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Roles of Glia in Developmental Neurotoxicity: Session VI Summary and Research Needs

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SESSION SUMMARY

The purpose of this session was to address the molecular, pathological, and functional responses of astroglia, microglia, and oligodendroglia to neurotoxicant exposure. Neurons are the signaling cells of the nervous system, and as such are responsible for the perception of sensory stimuli and the coordination of cellular, tissue, and organismal responses to stimuli from the environment. Neuronal function and nervous tissue structure requires the participation of neuroglia, or glia, including astroglia (which participate in neurotransmitter metabolism and responses to stress and injury), radial glia and Bergmann glia (which provide scaffolding for neuronal migration during development), oligodendroglia and Schwann cells (which myelinate axons in the central and peripheral nervous systems, respectively), and microglia (which mediate inflammatory responses in the central nervous system). The roles of each of these cells as target cells and participants in various aspects of developmental neurotoxicity was discussed.

ASTROGLIA AS METAL DEPOTS

The first talk, by Evelyn Tiffany-Castiglioni, was entitled "Astroglia as Metal Depots: Molecular Mechanisms for Metal Accumulation, Storage and Release, and Neurotoxicity". Dr. Tiffany-Castiglioni gave a brief background for the importance of metal physiology in the brain, listing five points: (1) the brain concentrates metals; (2) normal brain contains potentially toxic levels of Cu, Fe, Zn, and Mn; (3) brain cells have protective mechanisms against accumulated metals; (4) metals may share some mechanisms for entry, distribution, and storage in brain cells; and (5) some metals accumulate in astroglia. A schematic model for metal detoxification by mammalian cells was presented showing that cells handle metals by metalloregulation via proteins such as metallothioneins, incorporation into metal-requiring enzymes, extrusion from the cell (diffusion or P-type ATPases), and/or mineralization in organelles (lysosomes, mitochondria, and nucleus). This schematic was subsequently used to illustrate the handling of accumulated lead (Pb), mercury (Hg), manganese (Mn), and copper (Cu) by astroglia. Astroglia possess several properties that would allow them to serve as depots for metal in the brain. Among them are: (1) the perivascular localization of astroglial endfeet and the juxtaposition of astroglia between the blood brain barrier and the neuronal cell bodies; (2) high levels of metallothioneins I and II; (3) high cytosolic levels of glutathione; (4) metal-dependent enzymes (glutamine synthestase, Mn-superoxide dismutase, and Cu, Zn-superoxide dismutase; and (5) putative metal transport or carrier proteins, such as ceruloplasmin and ATP7a (Cu-ATPase or Menkes protein).

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Xenobiotic neurotoxic metals (Pb and Hg) were the first metals discussed. A brief survey of in vivo and in vitro work supporting David Holtzman's "lead sink hypothesis" was given. This hypothesis states that mature astroglia accumulate Pb in brain, whereas mature neurons and immature astroglia do not. Putative mechanisms for Pb entry into mammalian cells included an anion exchanger, L-type Ca^{2+} channels, and a cation channel activated by the depletion of intracellular Ca^{2+}. Pb deposition in astroglia was proposed to be associated with Pb binding to glucose regulated protein 78 (GRP78), interactions with several enzymes, probable deposition in organelles, and efflux, possibly by P-type ATPases. The next metal addressed was Hg, which can enter astroglia by diffusion as MeHg or Hg^0, or via an unknown transporter as Hg^{2+}. Hg is deposited in lysosomes in astroglia. Controversy exists concerning the cellular localization of Hg in exposed mammals, based in part on chemical form of Hg, species (rat, mouse, monkey, or human), exposure paradigm (concentration, duration, and recovery time), and precision of methodology (standardized autometallographic method). In general, rats, mice, and humans exposed to Hg^{2+} have been shown by various workers to be localized primarily to motor neurons. On the other hand, MeHg is widely distributed among neuronal types with little glial distribution in rats and mice. Squirrel monkeys treated with elemental mercury vapor show Hg localization in large neurons of the cerebellum, as well as astroglia. In contrast, a series of chronic studies with macaques indicates that Hg is primarily localized to astroglia and microglia when animals are exposed to mercury vapor or $HgCl_2$. The latter evidence has given rise to a glial buffering hypothesis for MeHg exposure whereby astroglia initially accumulate Hg and may subsequently either die and be phagocytized by microglia, which then accumulate the Hg, or undergo astroglioisis and proliferation. Much later, neurons may accumulate lessor amounts of Hg.

