a synergistic effect. At the higher dose (1000 U), IL-1β, IL-6, and TNFα caused a significant reduction in each parameter of dendrite morphology. These findings are consistent with a recent study reporting that TNFα significantly decreased neurite outgrowth and branching of hippocampal neurons in culture (Neumann *et al*, 2002). Interferon-γ also inhibits dendritic growth in hippocampal neurons (Kim *et al*, 2002). The synergistic effect of IL-1β and TNFα on dendrite morphology is also consistent with a previous study that found that IL-1β and TNFα had an additive effect on decreased cortical neuron survival (Jeohn *et al*, 1998).

While it is difficult to compare directly studies due to variations in assay methods and in the unit activity of recombinant cytokines, the concentrations of cytokines used in this experiment are within physiologic ranges. A measure of 100 U is approximately 2000 pg/ml of TNFα, 8000 pg /ml of IL-6, and 1666 pg/ml of IL-1β. In a previous

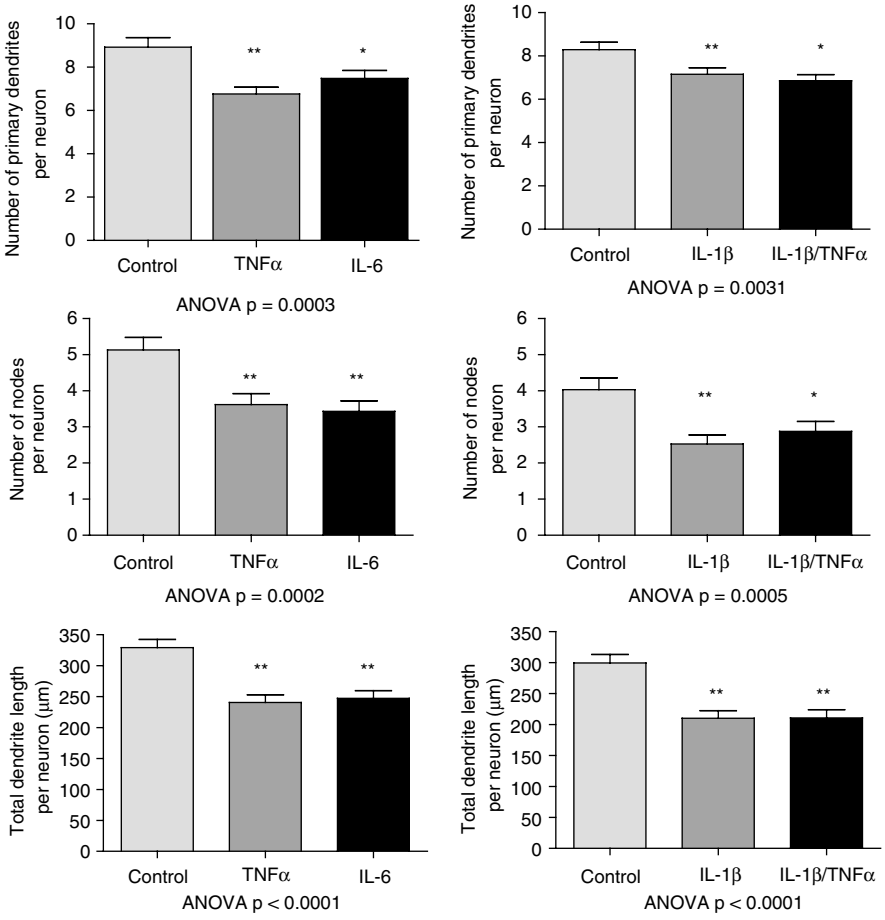

Figure 3 Morphometric analysis of cortical neurons in primary culture treated with 1000 U of IL-1β, IL-6, TNFα, or IL-1β + TNFα for 44 h. Each cytokine significantly decreased primary dendrite number, number of nodes, and total dendrite length. Results of ANOVA are presented with each experiment. *Post hoc* Dunnett's test: $*p<0.05$; $**p<0.01$.

