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Review

Cytokines and CNS Development

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Cytokines are pleotrophic proteins that coordinate the host response to infection as well as mediate normal, ongoing signaling between cells of nonimmune tissues, including the nervous system. As a consequence of this dual role, cytokines induced in response to maternal infection or prenatal hypoxia can profoundly impact fetal neurodevelopment. The neurodevelopmental roles of individual cytokine signaling pathways are being elucidated through gain- and loss-of-function studies in cell culture and model organisms. We review this work with a particular emphasis on studies where cytokines, their receptors, or components of their signaling pathways have been altered in vivo. The extensive and diverse requirements for properly regulated cytokine signaling during normal nervous system development revealed by these studies sets the foundation for ongoing and future work aimed at understanding how cytokines induced normally and pathologically during critical stages of fetal development alter nervous system function and behavior later in life.

Introduction

Cytokines are small, mostly secreted proteins that were originally characterized as immune modulators but have subsequently been found to mediate a diverse array of functions in nonimmune tissues, including the central nervous system (CNS). Cytokines are of particular importance during neural development and function at all stages, starting with induction of the neuroepithelium. Subsequently, cytokines, in particular the neuropoietic, or gp130 family cytokines, regulate the self-renewal of the neuroepithelial/radial glia cells (RGC), which function both as the scaffolds for radially migrating neurons and as the precursors for all neurons, macroglia (astrocytes and oligodendrocytes), and adult progenitors. RGCs give rise to neurons first and then glia, and several cytokines, including the gp130 cytokines and the bone morphogenetic proteins (BMPs), have a central role in this shift to gliogenesis. In addition, chemokines, a subclass of small cytokines with chemoattractant properties, function as cues for the migration of newly generated neurons and glia and are modulators of axon pathfinding. Furthermore, as a general rule, neurons and glia are produced in excess, and cytokines have roles as both neurotrophic factors that promote the survival of cells that make the appropriate connections and as signals that trigger the apoptosis of cells that fail to compete for these connections.

Not all cells in the CNS have neural origins. Microglia, which are immune surveillance cells of the hematopoietic lineage, colonize the early embryonic neuroepithelium and phagocytose developmental apoptotic debris. These immune cells also modulate vascularization, neuronal survival, and synapse formation and function, in part through their response to, and secretion of, a wide repertoire of cytokines.

Although cytokines primarily act locally, they can also have endocrine effects. Thus, cytokine induction in response to maternal infection or fetal injury may adversely affect neurodevelopment. Indeed, epidemiological evidence points to maternal infection as a cause of neurodevelopmental abnormalities that increase the risk for schizophrenia, autism, and cerebral palsy in the offspring (for more information, see accompanying article from Ellman and Susser, 2009 [this issue of *Neuron*]). Recent findings from animal models of maternal infection support this hypothesis and provide evidence that dysregulation of individual cytokines can induce striking behavioral deficits in the offspring. Further insight into how cytokine dysregulation interferes with normal neural development will come from animal models of maternal infection as well as a continued focus on the normal roles that cytokines have during CNS development.

Neural Induction and Primitive NSCs

Neural induction in the vertebrate embryo is repressed by BMPs, which are members of the transforming growth factor beta (TGFβ) cytokine superfamily (reviewed in Gaulden and Reiter, 2008). Active inhibition of BMP signaling is required for normal neural development in mice as demonstrated by the lack of forebrain development in the absence of Noggin and Chordin, two BMP antagonists (Bachiller et al., 2000). These findings have been largely recapitulated in culture. Embryonic stem cells (ESCs) can give rise to cells with the characteristics of primitive neural stem cells (NSCs) in the absence of exogenous factors, but do so more efficiently in the presence of Noggin, or if BMP signaling is blocked by deletion of SMAD4, the common downstream signaling effector for the TGFβ family of cytokines (Tropepe et al., 2001). In addition, leukemia inhibitory factor (LIF), a gp130 family cytokine well known for its role promoting mouse ESC self-renewal, functions as a permissive survival factor during the transformation of ESCs into cells with the characteristics of embryonic NSCs. This role for LIF is likely culture-specific since the neuroepithelium forms in vivo in the absence of LIF or gp130, the common receptor subunit required for signaling by LIF and related gp130 cytokines (Escary et al., 1993; Yoshida et al., 1996).

Maintaining the Neural Progenitor Pool

Neuroepithelial cells directly or indirectly give rise to all of the neurons, astrocytes, and oligodendrocytes in the adult brain. Initially, neuroepithelial cells divide symmetrically, expanding

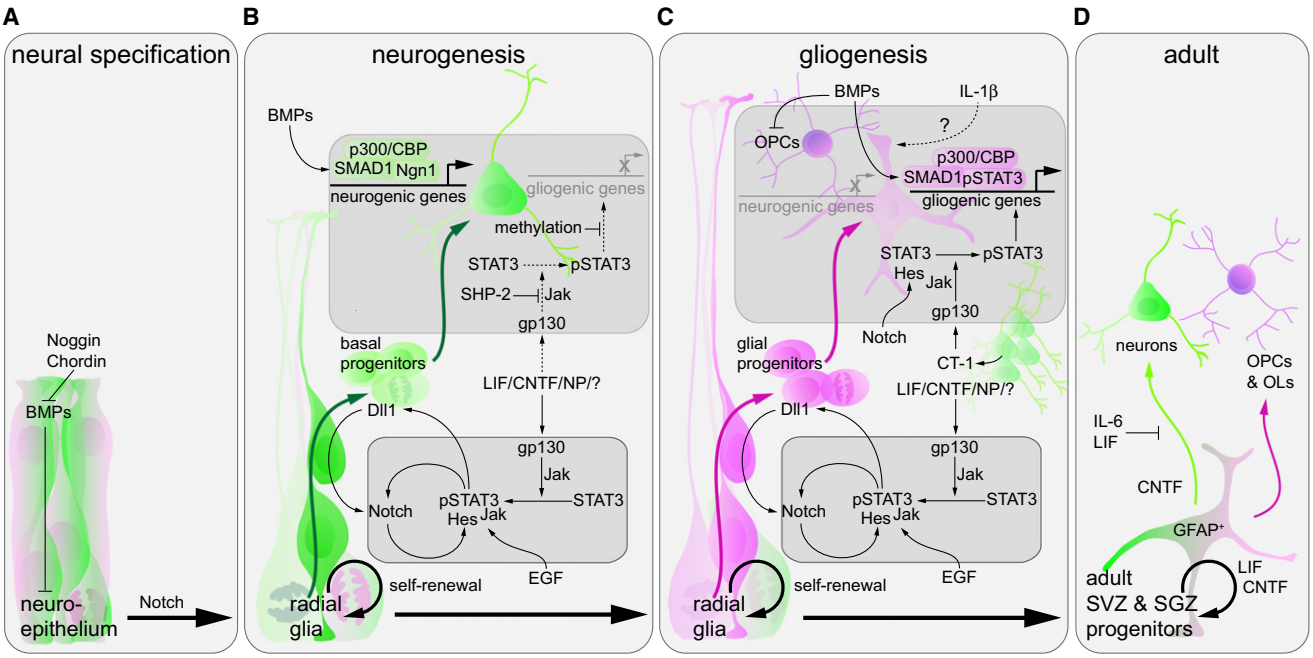

Figure 1. gp130 Family Cytokines Cooperate with Notch and BMP Signaling to Regulate Radial Glial Cell Self-Renewal and Progenitor Differentiation

(A) BMP antagonists Noggin and Chordin are required for neural specification. At the onset of neurogenesis, neuroepithelial cells acquire radial glial cell (RGC) characteristics in a Notch-dependent manner.

(B) gp130-STAT3 signaling cooperates with Notch to promote RGC self-renewal (bottom box). Activation of STAT3 can promote Notch activation through the upregulation of expression of both Notch1 and the Notch ligand, Dll1. In addition, Notch promotes STAT3 phosphorylation (pSTAT3) through the Notch effector proteins HES1/5, which facilitate JAK mediated-STAT3 phosphorylation in response to EGF. During neurogenesis (top box), gp130/STAT3 signaling components are expressed, but STAT3 activation is inhibited by the SHP-2 phosphatase. STAT3-mediated transcriptional activation of gliogenic genes is further kept in check by promoter methylation and by competition with the neurogenic Ngn transcription factor for binding the p300/CBP-SMAD1 coactivator complex.

(C) Newly generated neurons (in green) produce the gp130 family cytokine CT-1, which signals through gp130 to induce the phosphorylation and activation of STAT3. pSTAT3, in the presence of BMP signaling, forms a complex with SMAD1, a downstream effector of the BMPs, and the transcriptional coactivator p300/CBP. This complex induces transcription of gliogenic genes such as GFAP and S100β (top box). While BMPs promote astrogliogenesis, they inhibit oligodendrocyte specification. IL-1β expression in the CNS peaks during gliogenesis, and IL-1 can promote astrocyte differentiation in vitro.

(D) A subpopulation of RGCs transforms into astrocyte-like cells that retain the capacity to make new neurons and glia throughout adulthood. These cells persist in the adult brain, and give rise to neurons and oligodendrocyte lineage cells (oligodendrocyte progenitor cells, OPCs; oligodendrocytes, OL). In the adult brain, LIF promotes the self-renewal of SVZ progenitors, and CNTF can enhance the proliferation of GFAP$^+$ SVZ progenitors and stimulate neurogenesis in vivo while LIF and IL-6 inhibit neurogenesis from the SVZ and subgranular zone (SGZ), respectively.

the population. They then take on characteristics of RGCs, a conversion that coincides with the onset of neurogenesis (E9–E10 in mice) and requires Notch activation (Hatakeyama et al., 2004). RGCs extend processes to the ventricle and to the pia, support the radial migration of developing neurons, and act as precursors for all macroglia, including adult neural stem cells (NSCs), and all but the earliest born neurons (reviewed in Pinto and Götz, 2007).