Essential metals (Mn and Cu) may also present neurotoxicity concerns if individuals are overexposed or if transport of the metals to appropriate cells is impaired. The third metal discussed was Mn, which is an essential metal and exists in trace amounts in normal brain. Mn neurotoxicity has been described in certain industrial settings and may be a problem in future as MMT is added to gasoline. Astroglia are a likely target of Mn-induced damage in overexposure, as 80% of the Mn in brain resides in glutamine synthetase which is localized to astroglia. Furthermore, chick glia in culture concentrate Mn, whereas neurons do not. Neonatal rat astroglia in culture have a specific, relatively high capacity transport system for Mn. One recent study has demonstrated that in an olfactory exposure model in rats, astroglia show loss of glial proteins with no effects to neurons or microglia, thus providing evidence that astroglia are indeed a manganese target. The fourth metal discussed was copper (Cu), a metal required for numerous brain enzymes. Evidence suggests that intracellular copper accumulation may be involved in Pb-induced neurotoxicity. Dr. Tiffany-Castiglioni's laboratory has shown that Cu levels transiently increase four-fold in cultured astroglia exposed to high Pb and Cu accumulates in fetal and neonatal guinea pig brain with low Pb exposure. Furthermore, Pb blocks Cu efflux from C6 cells and Pb binds to the heavy metal binding region of the Cu-ATPase ATP7a. Thus, Pb may induce an increase in intracellular Cu in astroglia and/or prevent proper Cu distribution to neurons in the same manner proposed for Menkes disease.

GLIA AND FETAL ALCOHOL SYNDROME

The second speaker was Dr. Consuelo Guerri, whose talk was entitled "Glia and Fetal Alcohol Syndrome". Dr. Guerri focused on the spectrum of fetal effects caused by maternal alcohol consumption during pregnancy. Thus, depending on the dose, time and conditions of exposure, prenatal alcohol exposure can induce abortion, low birth weight, mental retardation, impaired motor development, and fetal alcohol syndrome (FAS). FAS is characterized by growth deficiency, a specific pattern of physical anomalies and, central nervous system (CNS) damage. However, CNS dysfunctions are the most devastating and permanent consequences of heavy and moderate maternal alcohol consumption and can occur in absence of gross morphological defects. Abnormalities in glial development including neuroglial heterotopias, smaller white matter mass, and ectoptic clusters of glia and neurons have been observed in FAS children, suggesting potential effects of ethanol on glial cells.

Dr. Guerri pointed out the exquisite sensitivity of the developing CNS to ethanol exposure in utero, identifying the types of damage produced in each critical period, and comparing human and rat developmental stages of the CNS. Organogenesis and neural tube formation occur, approximately, in the first 30 days of human gestation, and about 5–11 days of gestation in the rat. Exposure to high levels of alcohol during this period results in major neural tube defects and can produce facial dysmorphology. Proliferation of radial

glia and neurons and neuronal migration occur beginning in the second trimester of human gestation and about 11–18 gestational days in the rat. EtOH exposure during this period leads to abnormal radial glial development and neuronal migration, as well as neural cell loss. Beginning in the third trimester and extending into the first two years of life in humans and the postnatal period in the rat, the brain undergoes myelinogenesis, astrogliogenesis, and synaptogenesis. EtOH exposure during the third trimester of pregnancy leads to pronounced microencephalopathy, altered neural connections, impaired astroglial development, and agenesis of the corpus collosum. Exposure to EtOH in the third trimester is also associated with astrogliosis. Chronic ethanol exposure during all critical periods of CNS development induces in the rat a wide range of brain abnormalities and alterations observed in children with FAS, including decreased fetal viability, reduced fetal and postnatal body and brain weight, and ultrastructural and functional liver alterations. Dr. Guerri also briefly reviewed the radial unit hypothesis and the role of radial glia in neuronal migration to apical positions in the developing cerebral cortex.

