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NEUROIMMUNE MECHANISMS IN FETAL ALCOHOL SPECTRUM DISORDER

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Abstract

Fetal alcohol spectrum disorder (FASD) is a major health concern worldwide and results from maternal consumption of alcohol during pregnancy. It produces tremendous individual, social, and economic losses. This review will first summarize the structural, functional and behavior changes seen in FASD. The development of the neuroimmune system will be then be described with particular emphasis on the role of microglial cells in the normal regulation of homeostatic function in the central nervous system (CNS) including synaptic transmission. The impact of alcohol on the neuroimmune system in the developing CNS will be discussed in the context of several key immune molecules and signaling pathways involved in neuroimmune mechanisms that contribute to FASD. This review concludes with a summary of the development of early therapeutic approaches utilizing immunosuppressive drugs to target alcohol-induced pathologies. The significant role played by neuroimmune mechanisms in alcohol addiction and pathology provides a focus for future research aimed at understanding and treating the consequences of FASD.

Keywords

Microglia; CNS development; Fetal alcohol syndrome; Fetal alcohol spectrum disorders; Toll-like receptor

FETAL ALCOHOL SPECTRUM DISORDERS

A. Overview

FASD is a non-diagnostic umbrella term that encompasses a continuum of prenatal alcohol effects including the diagnostic conditions of fetal alcohol syndrome (FAS), partial FAS, alcohol-related neurodevelopmental disorder (ARND), and alcohol-related birth defects (ARBD). The impact on the individual is lifelong and includes a mild to severe spectrum of consequences beginning with low birth weight, growth retardation, birth defects, cognitive delay, and developmental delay (Riley and McGee, 2005). FAS produces the most serious consequences including persistent cognitive deficits, extensive developmental delays, major defects in CNS structure and function, musculoskeletal disabilities, and defects in other organ systems including visual, auditory, cardiac, and urogenital (Jones and Smith, 1973). The full array of CNS aspects of FAS can occur without physical defects as the disorder ARND. The lasting consequences of FASD include increased risk of major behavioral problems, psychiatric disorders, abuse and addiction to alcohol and other drugs, adverse encounters with law enforcement, and incarceration.

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The most devastating and lasting effects of prenatal alcohol exposure occur in the developing brain, causing an array of neuropsychological and behavioral consequences that is characteristic of FASD (Mattson et al., 2010). Cognitive impairments include deficits in intellectual performance, executive function, learning and memory, language, visual-spatial ability, motor function, attention, and activity levels (Mattson et al., 2011). Behavioral issues include increased rates of psychiatric disorders, substance abuse, adaptive dysfunction, academic and legal problems, and difficulty with communication, social interaction, and independent living. These neurological sequelae are present in individuals across the spectrum of FASD although the magnitude of the severity of the deficits varies with alcohol dose, pattern of exposure, and timing of exposure during gestation (Streissguth et al., 1989; Sood et al., 2001; Bailey et al., 2004; Guerri et al., 2009).

FASD is a major health problem worldwide as revealed by the epidemiology and economic impact (Olson et al., 2009; Popova et al., 2011; Riley et al., 2011). The prevalence of FAS in the U.S. is estimated at 2–7 per 1000 children and FASD prevalence may be as high as 2–5% of children (May and Gossage, 2001; Sokol et al., 2003; May et al., 2009). High prevalence of FAS and FASD have also been documented in other countries and can be particularly elevated in specific cultural groups (May et al., 2006; May et al., 2007; Petkovic and Barisic, 2010). The economic cost of FAS is daunting, with direct costs in the U.S. estimated at \$3.6–3.9 billion per year (Lupton et al., 2004; Olson et al., 2009; Popova et al., 2011). The cost of FASD to the national and international health care system and economy is astounding.

B. Structural and functional imaging in humans

The advent of various structural and functional imaging modalities has greatly increased our understanding of the teratogenic effects of prenatal ethanol exposure. Magnetic resonance imaging (MRI) studies indicate widespread structural brain abnormalities that result from maternal alcohol consumption during pregnancy (Astley et al., 2009; Lebel et al., 2011). The most consistently reported effect is a global reduction in total brain volume with additional region-specific reductions in volume (e.g., cerebral cortical, cerebellar, and various subcortical structures) that persist after correction for the reduction in total brain volume. The ethanol induced changes in cerebral cortex encompass all cortical regions with the frontal cortex being the most consistently affected while the occipital cortex is relatively spared (Lebel et al., 2011). For instance, ethanol exposure appears to reduce the overall size of the frontal cortex while increasing its thickness (Sowell et al., 2002; Sowell et al., 2008). Frontal cortex atrophy and polymicrogyria have also been reported (Riikonen et al., 1999; Reinhardt et al., 2010). The cerebellum is another structure that consistently exhibits a reduced volume, especially its anterior vermis (Sowell et al., 1996; O'Hare et al., 2005). Decreased volumes are also seen in various subcortical structures including the hippocampus, basal ganglia, amygdala, and thalamus (Lebel et al., 2011).