In cooperation with Notch and growth factors such as fibroblast growth factor 2 (FGF2), signaling by the gp130 family of cytokines maintains the RGC pool by promoting RGC self-renewal (Figure 1). Importantly, key components of the signaling pathway, including the receptor subunits LIFRβ, ciliary neurotrophic factor receptor (CNTFRα) and gp130, and the signal transducer and activator of transcription 3 (STAT3), a transcription factor that is a primary mediator of gp130 signaling, are all expressed in the early ventricular zone (VZ) populated by RGCs (Alfonsi et al., 2008; Gregg and Weiss, 2005; Hatta et al., 2002; Ip et al., 1993; Yoshimatsu et al., 2006). Several gp130 family cytokines are expressed in the embryonic brain, including cardiotrophin-1 (CT-1) in newly generated neurons, neuropoietin (NP) in the early neuroepithelium, and LIF and CNTF in the choroid plexus (Barnabé-Heider et al., 2005; Derouet et al., 2004; Gregg and Weiss, 2005), although the latter two have not been universally detected in the embryonic brain (Barnabé-Heider et al., 2005; Stockli et al., 1991). Multiple studies point to an important role for gp130 family cytokine signaling in the regulation of RGC self-renewal during embryogenesis. First, the number of mitotic RGCs in the VZ is reduced in the forebrain of gp130 KO mice at E15 (Hatta et al., 2002), and mice null for LIFRβ, a coreceptor used by a subset of gp130 cytokines including LIF, CNTF, and CT-1, show a similar deficit at E12.5 (Gregg and Weiss, 2005). Second, treatment of E14-E15 brain explant cultures with CNTF or LIF increases VZ cell division (Gregg and Weiss, 2005; Hatta et al., 2002). Third, deletion of the downstream signaling molecule STAT3 in E14.5 progenitors by in utero electroporation of vectors expressing Cre into *STAT3$^{fl/fl}$* embryos increases the expression of a neuronal marker (βIII-tubulin) and decreases expression of several RGC/progenitor markers (Yoshimatsu et al., 2006).

In vitro studies using the neurosphere assay further support the hypothesis that gp130 family cytokines enhance embryonic forebrain NSC self-renewal. Both exogenous LIF and CNTF potentiate secondary neurosphere formation (Gregg and Weiss, 2005; Pitman et al., 2004), an in vitro correlate of self-renewing potential, and LIF maintains the long-term growth of human embryonic NSCs (Carpenter et al., 1999). Interestingly, LIF is secreted by neurospheres, suggesting that LIF may promote self-renewal through an autocrine/paracrine mechanism (Chang et al., 2004). On the other hand, primary and secondary neurospheres can be generated from the E14.5 forebrain of *LIFRβ KO* and *WT* mice with equal efficiency, and the same appears to be true of cells from *gp130* mutant mice (Gregg and Weiss, 2005; Ohtani et al., 2000; Pitman et al., 2004; Shimazaki et al., 2001). Surprisingly, cells dissociated from *LIFRβ KO* mice can be maintained in epidermal growth factor (EGF) and FGF2 as multipotent, neurosphere-forming cells for at least 15 passages (Pitman et al., 2004). While the cause of this apparent contradiction with the in vivo findings is not clear, it is important to note that the sphere-forming potential of the *LIFRβ KO* cells, as compared to *WT* cells, is reduced when cultured at low density (Pitman et al., 2004) and is lost after several passages when grown in EGF-supplemented media lacking FGF2 (Shimazaki et al., 2001). Thus, perhaps only under in vivo conditions, where growth factor availability, paracrine effects and cell-cell interactions are tightly controlled, are the full consequences of disrupted LIFRβ/gp130 signaling apparent.

Investigation into the mechanism by which gp130 cytokine signaling promotes cortical RGC self-renewal has focused largely on interactions with the Notch pathway. Notch is necessary for RGC self-renewal and its activation occurs, at least in part, by cell-cell contact with newly generated neurons that express the Notch ligands Dll1 and Dll3 (Campos et al., 2001). gp130-STAT3 activation by CNTF increases Notch1 expression in cooperation with EGF, although whether this upregulation is required for the CNTF-induced enhancement of neurosphere formation was not demonstrated in this study (Chojnacki et al., 2003). In addition, STAT3 activation upregulates Dll1 in neurospheres, and knockdown of Dll1 by RNAi blocks the potentiation of neurosphere formation by activated STAT3, suggesting that the promotion of self-renewal by STAT3 requires Dll1-mediated Notch activation (Yoshimatsu et al., 2006). Interestingly, the upregulation of Dll1 by STAT3 provides a potential explanation for the surprising finding by these authors that enforced STAT3 expression in vivo promotes the RGC fate in a cell-non-autonomous manner. Together these results suggest that STAT3-induced Dll1 expression signals to adjacent RGCs to promote their self-renewal and prevents their precocious neuronal differentiation by maintaining Notch activation, which is known to enforce the RGC fate at the expense of neurogenesis. Furthermore, the crosstalk between STAT3 and Notch signaling appears to be bidirectional. The maintenance of the RGC phenotype by activated Notch is blocked in vivo by coexpression of a dominant-negative mutant of STAT3 (Kamakura et al., 2004). This may be related to the finding that the Notch effectors HES1 and HES5 enhance STAT3 phosphorylation, at least in culture (Kamakura et al., 2004). Thus, gp130-STAT3 signaling inhibits neurogenesis and maintains a pool of self-renewing RGCs through both cell-autonomous and cell-non-autonomous mechanisms that involve Notch pathway activation and cooperation with growth factor signaling.

In contrast to the gp130 cytokines, little is known about the role that other cytokines might have on RGC precursors, although other cytokines and cytokine receptors are expressed in the VZ. For example, the granulocyte colony stimulating factor (G-CSF) receptor is expressed in RGCs from E11 onward (Kirsch et al., 2008). Intraventricular infusion of G-CSF in the adult brain enhances neurogenesis from SVZ and subgranular zone (SGZ) progenitors (Jung et al., 2006), but whether this cytokine has similar effects on embryonic progenitors in vivo is not known. Another cytokine, interleukin-1β (IL-1β) is expressed in the embryonic spinal cord neuroepithelium in both rats and chicken, and ectopic delivery of IL-1β to the chick spinal cord in vivo increases the number of $BrdU^+$ proliferating cells in the dorsal spinal cord, while decreasing the number in the ventral cord (de la Mano et al., 2007). Conversely, delivery of an IL-1β blocking antibody induces a slight but significant reduction in proliferation in the dorsal spinal cord, suggesting that endogenous IL-1β regulates progenitor proliferation in vivo. In the adult, IL-1β and other inflammatory cytokines also act on neural stem and progenitor cells influencing proliferation and neurogenesis in response to stress, disease and injury (see Carpentier and Palmer, 2009 [this issue of *Neuron*]).

The Temporal Regulation of Neurogenesis and Gliogenesis

Neurogenesis largely precedes gliogenesis during mammalian brain development. Specific subtypes of neurons and then astrocytes and oligodendrocytes are generated from spatially and temporally segregated pools of RGCs that become progressively more restricted with time. Key aspects of this shift from producing neurons to glia are recapitulated in vitro using progenitors isolated from the early rodent cortex (Qian et al., 2000). Initially, during expansion in FGF2, these cells generate clones that give rise to neurons and a small population of multipotent progenitors. Only after 10 days in vitro (DIV) do they begin to produce astrocytes and oligodendrocyte progenitors. Results from studies of cultured embryonic progenitors implicate both intrinsic and extrinsic factors in the timely shift to gliogenesis, and particular attention has been focused on the role of gp130 family cytokines.

Exogenous LIF or CNTF can induce premature astrocyte generation, as assessed by upregulation of glial fibrillary acidic protein (GFAP) expression, in late (after E15), but not early (E12), cultures of embryonic cortical progenitors through the activation of the JAK/STAT pathway (Bonni et al., 1997; He et al., 2005; Molné et al., 2000). The change in competency to interpret LIF/CNTF-induced gp130-STAT3 signaling as astrogliogenic has been attributed to several factors: (1) the inhibition of STAT3 signaling by the protein tyrosine phosphatase SHP-2 during neurogenesis (Gauthier et al., 2007), (2) the competition between STAT3 and the proneuronal bHLH protein neurogenin 1 (Ngn1) for binding to the CBP-Smad1 transcriptional coactivator complex (Sun et al., 2001), (3) the timely developmental increase in EGFR expression, the forced expression of which accelerates the ability of precursors to interpret gp130 cytokines

as astrogliogenic signals (Viti et al., 2003), and (4) inhibitory methylation in the vicinity of the STAT3 binding sites within the promoters of the glial-specific genes, GFAP and S100β, which prevents their precocious expression (Song and Ghosh, 2004). In addition, STAT3 signaling directly induces the expression of several components of the JAK-STAT pathway, creating a positive, autoregulatory loop that potentiates JAK-STAT induced astrogliogenesis over time (He et al., 2005). Furthermore, gp130-STAT3 signaling cooperates with both the Notch pathway and BMP signaling to further reinforce the commitment to glial fate (Ge et al., 2002; Nakashima et al., 1999b; Taylor et al., 2007).

In vivo, deletion of LIF or CNTF has little effect on astrogliogenesis, although adult *LIF KO* mice have regional decreases in GFAP expression but not astrocyte number (Bugga et al., 1998). In contrast, astrogliogenesis, as measured by GFAP expression, is severely impaired in late-stage embryonic *gp130* or *LIFRβ* KO mice (Koblar et al., 1998; Nakashima et al., 1999a), and electroporation of siRNA against gp130 in the E14/E15 embryonic cortex reduces the percentage of transfected cells that express GFAP (Barnabé-Heider et al., 2005). Thus, while LIF and CNTF are the most widely used gp130 cytokines for in vitro gliogenesis studies, they may not be the key family members involved in astrogliogenesis in vivo. Indeed, this role appears to be filled in part by the related gp130 family member CT-1, as early postnatal *CT-1 KO* mice exhibit reduced expression of GFAP and the early astrocyte marker CD44 (Barnabé-Heider et al., 2005). Interestingly, CT-1 is secreted from newly generated neurons, suggesting that astrogliogenesis is triggered in part by the accumulation of CT-1-expressing neurons. Other gp130 family cytokines including NP and the cardiotrophin-like cytokine (CLC; also known as novel neurotrophin 1 and B cell stimulating factor 3), which binds CNTFRα together with cytokine-like factor 1 (CLF1) (Elson et al., 2000), could also have a role in gliogenesis since the gliogenesis phenotype in the CT-1 mice is apparently not as severe as that seen in *gp130* or *LIFRβ* KO mice.