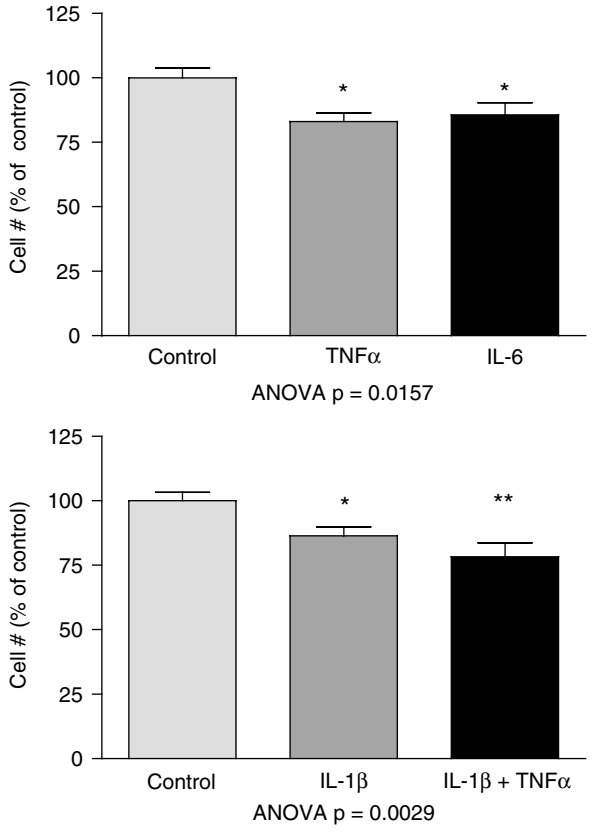

Figure 4 Neuron survival after exposure to 1000 U of IL-1β, IL-6, TNFα, or IL-1β + TNFα for 44 h. Each cytokine significantly decreased overall neuron survival. Results of ANOVA presented with each experiment. *Post hoc* Dunnett's test: *$p<0.05$; **$p<0.01$. A measure of 100 U of each cytokine had no significant effect on neuron survival.

study, we found levels of TNFα, IL-6, IL-1β in the fetal rat brain to be 1500 pg/g, 1500 pg/g, and 5000 pg/g tissue, respectively (Urakubo *et al*, 2001). Brain IL-1 β levels in adult rats and mice after peripheral LPS exposure reach levels of 50 000 pg/g (Nguyen *et al*, 1998) and 5000 pg/g of tissue for IL-6 (Meyer *et al*, 1997). Levels in human CSF in the setting of infection range as high as 8000 pg/ml for IL-1β in CSF after aseptic meningitis (Ramilo *et al*, 1990) and 26 000 pg/ml of TNFα in CSF after bacterial meningitis (Mustafa *et al*, 1989). Local concentrations of cytokines at a neuronal level are likely to be much higher.

The decreased dendrite development of cortical neurons observed after cytokine exposure offers a tangible link between an important environmental risk factor for schizophrenia and the cortical neuropathology observed in schizophrenia. Prenatal exposure to maternal infection, through the generation of systemic cytokines, could decrease dendritic development of cortical neurons, which would ultimately lead to the 'miswiring' and 'dysconnectivity' of cortical circuits thought to underlie the clinical and cognitive symptoms of schizophrenia. Especially relevant is the lowest concentration tested, in which 100 U TNFα and the combination of IL-1β and TNFα caused significant decreases in dendrite development while having no effect on overall neuron survival. This finding is consistent with the cortical neuropathology of schizophrenia that involves decreased neuropil (Selemon *et al*, 2003), dendrites and dendritic spines (Glantz and Lewis, 2000; Broadbelt *et al*, 2002), and synaptic markers (Glantz and Lewis, 1997), but no neuronal loss (Selemon *et al*, 2003).