The effects on the major white matter tract of the brain, the corpus callosum, are likely representative of similar effects in other white matter tracts and range from partial or complete agenesis to structural changes in volume, length, thickness and displacement. In addition, diffusion tensor imaging (DTI) studies indicate that more subtle microstructural abnormalities occur throughout the corpus callosum especially in posterior regions of the isthmus and splenium that project to parietal and temporal cortices (Wozniak and Muetzel, 2011). Ethanol induced changes in the corpus callosum appear to correlate with disease severity and functional deficit. For example, displacement of the corpus callosum correlates with changes in verbal learning (Sowell et al., 2001), while changes in callosal size correlate with motor and executive functions (Bookstein et al., 2002; Roebuck et al., 2002). Functional magnetic resonance imaging (fMRI) reveals significant differences in regional brain activity when comparing ethanol exposed and age-matched control subjects (Coles and

Li, 2011). Prenatal exposure to ethanol results in a range of effects including specific changes in working memory, verbal paired associative learning, math processing, inhibitory control, and visual sustained attention. These changes include task-specific increases or decreases in regional brain activity. For instance, FASD patients exhibit increased and more widespread frontal cortical activation during the spatial memory task (Malisza et al., 2005; Spadoni et al., 2009), but decreased cortical activation during working memory facial recognition (Astley et al., 2009) and number processing (Santhanam et al., 2009) tasks compared to controls. The general conclusion from these imaging studies is that gestational exposure to ethanol results in widespread structural and functional changes that appear to be correlated across the spectrum of the severity of ethanol diagnosis.

C. Neuropathology revealed in rodent models of FASD

Animal models of FASD have been instrumental to establishing the neuropathology caused by prenatal alcohol exposure and correlating the neuropsychological and behavioral deficits in FASD individuals with structural and functional abnormalities. Important questions in the alcohol field have been most accurately approached with animal models including the involvement of dose-response relationships, threshold relationships, critical temporal periods, and exposure patterns. Equally significant is the recent use of animal models for preclinical testing of preventative and therapeutic strategies for FASD. These are the models that are being employed to define the impact of alcohol on the immune system in the developing CNS.

Rodents have been heavily investigated as effective models of gestational exposure. Exposure of pregnant mice to alcohol on gestational day 7 produces facial dysmorphism and structural brain defects similar to that found in children with FAS. Midline features of the face and forebrain are particularly affected at this early stage of development (O'Leary-Moore et al., 2011). MRI studies of mouse embryos exposed to alcohol on gestational day 7 demonstrate forebrain abnormalities in the ganglionic eminences, hypoplasia or agenesis of the corpus callosum, defects in development of the cerebral cortex, hippocampus, and basal ganglia, and enlargement of the lateral ventricle (Schambra et al., 1990; Godin et al., 2010). Exposure at later stages of development causes defects in development of midbrain or hindbrain structures (Parnell et al., 2009; O'Leary-Moore et al., 2010). For example, exposure on gestational day 8 produces apoptosis in the hindbrain and in cranial neural crest cells, enlargement of ventricles, cranial nerve defects with loss of ganglia and abnormal fiber tracts, and reduced hippocampal and cerebellar volume with the cerebellum being the most severely affected (Dunty et al., 2002). Exposure on gestational day 10 produces global loss of brain volume and enlargement of ventricles (O'Leary-Moore et al., 2010).

Fiber tract defects are present in both humans and animals following prenatal alcohol exposure. DTI examination of mice exposed to alcohol on gestational day 7 demonstrates defects in the internal and external capsule and the fimbria/fornix. Fibers abnormally project across the midline and in the superior/inferior direction in the midline. DTI consistently reveals minor to major defects in the corpus callosum of embryos exposed to alcohol during early gestation even in the absence of distinguishable facial dysmorphism (O'Leary-Moore et al., 2011).

The developing cerebellum and hippocampus are particularly vulnerable to alcohol neuropathology as revealed in both human studies as described above (Riikonen et al., 1999; Berman and Hannigan, 2000; Autti-Ramo et al., 2002; Willoughby et al., 2008) and rodent models (Barnes and Walker, 1981; Maier et al., 1999; Maier and West, 2001; Parnell et al., 2009). Purkinje cells are the most vulnerable neuron in the developing cerebellum (Pierce et al., 1989; Goodlett et al., 1990; Bonthius and West, 1991; Hamre and West, 1993). In the rat, for example, exposure to a single dose of ethanol on postnatal day 4 produced a rapid

wave of extensive apoptosis in Purkinje neurons that both initiates and completes within a few hours (Light et al., 2002).

The strikingly vulnerability of the immature cerebellum to alcohol pathogenesis occurs when alcohol exposure coincides with the critical period of Purkinje neuron development (Goodlett and Horn, 2001). This period is in the third trimester of human pregnancy, which corresponds to the first two postnatal weeks in the rodent. The temporal and regional-specificity of alcohol-induced neuronal loss is best characterized in the cerebellum where exposure during the period of postnatal days 4–6 routinely produces 30–70% Purkinje neuron loss in the anterior lobules I–IV and posterior lobules VIII–X of the vermis (Pierce et al., 1989; Goodlett et al., 1990; Bonthius and West, 1991; Hamre and West, 1993; Napper and West, 1995; Pierce et al., 1997; Maier et al., 1999; Light et al., 2002). In these studies, significant loss of cerebellar granule neurons was also reported but these cells are significantly more resistant to alcohol than the Purkinje neurons. A similar situation occurs in the hippocampus where granule cells of the dentate gyrus display temporal and absolute differences in their sensitivity to cell loss compared to the pyramidal cells in CA1 and CA3 regions. The specific results seen in the hippocampus depend on the paradigm employed for alcohol exposure (e.g., gestational, combined prenatal and postnatal, or postnatal exposure alone).