Surprisingly, other evidence suggests that gp130 is dispensable for astrogliogenesis. Conditional deletion of gp130 in late RGC and astrocytes, by crossing *gp130* floxed mice to mice expressing Cre from the human GFAP promoter, does not lead to an obvious loss of astrocytes or GFAP expression in the adult, although *gp130* deletion does increase the sensitivity of these mice and their astrocytes to adult Toxoplasma encephalitis (Drögemüller et al., 2008). While the role of gp130 signaling during astrogliogenesis was not the focus of this study, the presence of apparently normal numbers of astrocytes in these mice suggests that gp130 is not necessary for their differentiation or adult GFAP expression. One potential explanation for the discrepancy between this finding and the dramatic loss of GFAP expression in the *gp130* and *LIFRβ KO* mice is that the loss of gp130 occurs later in the conditional KOs, raising the possibility that an early function of gp130 (e.g., the promotion of RGC self-renewal) underlies the deficit in astrogliogenesis. The reduced number of GFAP$^+$ cells observed after knockdown of gp130 later during development (E14/E15) by in utero electroporation of gp130 siRNA argues against this hypothesis. However, a caveat of this and other experiments using electroporation or viral vectors to manipulate gene expression in utero is the possibility that these perturbations induce an injury response involving altered cytokine expression. Since injury induces GFAP expression in the adult in a STAT3-dependent manner (Herrmann et al., 2008), injury-induced gp130-STAT3 cytokine signaling might contribute to the level of GFAP expression in the control transfected embryos. Alternatively, astrocyte differentiation and GFAP expression may be delayed in the absence of gp130, a possibility that could not be investigated in the *gp130* KO mice, which die perinatally. Thus, the requirements for gp130 cytokine signaling during astrocyte specification and differentiation are still unclear and warrant further developmental studies using conditional *gp130* KO mice.

Furthermore, while it is clear that gp130-STAT3 signaling stimulates GFAP expression, this does not necessarily represent a switch to a committed astrocyte fate since cells stimulated to express GFAP by LIF remain multipotent and self-renew, at least in vitro (Bonaguidi et al., 2005). This is in stark contrast to the GFAP-expressing cells generated in the presence of BMPs, which cease dividing and develop a stellate morphology similar to adult astrocytes (Bonaguidi et al., 2005). Thus, distinguishing between differentiated astrocytes and multipotent progenitors requires more than an analysis of GFAP expression. LIF/CNTF-stimulated astrogliosis may represent a step in the progression to a more adult-like, GFAP$^+$, multipotent astroglial population, similar to that which persists in the SVZ throughout life. Interestingly, these adult GFAP$^+$ SVZ progenitors are direct descendants of RGCs (Merkle et al., 2004), and it is possible that gp130 signaling is required for maintenance of SVZ progenitors throughout development and into adulthood, a possibility that has yet to be demonstrated, but is suggested by the finding that LIF can support their self-renewal in the adult as well (Bauer and Patterson, 2006). In addition, transgenes driven by the human GFAP (hGFAP) promoter are expressed in late, but not early, RGCs in the mouse (Anthony and Heintz, 2008) and hGFAP-driven, cre-dependent lineage tracing labels cortical projection neurons as well as astrocytes, oligodendrocytes and adult SVZ progenitors (Malatesta et al., 2003) demonstrating the multipotentiality of the GFAP$^+$ RGCs. Considered together, these findings indicate that gp130 family cytokines such as LIF and CNTF induce GFAP expression, but not astrocyte differentiation. Instead, these cytokines support the maintenance of a pool of self-renewing, multipotent progenitors that exhibit certain astroglial characteristics, while other cytokines such as the BMPs may promote the generation of stellate parenchymal astrocytes. Indeed, mice lacking the BMP receptors BMPR1a and BMPR1b in the CNS have reduced GFAP$^+$ and S100β$^+$ astrocytes in the P0 spinal cord (See et al., 2007).

While most studies have focused exclusively on the role of gp130 family cytokines as promoters of astrocyte-specific genes, comparatively little is known about how these cytokines influence oligodendrocyte specification during gliogenesis. In antagonism with BMP-2, LIF promotes oligodendrocyte lineage elaboration in vitro from fetal cortical progenitors (Adachi et al., 2005), and several gp130 family cytokines (e.g., LIF, CNTF, and IL-11) promote oligodendrocyte differentiation and survival (Barres et al., 1993; Mayer et al., 1994; Zhang et al., 2006). Whether gp130 family cytokines have a role in specifying astrocyte versus oligodendrocyte lineage fate in vivo is unclear.

Neuronal Fate Specification and Differentiation

While it is known that cytokines such as LIF can control neurotransmitter and neuropeptide phenotype in the adult nervous system (Bauer et al., 2007) and that many cytokines can influence neural identity in vitro (Mehler and Kessler, 1995), only a few studies have identified roles for cytokines in the control of neuronal identity in the embryonic brain. One recent study implicates LIFRβ signaling in the determination of facial branchiomotor (fbm) neuron subtype identity. LIFRβ expression is first detected at E11.5 in the rhombencephalon VZ, where fbm neuron progenitors arise, and it is also expressed, starting 1 day later, in postmitotic fbm neurons after they have reached the developing facial nucleus (Alfonsi et al., 2008). Fbm neurons, marked by Isl1 expression, are present in equivalent numbers in *LIFRβ* KO and WT mice, but expression of Phox2b, another marker of all fbm neurons, is strongly reduced in the KO. In addition, fbm neurons segregate into several subnuclei that innervate specific muscle targets. One subpopulation that expresses the ETS gene family transcription factor ER81 is expanded by 85% at E16.5 in *LIFRβ* KO mice, while another subpopulation marked by Lhx4, a LIM homeobox-containing transcription factor, is unchanged (Alfonsi et al., 2008). These findings suggest that LIFR signaling influences fbm neuronal subtype identity but not initial fbm specification.

Another example is TGFβ signaling, which is required for the differentiation of mouse mesencephalic progenitors into tyrosine hydroxylase $(TH)^+$ dopaminergic neurons in vivo (Roussa et al., 2006). *TGFβ2/TGFβ3* double KO mice have reduced numbers of TH^+ neurons in the ventral mesencephalon, but not in the locus coeruleus, demonstrating the importance of TGFβ signaling for ventral midbrain dopaminergic neuron development. In support of this, exogenous TGFβ induces ectopic TH^+ cell generation from dorsal mesencephalic neurospheres in a Shh- and FGF8-independent manner, an effect that results from the promotion of the TH^+ phenotype by TGFβ rather than from an increase in survival or proliferation (Roussa et al., 2006). Moreover, ventral dopaminergic neurons do not develop in zebrafish with mutations that disrupt TGFβ/nodal signaling (Farkas et al., 2003; Holzschuh et al., 2003).

Chemokines in Progenitor Migration, Proliferation, and Axon Pathfinding

Chemokines, a family of small proteins that are best known for their control of cell trafficking during immune surveillance and for inflammatory cell recruitment following infection or injury, are dynamically or constitutively expressed in the developing and adult CNS, and several are implicated the migration, proliferation, or differentiation of neurons and glia. Most chemokines and their receptors are promiscuous with respect to their binding partners; many receptors bind multiple chemokine ligands, and conversely, many chemokines bind several receptors. This promiscuity likely results from the extensive expansion of this family of cytokines by gene duplication during vertebrate evolution (DeVries et al., 2006). As a result, the lack of binding specificity has limited the understanding of the individual role that each chemokine and its receptor has during development and in the adult. In contrast, analysis of animals deficient for the chemokine CXCL12 (SDF-1) and its receptor CXCR4, which function largely as exclusive partners, has revealed a wide range of functions in multiple organs including the CNS, where they have critical roles in neuronal migration, proliferation, and axon pathfinding.

Initial characterization of *CXCR4* and *SDF-1* KO mice revealed that, in addition to hematopoietic and cardiac defects, these two lines exhibit largely overlapping defects in cerebellar development (Ma et al., 1998; Zou et al., 1998). Normally, rhombic lip-derived proliferating granule cells migrate tangentially along the cerebellar surface and form the external granule cell layer (EGL) (see Figure 2A). Dividing cerebellar granule cells express CXCR4, and it has been hypothesized that meningeal cells, which secrete SDF-1 as a chemoattractant (Klein et al., 2001; Zhu et al., 2002), maintain the granule cells in the EGL and facilitate their proliferation by exposing them to local sources of Sonic hedgehog (Shh), a mitogen for immature granule cells. Granule cells eventually lose responsiveness to SDF-1, possibly by downregulating CXCR4, and migrate to the internal granule cell layer (IGL), a process controlled by other chemoattractants and repellants (Zhu et al., 2002). In mice deficient for CXCR4 or SDF-1, the temporospatial formation of the EGL and IGL is disrupted. Groups of ectopic granule cells are found in the IGL and Purkinje cell layers during embryogenesis, suggesting that SDF-1 and CXCR4 are necessary to prevent the precocious migration of granule cells to the IGL. Alternatively, SDF-1 may direct the tangential migration of granule cells along the cerebellar surface from their germinal zone in the rhombic lip, where CXCR4 is highly expressed, and if so, this earlier disrupted tangential migration of granule cells would also be expected to contribute to the altered layering observed in the *SDF-1* and *CXCR4* KOs.