Studies in Dr. Guerri's laboratory focus on the effects of EtOH on early postnatal brain development in a rat model and complementary cell culture models. The work she presented addressed the hypothesis that astroglia, rather than neurons, are the primary cellular target of damage in EtOH neurotoxicity induced during late gestation. Previous studies showed altered glial fibrillary acidic protein (GFAP) expression and reduced expression of glutamine synthetase (GS) in a rat model of FAS, both indicating damage to astroglia. Accordingly, an in vitro model of astroglia isolated from the fetuses of chronic alcohol-fed rats and cultured in the absence of ethanol was examined for alterations that corresponded with in vivo effects. Among the alterations exhibited by astroglia cultured from the FAS rats compared to controls were: decreased DNA, RNA and protein synthesis, ultrastructural and cytochemical alterations, altered content and distribution of cytoskeletal proteins (e.g. GFAP), reduced levels of membrane-bound enzymes and membrane glycoproteins, decreased release of NGF and altered receptor expression, and altered expression of neural cell adhesion molecules (NCAMs) and their transport to the plasma membrane. Furthermore, several reports demonstrate that addition of ethanol to astroglia in culture results in accumulation of cells in the G_0/G_1 phase of the cell cycle, blocked activity of mitogenic growth factors (e.g. FGF, PGF, IGF, etc.), potentiation of growth inhibiting factors (e.g. TNF), increased free radical generation and induction of cytochrome P450 (2E1), decreased content of cytoskeletal proteins (e.g. GFAP), and decreased cytosolic GSH content. GFAP expression was discussed in detail. GFAP immunofluorescence of astrocytes in culture exposed to EtOH prenatally is reduced compared to controls. Furthermore, Dr. Guerri showed that radial glia cultured from rat fetuses prenatally exposed to ethanol are also affected. Cytoplasmic processes were shorter, transformation into the astrocyte phenotype was delayed, and GFAP expression was altered. Methylation-mediated repression of GFAP transcription could be a mechanism involved in EtOH-induced reduction of GFAP expression.

Evidence from in vivo and in vitro studies presented by Dr. Guerri clearly supports the idea that ethanol alters astrogliogenesis in humans and experimental animals by affecting key astroglial functions. Alterations in astroglial proliferation and differentiation can lead to decreased production of astroglial factors involved in neuronal survival (e.g. neurotrophins) or interactions required for neuronal migration (e.g. radial glial morphology, NCAM expression) which would influence neurogenesis, migration, and axonal guidance. Whereas astroglia participate in brain processes such as the supply of precursors for neuronal energy production, neurotransmission, and detoxification, ethanol-induced damage in glial cells would be likely to have profound effects in these processes, influencing the normal functional development of the CNS. Therefore, ethanol-induced astrocyte damage could be a potentially important mechanism involved in CNS dysfunctions observed after in utero EtOH exposure and in fetal alcohol syndrome.

ETHANOL EFFECTS ON ASTROGLIAL FUNCTIONS

The third talk, entitled "Effects of Ethanol on Selected Astrocyte Functions", was given by Dr. Michael Aschner. Dr. Aschner provided a brief review of the relationship between alcoholism and tonicity as the basis of the hypothesis for the work he would present. While intoxicated, the plasma of alcoholics is hyperosmotic. This phenomenon is attributable to the transient suppression by EtOH of the release of vasopressin (AVP; also called anti-diuretic hormone or ADH) from the posterior pituitary, producing diuresis and hypernatremia. When alcoholics are acutely withdrawn from EtOH, AVP release is stimulated, resulting

in water retention (anti-diuresis) and dilutional hyponatremia. These events imply that astrocytic and neuronal intracellular fluid would be hypertonic relative the extracellular fluid after EtOH withdrawal.