Gestational exposure to alcohol in the rat produces dramatic loss of neurons in the hippocampus when measured at approximately two months of age (Barnes and Walker, 1981; Perez et al., 1991). Loss occurs in all CA regions with reported decreases of approximately 20–45% in CA1 and approximately 30% in CA3. Comparatively, less loss of granule neurons is observed in the dentate gyrus at this age. However, following prenatal or prenatal plus postnatal alcohol exposure, a 10% loss of granule neurons was observed at three weeks of age accompanied by a similar reduction in CA1 pyramidal cells (Wigal and Amsel, 1990). In many studies in the hippocampus, rodents are treated with alcohol in the neonatal period to mimic maternal drinking during the third trimester. When given during this period, alcohol produces an approximately 10% loss of pyramidal neurons at three weeks of age and a 20–25% loss of both pyramidal and granule neurons at two months of age (Greene et al., 1992). CA1 pyramidal neuron loss but not granule neuron loss was found with postnatal alcohol exposure when analyzed at three months of age (Bonthius and West, 1991). Reduction in the size of neuronal populations in the hippocampus may reflect delay and/or inhibition of neurogenesis. In another study, both prenatal and postnatal exposure to alcohol in the rat reduced the number of pyramidal neurons in CA1 approximately 20% and postnatal exposure reduced the number of granule neurons (Miller, 1995). Analysis of neuronal precursor proliferation in the same study suggested that neurogenesis was delayed in the hippocampus due to alcohol exposure. Alcohol exposure potently inhibits hippocampal neurogenesis in adolescent and adult animals and similar mechanisms may be at play by analogy in the developing hippocampus. In all of these studies as well as others in the rodent hippocampus, the dose and timing of alcohol treatment markedly determines whether there is loss of pyramidal or granule neurons and the regionality and magnitude of the effect.

D. Behavioral measures correlate with neuropathology

FAS, the most severe disorder in FASD, is identified as a leading cause of mental retardation (Abel and Sokol, 1987; Pulsifer, 1996). Not all individuals with FAS have an intellectual disability as defined by an IQ below 70 with adaptive disability. However, neurocognitive deficits are present in individuals with FAS or ARND even in the absence of physical defects. The extent of the classical facial dysmorphology and growth retardation corresponds to the severity of intellectual impairment in children exposed to alcohol prenatally (Mattson et al., 1997; Ervalahti et al., 2007). Individuals with FAS, which

includes facial dysmorphology, have IQs of approximately 70 (Streissguth et al., 1991) and individuals with FASD without facial dysmorphology have IQs of approximately 80 (Mattson et al., 1997).

The consequences of damage to the hippocampus are easily observed in behavioral and neuropsychological correlates (Mattson et al., 2011). The hippocampal circuitry and functionality is crucial to learning and memory as well as visual-spatial abilities. Learning and memory deficits are often reported in children with FAS and ARND. Speech and language skills as well as visual and spatial perception and construction are also impaired in children exposed to alcohol during gestation.

Cerebellar structural and functional impairment is evident in the motor function problems commonly reported in children exposed to alcohol prenatally. In addition, damage to the basal ganglia (Archibald et al., 2001; Lebel et al., 2011) is evidenced as impairment of motor function. Both fine and gross motor skills are impaired by prenatal alcohol exposure, as observed in the earliest reports of FAS children (Jones and Smith, 1973) and many subsequent investigations.

There are marked deficits in mathematical abilities in individuals with FASD independent of their IQ level. In addition to lower overall mathematical academic achievement there are key insufficiencies in basic numerical processing skills. Interestingly, this neuropsychological feature may correlate with the abnormalities found in the brain of FASD children. Structural and function abnormalities exist in the parietal region and medial frontal gyrus (Santhanam et al., 2009; Lebel et al., 2010), regions central to mathematical processing.

Some of the disabilities characteristic of FASD have not been mapped to specific structural correlates. One arena in which individuals with prenatal alcohol exposure exhibit significant impairment is in executive function. Problems with planning, set shifting, fluency, response inhibition, and working memory are well documented (Mattson et al., 2011). The majority of children with FASD exhibit hyperactivity and attention deficits. Problems exist in vigilance, reaction time, information processing, and impulse inhibition (Mattson et al., 2011). Both internalizing and externalizing behavioral disorders occur in FASD. In addition, there are psychiatric disabilities in individuals with FASD that start as early as childhood including mood disturbance with negative affect and major depressive disorder. Structural and functional abnormalities in the cortex, hippocampus, and corpus callosum likely underlie several of these features of FASD. However, further work is needed to fine map these functional disabilities with specific structural correlates in individuals with prenatal alcohol exposure.