As noted above, studies in human pregnancies (Hillier *et al*, 1993; Fortunato *et al*, 1996; Yoon *et al*, 2003) and in animal models (Fidel *et al*, 1994; Urakubo *et al*, 2001) indicate that maternal infection increases systemic levels of IL-1β, IL-6, and TNFα. The source of systemic cytokines can be the maternal immune system, the placenta, and even the fetus itself. Prenatal exposure to maternal infection can also activate microglia and astrocytes in the developing cortex (Cai *et al*, 2000; Fatemi *et al*, 2002). These activated glial cells would be a potential local source of cytokines and may play a role in the transduction of the immune response to maternal infection to developing neurons. We have recently shown that maternal infection can also regulate the expression of brain-derived neurotrophic factor and nerve growth factor in the fetal and neonatal brain (Gilmore *et al*, 2003). Neurotrophic factors are expressed by glial cells and represent an additional mechanism through which maternal infection can regulate neuronal development.

Apoptosis represents another potential molecular mechanism by which altered expression of cytokines and neurotrophic factors could alter cortical neuronal development. Proinflammatory cytokines are well-recognized activators of neuronal apoptosis (Hu *et al*, 1997) and recent data implicate apoptosis in several disorders of neurodevelopment including schizophrenia (Jarskog *et al*, 2004). While activation of apoptosis is often associated with cell death, emerging evidence indicates that apoptotic activity can also underlie neuronal atrophy (Ona *et al*, 1999), neuritic degeneration (Ivins *et al*, 1998), and synaptic loss (Gilman and Mattson, 2002) without causing cell death. Certain apoptotic regulatory proteins are developmentally expressed in patterns that appear to increase the susceptibility to pathological apoptosis during early neurodevelopment (Hu *et al*, 2000; Jarskog and Gilmore, 2000; Yakovlev *et al*, 2001). Taken together, these data suggest that an apoptotic mechanism can contribute to cytokine-mediated effects on altered cortical development in schizophrenia and other neurodevelopmental disorders.

β-catenin is a member of the Wnt signaling pathway and has recently been shown to also regulate dendrite morphology, an action that involves interactions between β-catenin, cadherin, the actin cytoskeleton, and the cell membrane (Yu and Malenka, 2003). IL-6 and TNFα can decrease β-catenin expression in hepatocarcinoma cells (Cervello *et al*, 2001) and in bronchial epithelial cells (Carayol *et al*, 2002), suggesting that cytokines may act through a mechanism that involves β-catenin in developing dendrites. As noted above, TNFα significantly decreased dendrite development of hippocampal neurons; this effect involved a Rho GTPase-dependent mechanism that also regulates the cytoskeleton (Neumann *et al*, 2002). These studies suggest that regulation of the cytoskeleton is a potential mechanism through which cytokines can inhibit the development of dendrites.

While little is known about the impact of infection on the developing cortex, there has been much interest in the role that cytokines play in the white matter damage associated with cerebral palsy. Intrauterine infection greatly increases the risk of periventricular leukomalacia (PVL) and cerebral

palsy in premature infants and term infants (Grether and Nelson, 1997; Wu and Colford, 2000; Wu, 2002), and cytokines have been implicated in this association (Dammann and Leviton, 1997). Elevated levels of IL-6 in fetal cord blood and of IL-6 and IL-1β in amniotic fluid are associated with white matter lesions and cerebral palsy in premature infants (Yoon *et al*, 1996; Yoon *et al*, 2000). Children with cerebral palsy have high neonatal blood levels of cytokines, including IL-1β and TNFα (Nelson *et al*, 1998). Finally, premature infants with cerebral lesions on MRI exhibit high levels of IL-1β, IL-6, IL-10, and TNFα in the cord blood at birth (Duggan *et al*, 2001). In addition to elevations of cytokines in fetal blood and amniotic fluid, there is evidence that IL-1β, IL-6, and TNFα are highly expressed in white matter around PVL lesions, especially in astrocytes and microglial cells (Deguchi *et al*, 1996; Yoon *et al*, 1996; Kadhim *et al*, 2001). A recent study indicates that there is increased expression of IL-1β and TNFα by cortical and subcortical neurons in the brains of infants with PVL (Kadhim *et al*, 2001), indicating that the same inflammatory processes that lead to PVL may also alter cortical neuron development.