Dr. Aschner presented the following hypothetical sequence of events to explain astrocytic changes in cell volume in EtOH exposure and withdrawal: first, as astrocytes adapt to chronic EtOH exposure in the presence of a hyperosmotic extracellular fluid, intracellular electrolyte and osmolyte levels will increase in the cell. Second, after EtOH withdrawal, EtOH, as well as intracellular electrolytes, will leave the cell rapidly. However, because of slower adaptation to the release of compensatory osmolytes that have accumulated to maintain cell volume, the intracellular space will remain hypertonic, resulting in swelling. Third, the swelling will lead to the release of endogenous excitatory amino acids (EAA, glutamate and aspartate), and regulatory osmolytes such as taurine, myoinositol, and K^+ and a regulatory volume decrease (RVD). Dr. Aschner then presented experiments from his laboratory in rat astroglial cultures that tested this hypothesis.

The results of the study were as follows. First, ‘‘chronic’’ EtOH exposure (96 h) produced increased accumulation of the osmoregulatory amino acids taurine and aspartate. Second, EtOH withdrawal resulted in taurine but not aspartate release. This amino acid release profile is different from that seen during hypotonic-induced swelling, suggesting a taurine release mechanism other than the volume sensitive organic/acidic amino acid channel (VSOAC). Third, despite increased taurine efflux, astrocytes ‘‘chronically’’ exposed to EtOH were unable to fully volume regulate when swollen, perhaps due to retention of other osmolytes such aspartate. Fourth, in response to hyponatremia and EtOH withdrawal, astrocytes increased their cellular volume to a greater extent than astrocytes exposed to hyponatremia alone.

The study outlines of the sequelae of EtOH exposure both in adult and developing brain, alluding to changes that might be expected to take place in situ. Exposure to EtOH is an osmotic stressor and stimulates accumulation of osmoregulatory amino acids. Long term consequences are unknown. It is possible that ‘‘chronic’’ EtOH exposure results in the inability of astrocytes (and neurons) to accommodate stress responses, which in turn, leads to cellular atrophy. Alternatively, release of intracellular taurine upon EtOH withdrawal may represent a neuroprotective effect, inhibiting neuronal excitability, both in the developing and mature CNS. For example, taurine-mediated osmoregulation and post-ischemic glutamate surge suppression (PIGSS) are established mechanisms in taurine neuroprotection.

PRIMARY DEMYELINATION AND REMYELINATION

Dr. Glenn Matsushima presented the fourth talk, entitled ‘‘Toxicant-Induced Primary Demyelination and Remyelination’’. Dr. Matsushima explained that the sequence of events and the critical factors involved in primary CNS demyelination induced by neurotoxicants are only partly understood. Equally important are investigations to the repair of the lesions. His work focuses on the neurotoxicant, cuprizone, which is a copper chelator that provides an excellent model to study demyelination and remyelination. Cuprizone reduces the levels of Cu-dependent enzymes, such as cytochrome c oxidase, monoamine oxidase, and carbonic anhydrase II, leading to mitochondrial abnormalities, decreased microtubules, and vacuolization in oligodendroglia. Dr. Matsushima's laboratory is investigating cuprizone-induced demyelination in C57BL/6 mice, which is characterized by a synchronized cascade of morphological, biochemical, and molecular events. He outlined processes associated with cuprizone-induced demyelination as well as remyelination, detailing responses by both oligodendroglia and microglia. In this animal model, cuprizone causes perturbation in oligodendrocytes that leads to their depletion by apoptosis. Microglia and astrocytes accumulate in the lesion to clear debris and provide cytokines and growth factors important in aiding oligodendrocyte precursors. It is these precursors that are involved in the remyelination process. Thus, insult to the CNS by the neurotoxicant, cuprizone, results in a cascade of complex cellular, morphologic, biochemical of molecular events in which microglia or astrocytes appear to function in facilitating the remyelination process. Despite cuprizone's not being an environmental neurotoxicant, it was shown to be a potentially useful tool in deciphering mechanisms of demyelination and remyelination of xenobiotics.

Previous studies by other laboratories have examined ultrastructural changes and some biochemical alterations during demyelination and remyelination in cuprizone-treated weanling mice. Studies presented here were conducted on C57BL/6 mice to facilitate comparisons to knock-out and transgenic mice on a similar background; however, due to differences in weight of the weanling mice, young adult mice at 8–10 weeks of age were used to ensure similar dosing.