ROLE OF MICROGLIA IN THE DEVELOPING NEUROIMMUNE SYSTEM

A. Overview

Alcohol abuse has significant consequences for the individual and for society. The effects of alcohol on the peripheral immune system are complex. For example, acute alcohol consumption can have different effects than chronic consumption. A striking additional dichotomy exists in that chronic alcoholics are immunosuppressed and thus are at an increased risk of developing infections but chronic alcoholics often develop fibrotic or sclerotic livers with associated inflammation (Goral et al., 2008). Relative to the effects of alcohol on the peripheral immune system, little is known concerning alcohol effects on neuroimmune function in the CNS (Crews et al., 2006; Blanco and Guerri, 2007; Crews and Nixon, 2009). A common characteristic of alcohol abuse is CNS inflammation which is believed to contribute to the neurodegeneration and impaired neurological function observed in individuals who abuse alcohol. Recent studies also suggest an association between genes

that encode immune molecules and alcohol addiction, which has opened new avenues relevant to the understanding and potential treatment of addiction (Crews et al., 2011; Cui et al., 2011). Previous studies have investigated the effects of alcohol on neurons as well as non-neuronal cells, glia, including astrocytes and microglia. Most studies have focused on the effects of alcohol on microglia, the resident macrophage-like cells of the CNS. In animal models, as in humans, the effects of alcohol on the CNS in vivo and on microglia in culture are complex. Alcohol has been demonstrated to either increase or alternatively decrease neuroimmune activity. The effects of alcohol appear to vary dependent on a variety of factors including the dose of alcohol, the method of administration, and the cell or tissue under investigation. The effects of alcohol on microglia and the developing CNS will be summarized below.

Microglia normally maintain health and homeostasis in the CNS by, for example, producing neurotrophic factors and removing neurotoxins and thus protecting neurons. Microglia are also the primary immune cell of the CNS, and are critical in the removal of pathogens as well as clearing debris resulting from CNS injury. Microglia perform these functions following cell activation which results in morphological and functional changes. The activated microglia exhibit increased phagocytic activity and produce inflammatory molecules including nitric oxide (NO), cytokines, and chemokines. However, when microglia are chronically activated these same molecules can be toxic to host CNS cells and contribute to the pathology associated with neurodegenerative and neuroinflammatory disorders.

B. CNS Development: Role of Microglia

Controversy regarding the origin of microglia has existed for many years. It now appears clear that microglia are myeloid lineage cells of hematopoietic origin. These cells are initially produced in the fetal yolk sac and early during development migrate into the CNS (Saijo and Glass, 2011). Microglia express a number of markers in common with macrophages including CD14, CD11b, and EGF-like module containing mucin-like hormone receptor-like 1 (EMR1) and are commonly classified as CNS macrophages (Saijo and Glass, 2011). The myeloid origin of microglia is also supported by the fact that, like macrophages, microglial differentiation requires the transcription factor PU-1 and the growth factor colony stimulating factor-1 (CSF-1), and that microglia do not develop in PU. 1 deficient mice which were previously established to lack macrophages of the myeloid lineage (McKercher et al., 1996).

During early stages of development when microglia migrate into the CNS, these cells exhibit an ameboid morphology (Brockhaus et al., 1993; Haas et al., 1996). Later, microglia mature into a ramified morphology commonly exhibited in the healthy, mature CNS. The factors which mediate the change in microglia from an ameboid to a ramified appearance have not been well defined. Additional controversy has existed regarding how microglia are replenished after the initial movement of these cells into the CNS during early development. First, microglia are long-lived cells and additional microglia can be generated through proliferation of microglia or through microgliosis under pathological conditions. Second, microglia may also be generated from microglial precursors present in the CNS. Although a clear possibility, there is limited experimental data to support this mechanism of replenishing CNS microglia at this time. A third mechanism by which microglia could be replenished in the CNS is through transmigration of monocytes or macrophages from the periphery into the CNS followed by differentiation into microglia. Initial bone marrow chimera studies suggested that bone marrow derived monocytes were capable of migrating into the CNS of irradiated animals and subsequently forming microglia (Hickey and Kimura, 1988; Hickey, 1991; Eglitis and Mezey, 1997; Priller et al., 2001; Ransohoff, 2007). However, more recent studies using parabiosis in which the circulation of donor and

recipient organisms are directly connected indicate that in the absence of irradiation very few donor cells enter the CNS of recipient animals (Ajami et al., 2007; Mildner et al., 2007). Monocytic cells do enter the CNS in significant numbers upon development of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis, which is characterized by a compromised blood brain barrier. Although these monocytic cells are believed to contribute to the development of EAE they are not believed to differentiate into microglia (Ajami et al., 2011). Thus, peripheral monocytes or macrophages which enter the CNS are not believed to significantly replenish resident microglia.

C. Function of Microglia under Normal and Pathological Conditions

Approximately 5–20% of CNS cells are microglia. These cells are distributed throughout the CNS, although regional differences in the prevalence and morphology of the cells have been documented (Lawson et al., 1990). For example, grey matter contains more microglia than white matter (Rivest, 2009). Although the basis of regional differences in microglial density and morphology has not been defined, these differences may reflect regional differences in CNS function. Morphologically, microglia in the healthy CNS generally exhibit a small soma and finely branched processes that give the cell a ramified appearance (Ransohoff and Perry, 2009). Microglia generally occupy territories that do not overlap with adjacent microglia. Microglia perform a variety of functions critical to homeostasis and maintenance in the healthy brain. Multiphoton microscopy studies demonstrate that microglia are dynamic in vivo. These cells extend and retract their processes to sample the environment and perform their critical CNS surveillance function (Davalos et al., 2005; Nimmerjahn et al., 2005). Microglia respond by altering metabolism and gene expression in response to a variety of neurohormones, neurotransmitters, and neuromodulators (Kettenmann et al., 2011). Microglia also protect neurons through secretion of a variety of growth factors critical to survival of CNS cells including neurons and thus maintain the health of these cells.