The CXCR4/SDF-1 pair similarly regulates the proliferation and/or migration of cells in several other brain regions. For example, CXCR4 is highly expressed in the migratory stream of proliferative cells that forms along the ventral surface of the hippocampus and gives rise to the dentate gyrus, where new granule neurons are generated throughout adulthood (Lu et al., 2002). As in the cerebellum, this stream of progenitors lies adjacent to meninges that secrete SDF-1 (Figure 2B). Mice lacking CXCR4 have abnormalities in the formation of the dentate gyrus due to deficits in the migration and proliferation of dentate granule cells and their precursors (Bagri et al., 2002; Lu et al., 2002). Likewise, SDF-1, expressed both by the meninges on the cortical surface (Borrell and Marín, 2006) and by cells in the cortical SVZ and intermediate zone (IZ), serves as a chemoattractant for GABAergic interneurons that migrate tangentially into the cortex from the ganglionic eminences during embryogenesis (see Figure 2C; Stumm et al., 2003; Tiveron et al., 2006). The SDF-1 produced by cortical meninges also affects the distribution of the transient population of marginal zone cells known as Cajal-Retzius (CR) cells (Figure 2D), which are critical for the inside-out laminar development of the mammalian cortex (Borrell and Marín, 2006). Intriguingly, treatment with the DNA-alkylating agent methylazoxymethanol (MAM) at E15, which is used as a model of developmental disruption with possible relevance for schizophrenia (Lodge and Grace, 2008), causes a redistribution of CR cells to deeper cortical layers that is similar to the defects seen in *CXCR4* and *SDF-1* KO embryos (Paredes

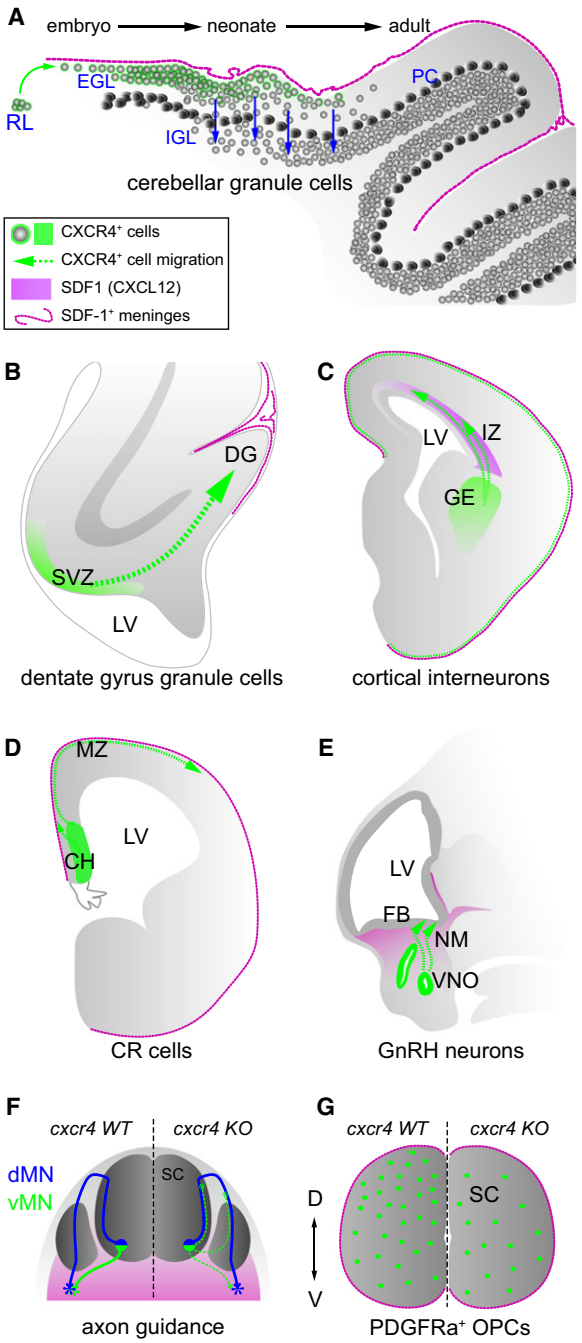

Figure 2. Signaling by the Chemokine SDF-1 and Its Receptor CXCR4 Mediates Numerous Developmental Events

Panels show schematics of embryonic and postnatal rodent brain and spinal cord with SDF-1 expression shown in purple and cells expressing CXCR4 shown in green.

(A) Granule cell migration during embryonic, neonatal, and adult stages is depicted from left to right. SDF-1 is secreted by the meninges (purple dotted lines), which attracts rhombic lip (RL)-derived, CXCR4-expressing granule cells that migrate tangentially along the cerebellar surface and proliferate, forming the external granule cell layer (EGL). Postnatally, granule cells cease proliferating, downregulate CXCR4, and migrate through the Purkinje cell (PC) layer to form the internal granule cell layer (IGL).

(B) In the hippocampus, SDF-1, secreted by the adjacent meninges, attracts CXCR4$^+$ granule cells, which migrate from their SVZ germinal zone to form the dentate gyrus (DG) during late fetal/early postnatal development.

(C) SDF-1, expressed by cortical cells within the intermediate zone (IZ), acts as an attractant for GABA$^+$ interneurons derived from the ganglionic eminence (GE).

(D) CXCR4-expressing Cajal-Retzius (CR) cells migrate tangentially within the marginal zone (MZ) from the cortical hem (CH). Meningial SDF-1 attracts the CH-derived CR cells and maintains their superficial position within the MZ.

(E) Gonadotropin-releasing hormone (GnRH) neurons migrate from the vomeronasal organ (VNO) into the basal forebrain on a gradient of SDF-1 produced by the nasal mesenchyme.

(F) SDF-1/CXCR4 affects axon pathfinding by modulating the responsiveness to repellants and/or attractants. CXCR4 is expressed on spinal cord (SC) vMN axons (green), and SDF-1 (purple) is expressed within the surrounding mesenchyme. In this model, SDF-1 renders these axons insensitive to ventral repellant cues. dMNs (blue), which do not express CXCR4 on their axons, are sensitive to the repellant cues, and exit the neural tube dorsally. In *CXCR4 KO* embryos, vMNs axons are more sensitive to repellants and often project dorsally (dotted green lines). (Adapted from Lieberam et al., 2005.)

(G) The number of PDGFRa$^+$ OPCs in the mouse E14 SC is reduced in the *CXCR4 KO* (right) as compared to the WT (left). The reduction is more obvious dorsally.

et al., 2006). This finding is surprising given that CXCR4$^+$ CR cells distribute throughout the marginal zone prior to the time of MAM delivery. A potential explanation comes from the finding that MAM administration induces meningeal injury and severely reduces SDF-1 expression, the disruption of which may cause a redistribution of CR cells away from the marginal zone. This hypothesis is further supported by the ability of exogenous SDF-1 to normalize the distribution of CR cells in slice cultures from MAM-treated embryos (Paredes et al., 2006).

SDF-1 and CXCR4 are also critical for embryonic migration of gonadotrophin-releasing hormone (GnRH)$^+$ neurons and some ensheathing glial precursors into the basal forebrain from the sensory epithelia in the vomeronasal organ (Schwarting et al., 2006). These neurons and glia express CXCR4 and migrate through the nasal mesenchyme that secretes SDF-1 in an increasing rostral to caudal gradient, which is most intense at the border with the forebrain (Figure 2E). Few GnRH neurons in *CXCR4* KO mice reach their ultimate destination in the hypothalamus, suggesting that SDF-1 is a chemoattractant for these neurons. Interestingly, GnRH neurons fail to reach the hypothalamus in Kallmann's syndrome, causing partial or complete loss of smell, suggesting a potential role for SDF-1/CXCR4 in this syndrome. Furthermore, a more recent analysis of *CXCR4* KO mice reveals a requirement for this receptor in the formation of pontine nuclei by tangentially migrating precerebellar neurons (Zhu et al., 2009). In all, these findings demonstrate a common requirement for SDF-1/CXCR4 chemoattractant signaling in several CNS regions to direct the migration of progenitors or maintain their position.

Beyond the regulation of neuronal progenitor migration, the development of the nervous system also depends on the establishment of the complex circuitry between neurons, and here too SDF-1/CXCR4 has a role in regulating axon pathfinding by modulating the response to axon guidance cues. SDF-1, acting through CXCR4, reduces the repellent activities of Slit-2, semaphorin 3A, and semaphorin 3C on cultured retinal ganglion cell axons, dorsal root ganglion sensory axons, and sympathetic axons, respectively (Chalasani et al., 2003). These effects appear

to be mediated by the elevation of cAMP levels. In zebrafish, knockdown of SDF-1 or CXCR4 results in aberrant axonal pathways within the retina, but as in the mouse, this finding does not stem from a direct loss of a SDF-1-mediated guidance cue, but by modulation of the response to Slit-2 (Chalasani et al., 2007). Zebrafish that have a partial functional loss of robo, the slit receptor, have axonal pathfinding errors in the retina that are rescued by a reduction in SDF-1 signaling (Chalasani et al., 2007). Finally, motor neurons sending axons through the ventral horn of the spinal cord transiently express CXCR4 on their growth cones, extending them toward mesenchymal cells that express SDF-1 (Figure 2F). Deletion of either SDF-1 or CXCR4 causes axon projection abnormalities, with some axons showing intraspinal trajectories similar to those of cranial dorsal motor neurons (Lieberam et al., 2005).