All assessments regarding cellular changes were conducted in the corpus callosum above the fornix. Histochemical evidence was presented to show that after 5–6 weeks of treatment with cuprizone, myelin was completely removed. A chronological map of histochemical events in demyelination and remyelination was presented, showing the following events: (1) exposure to cuprizone results in oligodendrocyte perturbation and death by apoptosis; (2) by five weeks of exposure, demyelination is nearly complete and correlated to a near absence of mature oligodendrocytes; (3) even in the presence of the neurotoxicant at week 6, remyelination has already commenced; (4) when cuprizone is removed at week 6, remyelination continues and by 12 weeks myelin recovers; (5) if cuprizone is not removed at week 6, there is a second episode of demyelination, and interestingly, this is followed by a second but weaker attempt to remyelinate at week 12. With the continued exposure to cuprizone, there is a final third episode of demyelination prior to the mice becoming moribund.

Molecular and cellular analyses of events coinciding with demyelination and remyelination showed that myelin-specific gene expression (myelin basic protein, and myelin-associated glycoprotein and ceramide galactosyltransferase) dramatically decreases after 2 weeks of cuprizone treatment. In addition ceramide decreases and reaccumulates in a manner coincident with demyelination and remyelination. Olidodendrocytes undergo apoptotic cell death, with a peak at week 3 corresponding to the depletion of mature oligodendrocytes as noted by GST–Pi (Pi isoform of glutathione-s-transferase) staining. The data suggest that most mature oligodendrocytes are depleted by apoptosis, implying that the remyelination is not due to the preservation by differentiation of mature oligodendrocytes, but rather to the influx and differentiation of oligodendroglial progenitor cells (detected with NG2 marker) into the lesion site. Their presence is noted at early stages of cuprizone exposure. Thus, very early on, events associated with remyelination take place simultaneously with the demyelinating processes.

In addition, microglia/macrophages are an intimate part of the demyelination and remyelination processes and begin to appear at 2–3 weeks, showing great accumulations at 4–6 weeks of cuprizone treatment, at which time they phagocytize myelin from degenerating mature oligodendrocytes. Astrocytes also appear at the lesion site, and unlike microglia which start declining after 6 weeks, some astrocytes remain continuously throughout the remyelination process. Interleukin-1 (IL-1) beta expression is associated with all of the microglia and about 50% of the astrocytes. Whereas IL-1 is toxic to oligodendrocytes and, thus plausibly might take part in demyelination, this hypothesis was tested by the use of a knock-out mouse for IL-1 beta. Cuprizone-induced demyelination in IL-1 beta$^{-/-}$ mice was equal to that of normal mice, thus indicating that IL-1 is not part of the demyelination process. However, IL-1 beta may be important for promoting regeneration and may induce insulin-like growth factor-1 (IGF-1) expression in astrocytes and microglia/macrophages. IGF-1 induction is absent in cuprizone-treated IL-1 beta knock-out mice, and the appearance of new mature oligodendroglia is also reduced in these mice. The hypothesis was presented that IGF-1 secretion by microglia/macrophages stimulates oligodendroglial precursors to mature and jump-starts the remyelination process.

TOXICANT-INDUCED REACTIVE ASTROGLIOSIS

The fifth talk, entitled "Toxicant-Induced Reactive Astrogliosis in the Developing Nervous System", was presented by Dr. James O'Callaghan. He defined reactive gliosis as a generic neurotoxic response and identified issues relating to the detection of neurotoxicity and the evaluation of reactive gliosis. Different insults to the CNS damage different targets, because brain regions and/or cells are selectively vulnerable, but in an unpredictable fashion. The sites of damage are not always revealed by classical histologic methods. For example, early postnatal hyperbilirubinemia results in selective damage to the cerebellum. In contrast, a single dose of cadmium on postnatal day (PND) 5 ablates the neostriatum, whereas trimethyltin damages the hippocampus. On the other hand, MPTP produces no cytopathology that is detectable by traditional histological means (Nissl staining/H&E), although it causes degeneration of dopaminergic nerve terminals. The physiological and/or biochemical bases for these selective neurotoxic effects often is unknown and, therefore, sites of damage are unpredictable. The CNS is composed of a vast array of morphologically diverse cells types, which is reflected in its mRNA. Most (65%) low relative abundance mRNAs in the body are found only in the brain. The dilemma of understanding and predicting neurotoxicty is that the brain presents multiple targets and multiple mechanisms for toxicity. Furthermore, large numbers and classes of neurotoxicants exist, but common features of neurotoxicity are yet to be established.