Microglia play important roles in the establishment, structure, function, and activity of synapses throughout life. CNS development is associated with extensive synaptic plasticity. This includes the formation and remodeling of synapses which occurs during activity-dependent synaptic pruning (Tremblay et al., 2010; Zettel et al., 2010). The role of microglia in synaptic plasticity is supported by observations that microglia are physically associated with developing and mature synapses (Perry et al., 1985; Dalmau et al., 1998; Fiske and Brunjes, 2000). In addition, microglial processes associate with dendritic spines (Wake et al., 2009; Tremblay et al., 2010). In the developing visual system, microglia contribute to complement-mediated elimination of CNS synapses (Stevens et al., 2007; Schafer DP et al., 2010). The idea that synaptic pruning by microglia, which results in synapse elimination, may be a common event in CNS development is supported by studies demonstrating microglial phagocytosis of synaptic structures in various brain regions (Tremblay et al., 2010; Paolicelli et al., 2011). Furthermore, microglial derived neuroimmune molecules including tumor necrosis factor (TNF)- α , interleukin (IL)-6, major histocompatibility complex (MHC)-I, pentraxins, and complement proteins play a critical role in synaptic development and function (Boulanger, 2009). In addition, the chemokine receptor CX3CR1, which is expressed specifically by microglia, binds to CX3CL1, a chemokine present on neurons. Mice deficient in CX3CR1 exhibit fewer microglia, deficient synapse formation, and altered plasticity in the developing hippocampus (Paolicelli et al., 2011). Collectively, these studies indicate that microglial interaction with neurons modulates synapse development and plasticity.

In the healthy CNS, debris including apoptotic cells is phagocytosed by microglia (Sierra et al., 2010). Notably, this can occur without microglial activation and associated inflammation. However, in instances of CNS infection or pathology, microglia become

activated resulting in enhanced phagocytic activity and the removal of pathogens and debris associated with neuroinflammatory or neurodegenerative conditions.

D. Inflammatory Response of Microglia

The CNS has traditionally been described as an immunoprivileged site. This is based on the observations that a blood brain barrier exists which limits movement of peripheral immune cells into the CNS, that lymphatic drainage is lacking in the CNS, and that tissue grafted into the CNS exhibits prolonged survival. However, activated immune cells are readily capable of crossing the blood brain barrier into the parenchyma where they actively performing immune functions. Also, resident CNS cells including microglia and astrocytes are capable of performing immune functions in the CNS (Carson et al., 2006). As noted previously, microglia perform a variety of maintenance and homeostatic functions in the healthy CNS. However, in response to CNS infection, inflammation, or injury microglia become activated, change morphology from a ramified to an amoeboid appearance, and express proinflammatory molecules including cytokines such as IL-1 β , IL-6, and TNF- α , chemokines including MCP-1, Cox-2, and NO. These activated microglia also become more phagocytic, become more effective antigen presenting cells, become more motile, and become proliferative. In this capacity, microglia modulate innate immunity within the parenchyma of the CNS. Microglia can also modulate adaptive immunity in the CNS by altering T cell function. Thus, although microglia are normally protective in the CNS, upon activation these cells can contribute to CNS pathology (Ransohoff and Perry, 2009; Saijo and Glass, 2011).

Microglia, like other cells of the innate immune system, principally respond to pathogens through activation of toll-like receptors (TLRs) which recognize conserved motifs present on the surface of pathogens. In addition to pathogens, TLRs can recognize endogenous danger signals including heat shock protein 70 and high mobility group box 1 protein which can be released from sequestration under conditions including inflammation or trauma. Following ligand binding to TLRs, TLR signaling pathways are triggered and microglia become activated with resulting changes to an amoeboid morphology, increased phagocytosis, proliferation, and antigen presentation, and increased expression of proinflammatory molecules. Microglial activation does not occur in an all or none manner. Instead, microglial activation can occur along a continuum which may be associated with distinct functional states (Streit et al., 1999; Schwartz et al., 2006; Carson et al., 2007; Hanisch and Kettenmann, 2007; Perry et al., 2007; Colton, 2009). Microglia can also undergo distinct forms of activation, such as classical or alternative activation, in response to distinct stimuli. This occurs in a manner similar to that originally characterized in peripheral macrophage populations which are characterized by different protein expression patterns with distinct functions (Ransohoff and Perry, 2009).

Microglia revert back toward a ramified morphology following removal of inflammatory stimuli. These cells may not be fully deactivated, however, because there are clear indications that these cells are more susceptible or primed to subsequent activation (Hanisch and Kettenmann, 2007). Although the mechanisms resulting in microglial activation have been extensively investigated, little is known concerning the mechanisms that result in deactivation of microglia. Candidate molecules involved in the suppression of microglia include TREM2, cytokines including TGF- β and IL-10, as well as steroid hormones and other ligands that interact with nuclear receptors (Saijo and Glass, 2011).