These studies with regard to the connection between cytokines and PVL offer clues as to how prenatal exposure to infection might affect the developing brain and increase risk for schizophrenia. Gross white matter damage is the only one of many possible outcomes of prenatal exposure to infection that likely includes more subtle alterations of cortical neuron and/or white matter development. Gross white matter lesions are not more frequently seen in schizophrenia (Rivkin *et al*, 2000), although more subtle abnormalities of white matter volume and diffusion tensor properties are present (Sigmundsson *et al*, 2001; Kubicki *et al*, 2002). Very little is known about the impact of prenatal exposure to infection on brain development beyond white matter, although maternal influenza infection can cause abnormal corticogenesis in mice (Fatemi *et al*, 1999). Our study provides additional evidence that maternal infection can alter cortical neuronal development as well.

The ultimate impact of infection on the developing brain is probably dependent on the severity and timing of the infection in relation to specific neurodevelopmental events, as well as the genetic susceptibility of the individual. For example, genes that regulate the immune response, especially in the setting of prenatal exposure to infection, would likely have an effect on early brain development. Studies have found associations between schizophrenia and polymorphisms of cytokine genes that increase cytokine production, including TNFα (Boin *et al*, 2001; Jun *et al*, 2003; Meira-Lima *et al*, 2003) and IL-1β (Katila *et al*, 1999). Further, there are associations between polymorphisms for IL-1β (Meisenzahl *et al*, 2001) and the TNF receptor-II (Wassink *et al*, 2000) and abnormal brain morphology in patients with schizophrenia, including lateral ventricle enlargement and cortical gray matter and white matter reductions.

Infection during pregnancy can be associated with other mechanisms that could also adversely affect prenatal brain development, including stress hormones (Trejo *et al*, 1995; Koenig *et al*, 2002), hypoxia (Altshuler, 1993), reduced blood flow to the fetus (Altshuler, 1993), malnutrition (Butler *et al*, 1994), and hyperthermia (Upfold and Smith, 1988). Ultimately, a full mechanistic understanding of the impact of maternal infection on the fetal brain development will have to integrate knowledge about the involvement of inflammatory, hormonal, and other potential mechanisms.

Finally, inflammatory cytokines play a mechanistic role in other forms of insults to the developing brain, including hypoxia–ischemia (Szaflarski *et al*, 1995; Grow and Parks, 2002) and excitotoxic injury (Silverstein *et al*, 1997). It has also been shown that infection sensitizes the immature brain to hypoxic–ischemic injury, suggesting a combined activation of inflammatory mediators in the brain (Eklind *et al*, 2001). Cytokines therefore may represent a final common pathway for the variety of perinatal complications that are associated with increased risk for schizophrenia (Cannon *et al*, 2002). The study of perinatal complications and schizophrenia is often criticized for being too nonspecific with regard to the mechanisms that underlie a diverse group of complications. Inflammatory cytokines offer an attractive unifying mechanism.

In summary, the inflammatory cytokines IL-1β, IL-6, and TNFα each decrease dendritic development of cortical neurons *in vitro*, consistent with the cortical neuropathology observed in schizophrenia. Schizophrenia is likely the result of a complex interaction between multiple genetic and environmental risk factors. If one assumes that schizophrenia is a disorder of synaptic connectivity, any molecule or process that regulates dendritic growth and synapse formation in the developing brain may contribute to risk. Given the evidence that prenatal exposure to maternal infection, hypoxia, and other insults activate an immune response in the developing brain, inflammatory cytokines are emerging as an important candidate mechanism in understanding pre- and perinatal risk factors for schizophrenia. The study of interactions between the immune system and the developing brain is just beginning, but offers a key to understanding one of the etiologic factors contributing to schizophrenia and other neurodevelopmental disorders. This line of investigation may ultimately offer targets for therapeutic strategies that may prevent or reduce the risk of abnormal brain development in the setting of infection and other perinatal insults, and may ultimately prevent or reduce the risk of schizophrenia.

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