Chemokines may also be important for glial cell development. For instance, OPCs express several functional chemokine receptors, including CXCR4, CXCR2, and CCR3 (Dziembowska et al., 2005; Maysami et al., 2006; Tsai et al., 2002). *CXCR4* KO mice have reduced numbers of platelet derived growth factor receptor alpha ($PDGFR\alpha^+$) OPCs in the E14 spinal cord, a reduction that is more obvious dorsally (see Figure 2G; Dziembowska et al., 2005). While SDF-1 functions as a chemoattractant for cultured neonatal OPCs, which express CXCR4 in vitro (Dziembowska et al., 2005), the authors did not demonstrate that spinal cord OPCs express CXCR4 in vivo, and it is not clear whether the deficit of OPCs in *CXCR4 KOs* stems from direct loss of CXCR4 signaling within OPCs or secondary effects due to alterations in other cells.

In mice lacking CXCR2, there is a deficit in the number and distribution of mature $CC1^+$-expressing oligodendrocytes in the spinal cord at postnatal day 7 (P7) (Tsai et al., 2002). Based on in vitro findings the authors hypothesize that CXCL1 (GRO-α), the ligand for CXCR2, holds OPCs in locations of GRO-α expression in order to enhance their response to local mitogenic factors such as PDGF. In vivo, GRO-α is expressed transiently by astrocytes, first near the ventral pial surface, and later in the dorsal spinal cord (Robinson et al., 1998), and thus could function as a ligand for OPC CXCR2 stimulation. However, OPC distribution is not affected in the *CXCR2* KO at P1, prior to maximal GRO-α expression, and the authors do not report how the later expression of GRO-α (or loss of CXCR2 expression) affects the distribution of OPCs, making it difficult to determine whether their hypothesis based on in vitro data also holds in vivo. Alternatively, the deficit in $CC1^+$ cells, together with apparently normal initial OPC generation and distribution in the absence of CXCR2, also seems consistent with a role for this chemokine receptor in oligodendrocyte maturation, a possibility that has not been examined. Thus, for both neurons and glia, chemokines appear to function not only as regulators of progenitor migration but also as modulators of mitogenic signaling and axon guidance cues. While less is known about the role that other chemokines and their receptors have during CNS development, the complexity and dynamics of chemokine/chemokine receptor expression within the developing brain makes it clear we are only beginning to understand their many roles during CNS development (see Figure 4; Horuk et al., 1997; Meng et al., 1999; Tran and Miller, 2003).

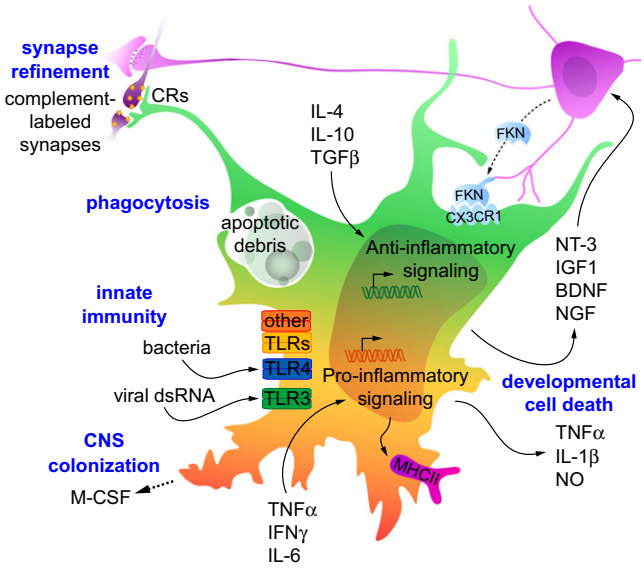

Figure 3. Cytokines and Microglial Function during Development
Microglia have been implicated in many aspects of neurodevelopment (blue text). M-CSF and other signals promote microglial proliferation and colonization of the CNS (bottom left). Microglia phagocytose apoptotic debris resulting from developmental cell death. Microglia express complement receptors (CR) and may remove complement-labeled synapses during synaptic refinement (upper left). Culture studies have shown that microglia respond to a wide array of pro- and antiinflammatory cytokines and chemokines as well as pathogens through the expression of toll-like receptors (TLRs) 1–9. In response, microglia can upregulate MHC class II surface expression and release cytokines, chemokines, nitric oxide, and several neurotrophins, which regulate developmental cell death. Neurons (in purple and not to scale) express fractalkine (FKN), in both membrane-bound and soluble forms, which attenuates microglial activation through its receptor CX3CR1.

Microglia

Microglia are CNS resident, macrophage-like cells of hematopoietic origin that function in the homeostasis of the healthy CNS and as immune surveillance cells in response to infection and injury. The ramified microglia present throughout the normal adult CNS parenchyma actively sample their surroundings by extending and retracting processes (Nimmerjahn et al., 2005), which make specific, transient contacts with synapses in vivo (Wake et al., 2009). Following injury or under neurodegenerative conditions, adult parenchymal microglia transform into a proliferative and motile amoeboid state in which they synthesize a large repertoire of cytokines and chemokines, produce reactive oxygen species (ROS) and exhibit phagocytotic activity. Likewise, as the primary immune sentinels in the CNS, microglia produce cytokines and other proinflammatory mediators in response to activation by variety of pathogens, which microglia recognize through a wide array of toll-like receptors (Falsig et al., 2008). Similar to their stimulated adult counterparts, fetal microglia are largely amoeboid in morphology, are active phagocytes, and are known to secrete cytokines including IL-1 and TNF-α (see Figure 3; Giulian et al., 1988; Munoz-Fernandez and Fresno, 1998).

Microglia are first observed in the neuroepithelium of rodents at, or before, the onset of neurogenesis (Alliot et al., 1999; Ashwell, 1991), and increase in number throughout embryogenesis

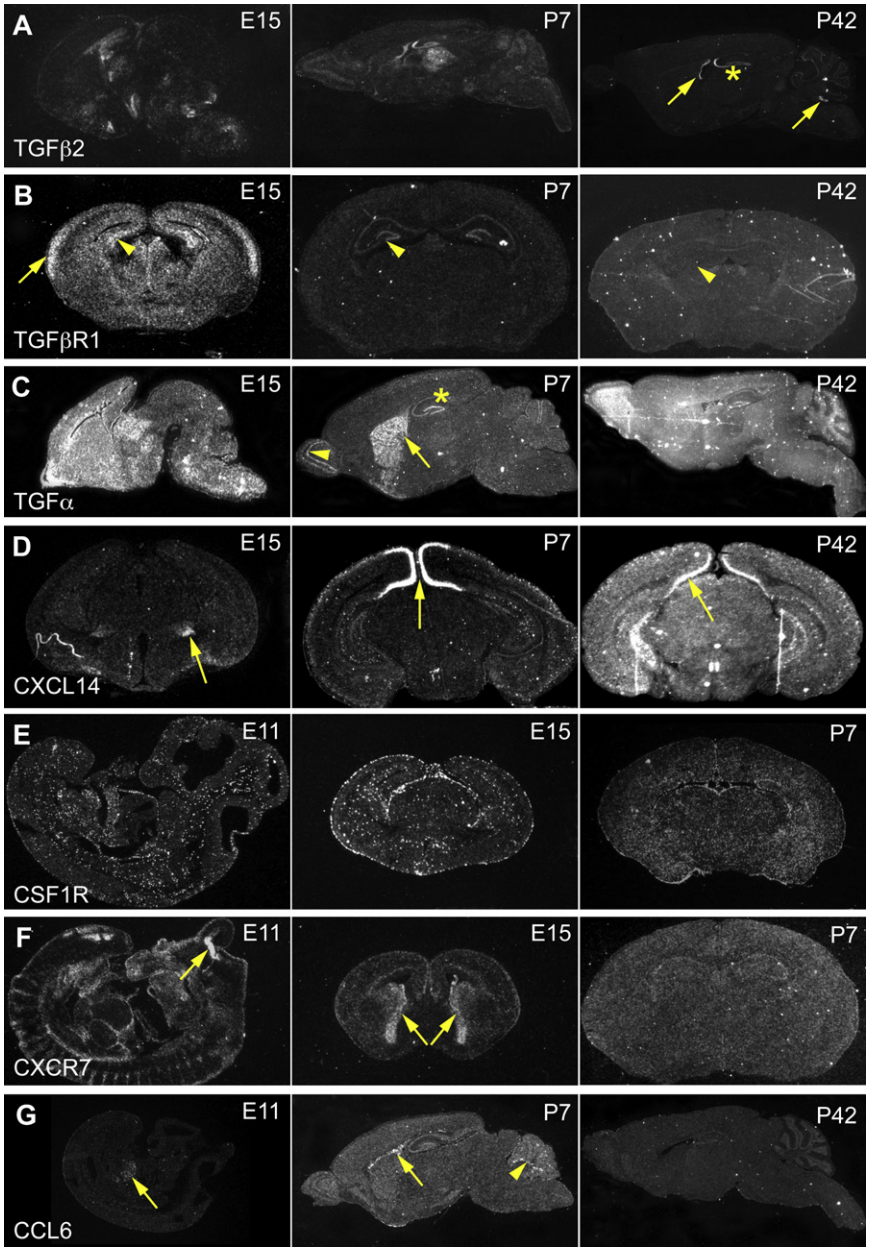

Figure 4. The mRNA Expression Patterns of Cytokine and Chemokine Receptors and Ligands Suggest Regionally-Specific Functions during Development

Panels show in situ hybridization data from C57Bl/6 mice across several developmental stages (E11, E15, P7, and P42/adult). Images were compiled from the developmental in situ hybridization database at www.stjudebgem.org (Magdaleno et al., 2006).

(A) TGFβ2 is expressed in multiple discrete regions throughout the brain at E15 and P7, while in the adult, TGFβ2 expression is most notable in the dentate gyrus, hippocampal CA2/3 region (asterisk), and choroid plexus (arrows).

(B) TGFβR1 is expressed in the developing lateral cortex (arrow) and in the hippocampus (arrowhead) at E15. At P7, TGFβR1 expression is most notable in the dentate gyrus (arrowhead) and pyramidal cell layer of the hippocampus but appears to be absent from the adult hippocampus (arrowhead).