THE LINK BETWEEN ALCOHOL CONSUMPTION AND INFLAMMATION

A. Human Studies

Previous studies demonstrate a clear link between alcohol consumption and CNS inflammation. Chronic alcoholics for example exhibit elevated serum levels of proinflammatory cytokines including IL-1 β , IL-6 IL-12, and TNF- α (Achur et al., 2010). Similarly, chronic alcohol consumption increases cytokine release from peripheral blood monocytes (Laso et al., 2007). Excessive alcohol consumption can result in liver pathology resulting in increased release of proinflammatory cytokines which enter the circulation. Hangover resulting from excessive alcohol consumption in humans is also associated with increased levels of cytokines in the circulation (Kim et al., 2003). Interestingly, these proinflammatory cytokines increase alcohol craving behavior which perpetuates the vicious cycle of alcohol abuse (Kiefer et al., 2002).

Studies utilizing human post mortem brain have been performed to analyze the effects of chronic alcohol consumption on gene expression. For example, changes in the expression of genes encoding immune molecules were demonstrated in the frontal cortex of alcoholic relative to non-alcoholic brains using microarray analysis (Liu et al., 2006). Expression of the transcription factor NF- κ B, which activates the expression of a variety of proinflammatory molecules, is elevated in the brains of alcoholics (Okvist et al., 2007; Yakovleva et al., 2011). Since NF- κ B expression is associated with synaptic plasticity as well as inflammation, these studies suggest that alcohol induced neuroinflammation may contribute to cognitive deficits common to alcoholics and possibly to alcohol dependence. In another study, MCP-1 levels were elevated 2- to 3-fold in the amygdala, hippocampus, substantia nigra, and ventral tegmental regions of alcoholic relative to control brains (He and Crews, 2008). Although the morphology of microglia was not altered in alcoholic brains, the levels of Iba-1 and Glut5 which are microglial specific markers were elevated, suggesting alcohol-induced changes in the microglia. A potential complication with this study which was raised by the authors was that smoking behavior was elevated in alcoholics relative to controls, and this potential confound could not be resolved due to a small sample size. Animal studies indicated that transgenic overexpression of MCP-1 alters synaptic transmission in the hippocampus (Nelson et al., 2011). These studies collectively suggest that NF- κ B and MCP-1 may alter synaptic plasticity in alcoholics.

Utilizing fetal cord blood and maternal blood isolated at birth, the effects of alcohol consumption on cytokine expression was evaluated (Ahluwalia et al., 2000). These studies demonstrated that IL-1 β , IL-6, and TNF- α were elevated in both neonatal and maternal blood in mothers who chronically consumed alcohol relative to those that moderately or did not consume alcohol. These studies did not distinguish fetal versus maternal expression of these cytokines, but clearly indicate that maternal consumption of alcohol increases cytokine exposure to the fetus.

B. Animal Studies

Fetal and neonatal rodent models of FASD have contributed extensively to our understanding of alcohol effects on the developing neuroimmune system. The neonatal rodent brain is similar developmentally to the third trimester human brain, a stage of high vulnerability to alcohol damage (Napper and West, 1995; Goodlett and Eilers, 1997; Pierce et al., 1997; Pierce et al., 1999; Clancy et al., 2001; Light et al., 2002). Recent studies from our laboratory indicated that exposure to alcohol on postnatal days 3–5 in the mouse results in loss of microglia and altered microglial phenotype in the developing cerebellum (Kane et al., 2011). Microglia that survived alcohol exposure exhibited altered morphology characterized by enlarged cell somas and shortened and less ramified processes. These

morphological changes suggest a partial activation of microglia and associated alterations in microglial function, which could result in developmental changes in the CNS. Given the repertoire of microglial activity in the developing CNS, the loss of microglia may be expected to have profound consequences. We hypothesize that alcohol effects on immature microglia may alter their developmental profile resulting in persistent changes in microglial function that last throughout life.

Alcohol-induced increases in cytokine expression in the neonatal rat brain can persist for extended periods following alcohol withdrawal (Tiwari and Chopra, 2011). For example, neonatal rats exposed to alcohol on postnatal days 7–9 exhibited persistent elevated expression of IL-1 β , TNF- α , and TGF- β in the hippocampus and cortex 19 days following withdrawal of alcohol. The levels of NF- κ B were also elevated in these brain regions. Persistent elevation of cytokine expression in response to alcohol may reflect the presence of primed microglia, as discussed previously.

Since alcohol treatment during the same developmental period results in neuron and glial loss (Napper and West, 1995; Goodlett and Eilers, 1997; Pierce et al., 1997; Pierce et al., 1999; Light et al., 2002; Kane et al., 2011) and deficits in cognition and behavior (Schneider et al.), it can be speculated that changes in the developing neuroimmune system contribute to the persistent neuropathologic CNS damage. Oxidative stress is known to contribute to neurodegeneration. Gestational or early neonatal exposure to alcohol has been demonstrated to increase oxidative stress and neuronal loss in rodents. For example, oxidative stress markers including superoxide dismutase, catalase, lipid peroxidation, reduced glutathione, NO, and acetylcholinesterase were elevated in the hippocampus and cerebral cortex of alcohol-treated animals (Tiwari and Chopra, 2011). These increases in oxidative stress occurred co-incidentally with increased cytokine expression and expression of the apoptotic marker caspase-3 in the same brain regions. In separate studies, a single exposure to alcohol on postnatal day 7 resulted in lipid peroxidation, caspase-3 activation and DNA fragmentation in the developing cerebellum (Kumar et al., 2011). Collectively, these studies suggest that oxidative stress plays a critical role in alcohol induced pathology in the developing CNS.