Astrogliosis, often referred to as glial activation, reactive gliosis or simply gliosis, is detectable by immunohistochemistry of the astrocyte intermediate filament protein, glial fibrillary acidic protein (GFAP). Astrogliosis can be quantified by assaying GFAP or its mRNA. Neurotoxicologists should be interested in GFAP because astrocytes undergo hypertrophy when damage occurs and accumulate glial filaments, the main component of which is GFAP. Hypertrophy has been widely documented by measurements of GFAP in a variety of disease states. Reactive gliosis is characterized by hypertrophy of the astrocyte cell body and lengthening and thickening of processes. Glial activation is rapid, and occurs below levels detectable by histologic methods. Dr. O'Callaghan mapped out astroglial reactions following treatment with a variety of prototypic neurotoxic compounds that target different cellular and subcellular targets and that encompass different underlying mechanisms of damage. Most compounds were tested in adult experimental animals. All types of chemically induced damage were shown to result in glial activation, validating GFAP as a marker of neurodegeneration and implying a common injury signal.

The signaling mechanisms underlying the astroglial response to injury remain largely unknown, but progress is being made through molecular analyses, e.g. by use of inducible transgenic mice and the application of expression arrays to injury models. Although microglial activation precedes astrocytic activation, proinflammatory cytokines are not likely signals for reactive gliosis. However, the JAK–STAT signaling pathway has a known role in gliogenesis, in vitro, and this kinase module and its upstream effectors may play a role in the induction of gliosis, in vivo. Supporting this notion is the fact that STAT3 binding to the GFAP promoter mediates transcription. In vivo, the known dopaminergic neurotoxicant (MPTP) causes dose- and time-dependent activation of astroglia as reflected by assays of GFAP. Activation of STAT3 occurs prior to the induction of gliosis by MPTP. Thus, STAT3 may be a sensitive biomarker and potentially a mediator of astrogliosis.

The current dogma is that astrogliosis is a dominant response of the adult central nervous system to all types of injuries. Historically, astrogliosis has been viewed as a permanent response involving cell division and scarring. Furthermore, the prevailing view holds that astrogliosis is diminished or absent in the developing CNS. Contrary to this notion, Dr. O'Callaghan and others have used dose-, time- and region-dependent analysis of GFAP to show a robust astroglial response to diverse toxic insults of the developing nervous system. All of these responses were target appropriate and suggested that, as in the adult, astrogliosis is a characteristic response to neurological damage regardless of the nature or the site of damage. Furthermore, astrogliosis is time dependent and impermanent. Signaling mechanisms are likely to be shared between the adult and developing CNS. Assessments of reactive gliosis should be incorporated into future developmental neurotoxicity testing paradigms.

REACTIVE MICROGLIOSIS IN THE DEVELOPING BRAIN

The final talk in this session, entitled "Reactive Microgliosis in the Developing Nervous System", was given by Dr. Wolfgang Streit. In a scientific field dominated by astrocytes, microglia are experiencing a renaissance because of two major advances: they can be detected by specific histochemical methods and they can be cultured. Rio del Ortega coined the term "microglia" and devised a silver carbonate method for their detection. The method was so unreliable that it was abandoned for decades. Now, however, it is known that microglia are ubiquitous in the CNS, as numerous as neurons, and possessing a high degree of morphological plasticity. Microglia are the most immunocompetent cells of the CNS, capable of cytokine production, antigen presentation, and phagocytosis. They are the only cells in the CNS to express major histocompatability proteins. Furthermore, microglia have the potential for self-renewal and can proliferate, especially in response to neuronal signals. Dr. Streit showed an example of histochemical labeling of microglia with the *Griffonia simplicifolia* lectin conjugated to horse radish peroxidase allowing visualization. The lectin binds specifically to sugar residues of a glycoprotein in the cell membrane of microglia. This method labels microglia at all stages of development.