(C) TGFα expression is widespread throughout the E15 telencephalon, with regional variation of expression apparent in the diencephalon and brainstem. At P7, TGFα expression is obvious in the striatum (arrow), olfactory bulb, basal forebrain, hippocampus, and cerebellum. In the adult, TGFα is expressed in the olfactory bulb, hippocampus and cerebellar white matter.

(D) CXCL14 expression appears to be localized to a specific thalamic nucleus at E15 and in the retrosplenial cortex at P7 and P42 (middle and right). CXCL14 is also expressed in the striatum (data not shown).

(E) CSF1R is expressed by macrophages/microglia distributed throughout the early embryo at E11, and in microglia in the brain at E15 and P7.

(F) CXCR7, a receptor recently found to bind SDF-1, appears to be expressed in ventral progenitor regions at E11 and E15 and is expressed at low or undetectable levels by P7.

(G) The chemokine CCL6 is expressed by cells within the fetal liver at E11, and clusters of $CCL6^+$ cells are observed in the corpus callosum and cerebellum at P7 but not in the adult. For simplicity, original multisection images were edited to show only one or two sections. The sections shown are unaltered other than minor adjustments to the brightness/contrast on several of the images, (applied equally for all developmental stages within a panel).

and postnatal stages. The mechanisms that recruit microglia into the mammalian CNS are not entirely clear, although colonization in zebrafish requires *fms*, the macrophage-colony stimulating factor (M-CSF) receptor (Herbomel et al., 2001). M-CSF is a major growth factor for microglia in mammals as well, and osteopetrotic (op/op) mutant mice, which lack M-CSF, have decreased microglia in the neonatal retina (Cecchini et al., 1994) and brain (Kondo et al., 2007; Sasaki et al., 2000; Wegiel et al., 1998), although a deficit in the brain was not observed in all studies (Blevins and Fedoroff, 1995; Chang et al., 1994). In addition, microglia colonization may also be stimulated by the widespread cell death that occurs during normal CNS development (Ferrer et al., 1990; Tseng et al., 1983), since microglia migrate toward injured/dying cells (Akiyama et al., 1994; Bodeutsch and Thanos, 2000; Kurpius et al., 2006) and phagocytose the cell debris (Wang et al., 2002).

The role of microglia in the phagocytosis of dying cells is not limited to the removal of debris; microglia also actively regulate the cell death process through the secretion of cytokines and other factors such as ROS. Indeed, selective depletion of microglia from neonatal cerebellar slice cultures reduces the phagocytosis of caspase-3-expressing Purkinje cells. Remarkably, this ultimately leads to an increase in Purkinje cell survival (Marín-Teva et al., 2004), suggesting that microglia have an active role in killing these neurons and that caspase activation in Purkinje cells does not necessarily represent a commitment to die.

In contrast to their role as phagocytes and executioners, microglia can also be neurotrophic. For example, in *M-CSF* mutant mice auditory and visual processing is impaired, and the newborn pups do not respond to external cues (Michaelson et al., 1996). These effects are thought to be the result of indirect effects mediated by microglia, as the M-CSF receptor is not found on neurons. This hypothesis is supported by culture studies showing that M-CSF acts as a neurotrophic factor for neurons, stimulating survival and neurite outgrowth indirectly through microglia (Michaelson et al., 1996; Pollard, 1997). Culture studies also highlight the vast repertoire of regulatory factors that microglia can produce, including the neurotrophins NGF, BDNF, NT-3, and GNDF, as well as cytokines with neurotrophic activity (reviewed in Garden and Möller, 2006; Kim and de Vellis, 2005).

Furthermore, by responding to and releasing a wide variety of cytokines and factors, microglia modulate several critical aspects of neurodevelopment beyond cell survival. For example, microglia secrete factors that are angiogenic and depletion of microglia in the neonatal retina reduces vascularization (Checchin et al., 2006). Microglia have also been reported to modulate axon pathfinding, perhaps through modification of extracellular matrix by thrombospondin (Chamak et al., 1994). Embryonic microglia produce IL-1, a known mitogen for astrocytes, with expression peaking during astrogliogenesis just before birth (Giulian et al., 1988), and TNFα, an important regulator of developmental apoptosis and synaptogenesis. Thus, microglia participate in many developmental processes, in part by responding to and releasing cytokines and chemokines. The roles that specific cytokines and chemokines have in the context of microglial activation and function are derived largely from culture studies and adult injury/disease models in which microglia are stimulated as part of the repair response (see Carpentier and Palmer, 2009 [this issue of *Neuron*]). Much remains to be done in understanding how microglia, and the cytokines that modulate their activities and mediate their functions, impact normal in vivo development.

Regulation of Cell Survival

During vertebrate CNS development, more neurons are generated than are required to form the appropriate number of connections. This sets up a selective process in which neurons compete for target-derived trophic signals to achieve the appropriate number of axons innervating a target of a particular size. In this way, the survival of neurons that acquire the proper connections is favored, while those that fail in this process are eliminated. Motor neurons are generated in excess and are pruned by apoptosis shortly after they innervate their myotube targets. Aspects of this process can be modeled in spinal cord explant cultures. If explants are made from the E13 rat, motor neurons undergo cell death after 2–3 days, similar to the timing in vivo in which approximately half of the motor neurons die (E15–17) (Harris and McCaig, 1984). If spinal cord explants are made from E12 embryos, however, motor neurons largely survive for at least 5 days in culture. This finding suggested that the timing of death in the absence of target-derived survival factors is not intrinsically programmed, but rather that a delayed death program is activated by an extrinsic factor that is not present, or later induced, in the E12 spinal cord explant cultures. Interestingly, TNFα is produced by macrophages in the adjacent somites specifically within the E12-E13 time window and is largely responsible for the induction of this delayed death program (Sedel et al., 2004). Treatment of E12 explants with TNFα mimics the somite-derived factor, and conversely, a TNFα-blocking antibody inhibits the delayed motor neuron death found in spinal cord cultures that also contain somite tissue. The importance of TNFα in this model was further confirmed in explants made from *TNFα* KO mice, which have reduced motor neuron death. TNFα appeared to mediate this effect through TNFR1, the TNF receptor that commonly transduces pro-death signals from TNFα. In addition, *TNFα KO* embryos have more surviving, and less pyknotic, sympathetic and sensory neurons (Barker et al., 2001).

Motor neuron survival is also modulated by gp130 family cytokines during development and in the adult. Mice lacking *CNTF*, *LIF*, or both *CNTF* and *LIF* (double KO mice), do not show significant motor neuron loss during development, although CNTF KO mice show progressive motor neuron degeneration in the adult (Sendtner et al., 1996). In contrast, loss of CT-1, which is normally expressed in developing skeletal muscle, results in increased motor neuron death in the spinal cord and brainstem nuclei starting at E14 and extending through the first postnatal week (Oppenheim et al., 2001). Since *CT-1* KO mice have no further loss of motor neurons after this period, CT-1 appears to have a specific role as a developmental, target-derived trophic factor. Interestingly, triple KO mice lacking *CT-1*, *CNTF*, and *LIF* display a further progressive loss of motor neurons that is not seen in double *CT-1*/*CNTF* KO mice, a finding that reveals a function for LIF in the postnatal maintenance of distal axons and motor endplates (Holtmann et al., 2005). However, even the triple KO mice have a less severe phenotype than mice lacking any one of the gp130, CNTFRα, or LIFRβ receptors, which all display severe reductions in motor neurons perinatally, suggesting the presence of another critical ligand(s) (DeChiara et al., 1995; Li et al., 1995; Nakashima et al., 1999a). Indeed, deletion of either component of the composite cytokine CLC/CLF, which activates gp130 signaling by binding CNTFRα, causes motor neuron loss and perinatal death resulting from a suckling defect similar to that seen in the CNTFRα KO (Forger et al., 2003; Zou et al., 2009).

Surprisingly, conditional deletion of *STAT3* in NF-L-expressing motor neurons does not result in developmental motor neuron death (Schweizer et al., 2002), suggesting that gp130 cytokines modulate motor neuron survival through a STAT3-independent mechanism, or alternatively, through a non-cell-autonomous mechanism. In support of the latter possibility, CNTF induces expression of Reg-2, a small, secreted protein that promotes the survival of motor neurons. Blocking Reg-2 expression abrogates the survival effect of CNTF (Nishimune et al., 2000). Thus, Reg-2 functions in an autocrine/paracrine loop to mediate the survival effect of CNTF and may explain the apparent STAT3-independent, CNTF-mediated survival.

In the cortex, neurons and their progenitors die by apoptosis in two waves during development. The first, embryonic wave involves proliferating progenitors and the second, perinatal wave targets postmitotic neurons. Recently, the cytokine IL-9

and its receptor have been implicated in the survival of postmitotic neurons (Fontaine et al., 2008). IL-9 and the IL-9R are both expressed in developing cortical neurons with a peak between P0 and P10. Mice lacking the IL-9 receptor have more caspase-3$^+$ cortical neurons (motor, somatosensory, and visual areas), while daily IL-9 injections in WT mice reduce the number of caspase-3$^+$ cortical neurons. Together, these findings suggest that IL-9 functions in an autocrine/paracrine manner to promote survival. This effect requires Jak/STAT, but not MAPK or NF-κB activation in culture, a finding further supported by the observed increase in STAT1 and STAT3 phosphorylation following IL-9 treatment (Fontaine et al., 2008).