Recent studies indicate that fetal exposure to alcohol may alter the immune response to injury or insult in adulthood (DeVito and Stone, 2001). The offspring of pregnant rats exposed in utero to alcohol were maintained until adulthood and then inflicted with a stab wound to the brain parenchyma. Four days later, the expression of GFAP, TNF- α , and ICAM-1 was determined to be diminished in animals exposed in utero to alcohol relative to control animals. Interestingly, however, expression of ED1 and VCAM-1 were elevated in these animals suggesting activation of microglia or macrophages as well as endothelial cells. Although the effects of fetal exposure to alcohol on the adult innate immune system are complex, these studies collectively suggest that fetal alcohol exposure increases the vulnerability of adult CNS to injury and disease. Together with the knowledge that an interplay exists between neuroinflammatory molecules and alcohol drinking behavior, this suggests the possibility that early exposure to alcohol alters vulnerability to alcohol abuse in adolescents and adults.

Alcohol can be toxic to neurons. Alcohol exposure prenatally, for example, is toxic to cortical neurons, hippocampal neurons, hypothalamic neurons, and cerebellar Purkinje and granule cell neurons (De et al., 1994; Miller, 1995; Maier et al., 1999; Jacobs and Miller, 2001; Light et al., 2002; Moulder et al., 2002; Sarkar et al., 2007). Primary cell culture models have also been used to evaluate the effects of alcohol directly on neurons or indirectly on neuron viability through effects on microglia, utilizing microglia-neuron co-culture paradigms (Boyadjieva and Sarkar, 2010). In these studies, alcohol increased the

expression of the cytokines IL-6 and TNF- α and the chemokines MIP-1 and MIP-2 in primary microglial cultures. Conditioned medium from alcohol-treated microglia increased the apoptosis of cultured mediobasal hypothalamic neurons. TNF- α was demonstrated to be a critical molecule in mediating neuron apoptosis in these studies, as determined by immunoneutralization techniques. Thus, culture studies of purified and co-cultured microglia and neurons from developing rodent brain suggests that alcohol treatment activates microglial cells that, in turn, facilitates alcohol-induced neuronal apoptosis.

C. Signaling Pathways Mediating Inflammation

Microglia respond to pathogens as well as endogenous danger signals through activation of TLRs present on the surface of these cells. The role of individual TLRs in mediating alcohol effects on inflammation in the CNS have recently been investigated in astrocytes, another glial cell capable of immune activity. Alcohol increased the expression of the proinflammatory molecules Cox-2 and iNOS in primary astrocytes through mechanisms involving phosphorylation of p38 MAP kinase, ERK1/2, SAPK/JNK, and IRAK. Alcohol was further demonstrated to activate the proinflammatory transcription factors NF- κ B and AP1 in these cells. Neutralizing antibodies specific for IL-1R and TLR4 were shown to block alcohol effects on production of these proinflammatory molecules and activation of these signaling pathways, suggesting that IL-1R and TLR4 are important mediators of these alcohol effects in astrocytes (Blanco et al., 2005). Also in astrocytes, alcohol was demonstrated to induce Cox-2 and Src phosphorylation through TLR4 mediated mechanisms (Floreani et al., 2010). IL-1R and TLR4 were shown to move into lipid rafts in alcohol-treated astrocytes (Blanco et al., 2008). This suggests that alcohol induction of proinflammatory molecules occurs through a mechanism involving translocation of IL-1R and TLR4 into lipid rafts which are subsequently endocytosed leading to stimulation of signaling pathways that ultimately increase the production of inflammatory molecules.

The role of TLR4 in mediating alcohol effects on CNS inflammation have also been determined utilizing TLR4-deficient mice and these studies complement studies utilizing TLR4 neutralizing antibodies. Alcohol was shown to increase the production of cytokines, iNOS, Cox-2, and activate signaling pathways involving p38 MAP, ERK, and JNK in microglia derived from wild-type but not from TLR4 knockout mice. Microglial conditioned medium from alcohol treated wild-type mice increased primary cortical neuron apoptosis, while microglial conditioned medium from TLR4 knockout mice did not induce apoptosis, indicating that TLR4 signaling in microglia indirectly alters neuron viability (Fernandez-Lizarbe et al., 2009). Other studies demonstrated that in the medial frontal cortex of wild-type mice, alcohol increased the expression of the microglial marker CD11b and the astrocyte marker GFAP, and that expression of these molecules was suppressed in TLR4 knockout mice. In addition, alcohol induced the expression of iNOS, Cox-2, IL-1 β , IL-6, and TNF- α in a TLR4-dependent manner. Alcohol induction of these inflammatory molecules was likely mediated by NF- κ B since alcohol suppressed I- κ B and induced NF- κ B expression in the cortex of wild-type but not TLR4 knockout mice. Furthermore, the studies indicated that alcohol induced expression of the apoptosis marker caspase-3 in a TLR4-dependent manner (Alfonso-Loeches et al., 2010). Collectively, these studies indicate that TLR4 is a critical mediator of alcohol induced CNS inflammation and neurodegeneration.