Microglial cells can be found early in the developing rodent nervous system at about mid-gestation (embryonic day 11) when the neuroepithelium is only a few cell layers thick and fetal macrophages invade the developing brain. As the primitive CNS matures and enlarges during the prenatal period, microglia become an integrated cellular component of the CNS microenvironment. By embryonic day 14, microglia have ramified processes. Most development of microglia occurs postnatally in rodents. At birth, microglial cells are abundant in the neonatal CNS, and some of them appear as rounded cells, commonly referred to as ameboid microglia. The common dogma is that microglia are the macrophages of the brain. This notion is

true in a sense in that microglia can become macrophages. However, resting microglia are not phagocytic, but have the potential to become phagocytic when neurons die. Microglia exist in three states that can be distinguished morphologically: resting microglia, activated or reactive microglia (which secrete cytokines, express MHC antigens, and proliferate) and phagocytic microglia (which are full-blown macrophages that appear with neurodegeneration).

Ameboid microglia form cell clusters in white matter regions in the postnatal CNS, particularly in the supraventricular corpus callosum (SVCC). The morphological resemblance of these ameboid microglia to macrophages has been highlighted in the literature and accordingly, ameboid microglia have been implicated in macrophage functions, including phagocytosis and production of proinflammatory cytokines, such as IL-1. However, studies from Dr. Streit's laboratory do not entirely support this view. Dr. Streit carried out a comparison of cultured microglia (which morphologically resemble brain macrophages) and ameboid microglia sampled from the SVCC with respect to their ability to secrete IL-1. He showed that while cultured microglia express high levels of mRNA for IL-1, microglia derived from the SVCC do not express detectable message for IL-1. Also, LPS/IF-1 stimulates microglia in culture to increase IL-1 expression. In vivo, IL-1 expression is undetectable in the developing brain and low in the adult brain.

Further evidence showed that while it is clear that microglia are engaged in phagocytosis, primarily of apoptotic bodies that are plentiful during postnatal development, these phagocytic microglia are not the same as the ameboid cells clustered in the SVCC. Instead, the microglia phagocytosing apoptotic cells are scattered throughout the CNS, and they do not have an ameboid morphology. An alternative possible role for ameboid microglia in the white matter of the developing brain is in axonal growth. Dr. Streit briefly presented work to show that transplantation of cultured microglia into a lesion cavity in the brain leads to enhanced neuronal growth, with many axons growing into the transplant. It is, thus possible that ameboid microglia might promote axonal growth and elongation.

RESEARCH NEEDS

The summary of Research Needs in Session VI for the Roles of Glia in Developmental Neurotoxicity:

1. Complete chronological fate of metals accumulated by astroglia (early versus late distribution in cell types).
2. Mechanisms of metal uptake, storage and release by astroglia.
3. Comparisons of differences in metal handling between immature and mature astroglia.
4. Interactions of non-physiologic toxic metals with essential metals.
5. Levels of alcohol affecting astroglial functions in the developing and mature brain.
6. In utero alcohol exposure and regulation of glutamate transporters in immature and mature astroglia.
7. Ethanol and calcium homeostasis in immature and mature astroglia.
8. Ethanol, neuron-glia-intersignaling and synaptic interaction.
9. Role of changes in osmolarity in vivo in long term effects of ethanol on the CNS.
10. Dynamics of astroglial cell volume change (prolongation, duration, regulation) as a result of daily binge alcohol.
11. Reactive gliosis as a biomarker in developmental neurotoxicity testing paradigms.
12. Analysis of glial function during developmental myelination compared to remyelination processes after neurotoxicant exposure.
13. Roles of microglia in post-injury and/or post-intoxication cellular repair.
14. Specific interactions of microglia with dying versus non-moribund injured neurons.
15. Validation of gene expression microarrays for mechanistic neurotoxicology and qualitative risk assessment.
16. Limits of adaptation and plasticity of the developing nervous system to neurotoxic insult.
17. Refinement of the concepts, advantages, and limitations of in vitro neurotoxicity models.