The overexpansion of progenitor pools and apoptosis of cells that fail to compete is not limited to neurons; OPCs are likewise generated in excess of their final numbers. Indeed, ~50% of OPCs that colonize and proliferate within the postnatal optic nerve undergo apoptosis during maturation into oligodendrocytes (Barres et al., 1992). OPCs migrate widely from their regions of origin to colonize the entire CNS and send out and retract processes along the way, seeking out unmyelinated axons (Kirby et al., 2006). Axons provide survival signals for differentiating oligodendrocytes (Barres and Raff, 1994) as do several classes of trophic factors (Barres et al., 1993) including the gp130 family cytokines. CNTF, LIF, IL-6, and IL-11 enhance oligodendrocyte survival in vitro (Barres et al., 1993; Louis et al., 1993; Zhang et al., 2006), and exogenous CNTF promotes optic nerve oligodendrocyte survival in vivo (Barres et al., 1993). Accordingly, *CNTF KO* mice have fewer OPCs in the developing optic nerve. However, CNTF also functions as a mitogen for OPCs in the presence of PDGF, which is present endogenously in the optic nerve, so the reduction in OPC number cannot be attributed solely to its effect on OPC survival (Barres et al., 1996). Oligodendrocyte number ultimately recovers in *CNTF KO* mice, suggesting that other factors compensate for the early OPC deficiency. It would not be surprising if mice lacking LIF or other gp130 family members exhibit similar deficits in oligodendrocytes early in development, a possibility that requires further testing.

In contrast to the survival function of gp130 family cytokines, other cytokines can be toxic to oligodendrocyte lineage cells. For example, transgenic expression of IFNγ in GFAP-expressing astrocytes results in severe hypomyelination of the cerebellum and corpus callosum, ataxia, and tremor (LaFerla et al., 2000), and myelin basic protein (MBP)-driven transgenic expression of IFNγ within oligodendrocytes induces similar results (Corbin et al., 1996). While IFNγ induces the death of oligodendrocytes and their progenitors in culture (Andrews et al., 1998; Baerwald and Popko, 1998), the hypomyelination observed in the IFNγ transgenic mice may not be due simply to oligodendrocyte death as, at least in the case of MBP-IFNγ transgenic mice, the mRNA levels for MBP and proteolipid protein (PLP), both of which are expressed exclusively by oligodendrocytes, are not reduced (Corbin et al., 1996). The effects appear to occur through direct IFNγ signaling to oligodendrocytes since transgenic expression of suppressor of cytokine signaling 1 (SOCS1), which inhibits Jak/STAT signaling downstream of IFN-γ, in oligodendrocytes prevents the hypomyelination observed in the IFNγ transgenic mice. One possible mechanism by which IFNγ expression may disrupt myelination is through the upregulation of class I MHC on oligodendrocytes. Class I MHC expression is upregulated in oligodendrocytes in IFNγ transgenic mice, and mice made to express class I MHC by a MBP transgene exhibit a hypomyelination phenotype similar to that seen in transgenic INFγ mice (Turnley et al., 1991).

Synapse Modulation and Elimination

Several cytokines have roles in developmental synaptic refinement. Synapse formation occurs with a significant delay after axons reach their target area, at a time that correlates with the appearance of ensheathing astrocyte processes (Ullian et al., 2001). Astrocytes produce several soluble factors that promote synaptogenesis, including thrombospondins and cholesterol (reviewed in Barres, 2008). However, the formation of postsynaptically active synapses requires the presence of additional, unidentified glial-derived factors. One contributing factor may be TNFα, which is likely produced by microglia that are often present in astrocyte cultures. Exogenous TNFα modulates synaptic strength in hippocampal neurons by rapidly promoting surface expression of AMPA-type glutamate receptors (AMPAR), and interfering with TNFα signaling using TNFα antibodies or a soluble form of the TNFR1 (sTNFR1) decreases AMPAR surface expression (Beattie et al., 2002). Recent findings demonstrate that TNFα mediates this effect on AMPARs through a process that involves the upregulation of β3 integrin expression (Cingolani et al., 2008).

Interestingly, prolonged inhibition of spontaneous neuronal activity in hippocampal cultures stimulates the release of TNFα from glia, while activity-regulated signals, such as glutamate, appear to decrease TNFα release (Stellwagen and Malenka, 2006). Therefore, glia sense overall network activity levels and modulate TNFα release to make compensatory changes in synaptic strength. This may represent a form of homeostatic plasticity known as synaptic scaling, in which the strength of all synapses on a neuron are modulated in response to changes in local network activity (Stellwagen and Malenka, 2006). Such changes are thought to provide stability to neuronal networks (Turrigiano and Nelson, 2004).

The functional relevance of this TNFα-mediated synaptic scaling during development was recently demonstrated using the monocular vision deprivation model. In cortical neurons receiving binocular input, deprivation of vision from one eye during a critical period of postnatal development leads to weakening of the response to input from the deprived eye and a delayed strengthening of the response to input from the open eye. In *TNFα* KO mice, the weakening of the response to the deprived eye occurs normally, but interestingly, the delayed strengthening of the response to input from the open eye is absent (Kaneko et al., 2008). Similar findings were observed after inhibition of TNFα signaling by cortical infusion of sTNFR1 during monocular deprivation, indicating that this effect is due to an acute activity of TNFα, not to effects on earlier aspects of circuit formation. These in vivo findings, together with the hippocampal slice culture studies, indicate that TNFα is critical for the homeostatic potentiation of synaptic strength that occurs following reduction in global activity and suggest that such homeostatic

mechanisms have functional roles during developmental synaptic refinement.

Other cytokines can also regulate synaptic strength early in development. For example, *TGFβ2* KO mice die at birth and do not show rhythmic respiratory activity. This does not result from neuromuscular junction dysfunction, but from deficits in both glutaminergic and GABAeric presynaptic function in the PreBotC area of the brain stem, which is part of the central respiratory rhythm-generating network (Heupel et al., 2008). Expression of TGFβ2 and other TGFβ cytokines is widespread embryonically and postnatally (see Figure 4; Burns et al., 1993; Constam et al., 1994; Pelton et al., 1991), raising the possibility that they may have similar functions in other circuits. Moreover, TGFβ signaling mediates diverse neuronal retrograde signals in the *Drosophila* CNS (Sanyal et al., 2004), and TGFβ signaling regulates neuromuscular synapse formation and function in *Aplysia*, *C. elegans*, *Drosophila* as well as in mammals (Feng and Ko, 2008; Vashlishan et al., 2008), suggesting a conserved role for the TGFβ pathway in synapse modulation.

Synapses and axons, like cells during development, are generated in supernumerary numbers, and here too, glia and glia-derived immune-related molecules have a critical role. Immature astrocytes induce the expression of the complement component C1q on retinogeniculate neurons (Stevens et al., 2007). C1q localizes to synapses, both in culture and in the postnatal CNS, and is critical for proper synapse elimination, as demonstrated by the finding that mice lacking C1q, or the downstream complement component C3, exhibit sustained defects in CNS synapse elimination (Stevens et al., 2007). The immature, astrocyte-derived, soluble factor that triggers C1q production has not been identified, although several cytokines would seem to be attractive candidates. Also unclear is how tagging synapses with complement components targets a subset of synapses for removal, although it is likely that microglia are involved in synapse phagocytosis, since they can express complement receptors for C1q and C3 (Gasque et al., 2002), and activation of these receptors stimulates phagocytosis.

Despite the profound deficits in synapse elimination observed in mice lacking C1q or C3, these mice still display some synaptic refinement and pruning, indicating the existence of complement-independent forms of CNS synapse elimination. Remarkably, an additional means of synapse strengthening and elimination makes use of another set of immune-regulated molecules: the class I MHC proteins. Mice deficient in class I MHC signaling have synaptic refinement deficits of the retinogeniculate projections to the lateral geniculate nucleus (LGN) and have synaptic plasticity abnormalities in the adult hippocampus (Huh et al., 2000). Class I MHC expression is present, not only in the LGN and hippocampus, but also in distinct neuronal populations throughout the CNS (Huh et al., 2000), raising the possibility that class I MHC signaling is involved in the synaptic refinement of additional circuits. In addition, class I MHC expression is developmentally regulated and can be further induced by changes in activity, injury, and cytokines, including TNFα and IFNγ (reviewed in Boulanger and Shatz, 2004; Boulanger, 2009 [this issue of *Neuron*]). Since IFNγ also inhibits dendrite outgrowth in cultured hippocampal and sympathetic neurons and can induce the retraction of existing dendrites, without affecting axonal outgrowth or neuronal survival (Kim et al., 2002) these findings suggest that cytokine dysregulation could have profound effects on the function of specific networks in part by modulating class I MHC expression during critical periods of development. In conclusion, both astrocytes and microglia make contact with synapses both during development and in the adult, and cytokines produced by these glia have critical effects on synaptogenesis and the modulation of synaptic strength, and may also contribute to the targeting of synapses for elimination.

Cytokines and the Fetal Origins of Brain Disorders

All neural and nonneural cell types (e.g., microglia and endothelial cells) within the developing CNS use cytokines for paracrine and autocrine signaling, and because many of these same cytokines also serve as immune modulators, normal cytokine-mediated developmental processes can be susceptible to disruption by immune dysregulation resulting from maternal infection (for an overview of cytokine perturbations that have been performed and their effects on neurodevelopment, see Table 1). Indeed, maternal infection is a risk factor for brain disorders such as periventricular leukomalacia (PVL, i.e., white matter damage), schizophrenia, and autism. PVL is a leading cause of cerebral palsy and cognitive deficits in low birth weight infants, and involves a cytokine imbalance and microglial activation that deplete OPCs and immature neurons in periventricular regions (reviewed by Cockle et al., 2007; Deng et al., 2008). In the case of schizophrenia, serological evidence of any of several types of maternal infection is associated with increased risk for the disorder in the offspring (Patterson, 2009; Penner and Brown, 2007). Moreover, elevated IL-8 or TNFα in maternal serum is also associated with an increased risk for schizophrenia in the offspring (Brown et al., 2004; Buka et al., 2001). In addition, other known risk factors for schizophrenia such as malnutrition and stress involve upregulation of inflammatory cytokines in maternal serum (reviewed by Patterson, 2009). Although the epidemiology is not nearly as extensive as it is for schizophrenia, there is evidence that maternal infection is also associated with a greatly increased risk for autistic symptoms in the offspring (Patterson, 2009). These association studies suggest that cytokine imbalance during embryogenesis can alter fetal brain development and subsequent behavior and/or cognitive function in the offspring.

The hypothesis that cytokine dysregulation induced by maternal immune activation (MIA) can alter neurodevelopment and subsequent behavior in the offspring is also supported by evidence from animal studies. Investigators have used infection with influenza virus, injection of the dsRNA poly(I:C) to mimic viral infection, or lipopolysaccharide (LPS) to mimic bacterial infection and PVL; and have employed rodents, ewes, and nonhuman primates as models. These various methods of MIA alter fetal brain development such that the offspring display a variety of behavioral abnormalities and neuropathologies that are consistent with those seen in mental illness (Meyer et al., 2005; Patterson, 2009). Maternal poly(I:C) or LPS treatment not only induces a cytokine cascade in maternal serum, but also increases IL-1β, IL-6, IL-10, and TNFα protein and mRNA levels in fetal brain (Meyer et al., 2005; Patterson, 2009).

Table 1. Neurodevelopmental Effects of Cytokine Perturbations In Vivo

Perturbation	Key Findings	References
Chordin/noggin dKO	Severe deficits in forebrain development.	Bachiller et al., 2000
CLC/CLF1 Kos	Severe motor neuron deficits in both individual KOs, die from a lack of suckling.	Forger et al., 2003; Zou et al., 2009
CNTF KO	Reduced OPC number in the optic nerve (ON).	Barres et al., 1996
Exogenous CNTF	Increased OPC survival in the ON with injection of CNTF-expressing cells into the subarachnoid space of postnatal rats.	Barres et al., 1993
E15 CNTF vector in utero electroporation	GFAP$^+$ cells increased after 3 days.	Barnabé-Heider et al., 2005
CNTFRα KO	Severe motor neuron (MN) deficiency.	DeChiara et al., 1995
CNTF/LIF/CT-1 tKO	Motor neuron deficiency, similar to CT-1 KO.	Holtmann et al., 2005
CT-1 KO	Deficiencies in motor neuron subpopulations. Reduced cortical GFAP and CD44 (early astrocyte marker) expression.	Oppenheim et al., 2001; Barnabé-Heider et al., 2005
CXCR2 KO	Reduced CC1$^+$ mature oligodendrocytes in the neonatal SC.	Tsai et al., 2002
CXCR4 KO	Altered migration of cerebellar granule cells, CR cells, cortical interneurons, dentate granule cells, precerebellar nuclei neurons, and GnRH neurons. Reduced SC OPC numbers, and axonal pathfinding defects.	Bagri et al., 2002; Borrell and Marín, 2006; Dziembowska et al., 2005; Lieberam et al., 2005; Lu et al., 2002; Ma et al., 1998
CXCR4b morpholinos in zebrafish embryos	CXCR4b knockdown rescues retinal axon guidance deficits caused by partial loss of function Robo2 mutation.	Stumm and Höllt, 2007; Zou et al., 1998; Chalasani et al., 2007
CXCR4b zebrafish mutants (ody/ody)	Displaced olfactory sensory neurons and axon guidance defects.	Miyasaka et al., 2007
E14/15 gp130 siRNA in utero electroporation	Decreased percentage of cells expressing GFAP among transfected cells.	Barnabé-Heider et al., 2005
GFAP-IFNγ or MBP-IFNγ Transgenic mice	Severe hypomyelination, ataxia, and tremor	Corbin et al., 1996; LaFerla et al., 2000
Exogenous IL-1β	Ectopic delivery to chick or rat spinal cord alters progenitor proliferation.	de la Mano et al., 2007
Maternal IL-6 i.p. injection at day 12.5	Prepulse inhibition (PPI) and latent inhibition behavioral abnormalities in the adult offspring.	Smith et al., 2007
IL-6 KO / IL-6 Ab inj.	Poly(I:C)-induced behavioral changes in the adult offspring are absent in IL-6 KOs or when IL-6 Ab is injected with poly(I:C).	Smith et al., 2007
IL-9R KO	Increased density of caspase-3$^+$ cells in the neonatal cortex.	Fontaine et al., 2008
IL-9, P1-P4 2X daily i.p. injections	Reduced the number of caspase-3$^+$ cortical neurons.	Fontaine et al., 2008
IL-10 transgenic (CD68 macrophage specific promoter)	Blocks maternal poly(I:C) treatment-induced behavioral changes in the offspring. IL-10 transgenic offspring display behavioral abnormalities in the absence of MIA suggesting cytokine imbalance.	Meyer et al., 2008
LIFRβ KO	Severe MN deficiency, perinatal death. Altered MN subtype identity in the facial nuclei. Reduced RGC self-renewal in the cortical VZ at E12.5.	Li et al., 1995; Alfonsi et al., 2008
M-CSF mutant (*op/op*)	Reduced microglia colonization of the CNS (not observed in some studies*). Auditory and visual processing impairments, pups fail to respond to external cues.	Gregg and Weiss, 2005; Kondo et al., 2007; Naito et al., 1991; Sasaki et al., 2000; Wegiel et al., 1998; Witmer-Pack et al., 1993; Blevins and Fedoroff, 1995*; Chang et al., 1994*; Michaelson et al., 1996
SDF1 (CXCL12) KO SDF1a morpholinos in zebrafish embryos	Ectopic cerebellar granule cell migration, cortical interneuron migration deficits. SDF1a knockdown rescues retinal axon guidance deficits caused by partial loss-of-function Robo2 mutation.	Ma et al., 1998; Stumm et al., 2003; Chalasani et al., 2007
STAT3$^{fl/fl}$ Cre vector in utero electroportation into E14.5 cortex	Decreased expression of several RGC/progenitor markers and increased the expression of neuronal markers, effect at least partially cell nonautonomous.	Yoshimatsu et al., 2006

Table 1. *Continued*

Perturbation	Key Findings	References
TGFβ2 KO	Die at birth from respiratory failure due to deficits in brain stem presynaptic function.	Heupel et al., 2008
TGFβ2/TGFβ3 dKO	Reduced tyrosine hydroxylase (TH)+ dopaminergic neurons in the ventral midbrain.	Roussa et al., 2006
TNFα KO	Loss of the homeostatic increase in response to the open eye following monocular visual deprivation. Reduced MN death in SC explant cultures. Reduced sympathetic and sensory neuron death.	Kaneko et al., 2008; Sedel et al., 2004; Barker et al., 2001

To examine the contribution of individual maternal cytokines to the alterations of brain development induced by MIA, various types of perturbation have been carried out: maternal cytokine injection, blocking cytokines with antibodies, using cytokine KO mice, and using transgenic mice overexpressing a cytokine in a particular cell type. Maternal injection of IL-6 in the mouse can mimic many of the effects of maternal infection or maternal poly(I:C), yielding offspring that display behavioral abnormalities consistent with those seen in schizophrenia and autism (Samuelsson et al., 2006; Smith et al., 2007). It is remarkable that a single, transient pulse of increased IL-6 can have such profound and long-lasting effects on behavior. This illustrates the embryonic brain's responsivity to relatively subtle changes in the maternal-fetal environment. In the converse experiment, maternal coinjection of an anti-IL-6 antibody with poly(I:C) blocks the effects of MIA on the behavior and brain gene expression of the offspring. Moreover, injection of poly(I:C) in pregnant *IL-6* KO mice yields offspring with normal behavior (Smith et al., 2007). Consistent with the importance of a pro-inflammatory/anti-inflammatory cytokine balance, macrophage-specific transgenic expression of the anti-inflammatory cytokine IL-10 suppresses the effects of maternal poly(I:C) on the behavior of the offspring (Meyer et al., 2008). The behavioral deficits may be prevented by the IL-10-induced reduction in the IL-6 and TNFα in maternal serum after poly(I:C) injection, or by the IL-10-induced shift in balance between the pro- and anti-inflammatory cytokine expression in the fetal brain. Also supporting the cytokine balance hypothesis is the observation that IL-10 overexpression in the absence of poly(I:C) treatment induces behavioral abnormalities in the offspring (Meyer et al., 2008). One caveat of these experiments is that the IL-10 transgene was not only present maternally, but also in the offspring, so the behavioral results may have stemmed from a postnatal, anti-inflammatory action of IL-10.

Future Directions

Our knowledge of the diverse roles that cytokines have during CNS development is in its infancy. Many well-characterized cytokines have profound effects on cultured neurons and glia, although the in vivo relevance of the majority of these findings remains to demonstrated. In addition, the expression patterns of many less well-characterized cytokines and chemokines as well as their receptors and signaling components show striking regional and developmental stage specificity (Figure 4), implying important developmental functions. Furthermore, beyond their roles in normal CNS development, disruption of the balance of cytokines in the maternal-fetal environment by MIA may severely affect fetal brain development as seen in PVL, schizophrenia, and autism. Although not covered in this review, cytokines also have profound effects on the peripheral nervous system that may likewise be disrupted by MIA with long-term consequences for the offspring. Discovering where the MIA-induced cytokines are acting by localizing the expression of key cytokine receptors as well as activation of downstream signaling pathways will begin to illuminate the cellular and molecular pathways involved in altering fetal neurodevelopment. Also of interest is the relative importance of maternal- versus fetal-derived cytokines. In the case of MIA, are maternally derived cytokines sufficient to trigger the behavioral and neuropathological abnormalities, or is embryonic cytokine dysregulation also required? Cytokine transgenic and KO mice can now begin to address this question.

The observation that MIA alters cytokines in the fetal brain may also be relevant in the context of the subsequent immune dysregulation that is observed postnatally in schizophrenia and autism. Cytokines are strongly elevated in several brain regions and in the cerebral spinal fluid of autistic subjects, ages 4–45 years old (reviewed by Pardo et al., 2005). A variety of immune-related genes are also dysregulated in autistic and schizophrenic brains (reviewed by Patterson, 2009). It remains to be determined if an embryonic cytokine imbalance drives such immune dysregulation in the adult brain, and if so, how this altered immune state is established and whether interrupting it would be therapeutically beneficial.

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