Recent studies indicated that TLR4 deficient mice consumed less alcohol than wild-type mice and exhibited less alcohol-mediated cognitive and anxiety-associated behavioral impairment (Pascual et al., 2011). Together with the observations that TLR4 deficient mice exhibit less alcohol-induced CNS inflammation, these studies further support a link between TLR4 mediated alcohol induced neuroinflammation and cognitive and behavioral deficits. These studies also demonstrated that alcohol administered chronically decreased histone acetylation in the cortex, striatum, and hippocampus of wild-type but not TLR4 knockout

mice. This suggests that alcohol may induce epigenetic modifications of chromatin configuration resulting in cognitive and behavioral dysfunction, and that these modifications occur in a TLR4-dependent manner. In other studies, TLR4 deficient mice demonstrated reduced sedation and impaired motor activity in response to alcohol compared to wild-type animals. Alcohol pharmacokinetics were similar in wild-type and TLR4 deficient mice suggesting that other TLR4 mediated mechanisms were responsible for this altered behavior (Wu et al., 2011). When TLR4 siRNA was infused into the central nucleus of the amygdala, binge drinking behavior in alcohol preferring rats was inhibited. Additional studies indicated that TLR4 mediated alterations in drinking behavior is controlled by GABA_A $\alpha 2$ (Liu et al., 2011). Collectively, these studies support the hypothesis that TLR4 plays a critical role in alcohol induced CNS neuroinflammation and associated behavioral and cognitive effects of alcohol.

Ligand binding to TLR receptors can activate two distinct signaling pathways, the MyD88-dependent and the MyD88-independent pathways. TLR4 is capable of activating both of these signaling pathways. In the MyD88-dependent pathway, TLR4 interacts with the adaptor protein MyD88 which results in the downstream activation of IRAK1/4 and TRAF6. NF- κ B is subsequently activated in the MyD88-dependent signaling pathway. In the MyD88-independent, or TRIF pathway, TLR4 interacts with the adaptor protein TRIF leading to activation of the kinase IKK α 1. The MyD88-independent pathway ultimately results in the activation of two transcription factors, IRF-3 and NF- κ B. IRF-3 induces the expression of type I interferons and interferon-responsive genes. It is clear from the above studies that TLR4 plays a significant role in alcohol induced CNS neuroinflammation and behavioral consequences of alcohol, but few studies have addressed whether this occurs through MyD88-dependent or MyD88-independent pathways. Recent studies demonstrated that alcohol effects on sedation and motor impairment were suppressed in MyD88 knockout mice which suggests that the MyD88-dependent pathway is critical in controlling these alcohol-mediated effects (Wu et al., 2011). In other studies, alcohol was demonstrated to increase both NF- κ B and IRF-3 activation in microglia (Fernandez-Lizarbe et al., 2009), which suggests that these processes involve the MyD88-independent as well as possibly the MyD88-dependent pathways. Studies utilizing MyD88 and TRIF knockout mice will be required in the future to more completely assess the role of MyD88-dependent and MyD88-independent signaling in alcohol induced neuroinflammation and associated behavioral consequences.

THERAPEUTIC TARGETING OF NEUROIMMUNE PROCESSES

There is abundant literature indicating that alcohol induces neuroinflammation and that proinflammatory molecules contribute to alcohol addiction and pathologies associated with alcohol abuse. This suggests that immunosuppressive drugs may be effective in treating these pathologies and recent studies have begun to address these issues. We have previously demonstrated that PPAR- γ agonists are effective in suppressing CNS inflammation (Drew et al., 2008). Recently, utilizing a neonatal mouse model of FASD, we determined that PPAR- γ agonists protected both microglia and cerebellar neurons from the toxic effects of alcohol (Kane et al., 2011). PPAR- γ agonists were also recently demonstrated to suppress alcohol consumption in alcohol preferring rats (Stopponi et al., 2011). In a rat model of FASD, the polyphenolic compound resveratrol was shown to protect cerebellar neurons (Kumar et al., 2011). Also using a rat model of FASD, another study demonstrated that resveratrol suppressed alcohol activation of cytokines, oxidative stress, caspase-3 and NF- κ B in the hippocampus and cortex, and also protected against alcohol induced memory impairment (Tiwari and Chopra, 2011). Using an adolescent binge drinking model the non-steroidal anti-inflammatory drug indomethacin was demonstrated to suppress the production of the molecules iNOS and Cox-2 as well as suppress alcohol-induced behavioral deficits

including cognitive and motor deficits that persisted into adulthood (Pascual et al., 2007). Minocycline, a tetracycline antibiotic, was previously demonstrated to suppress immune responses and was recently demonstrated to also reduce alcohol consumption in mice (Agrawal et al., 2011). Minocycline also decreased alcohol-induced sedation, but interestingly increased motor impairment in mice. Collectively, these studies demonstrate the therapeutic potential of immunosuppressive compounds to prevent alcohol-induced pathologies.

CONCLUSION

FASD have a devastating effect on society and on the individual. Human imaging studies have revealed the presence of structural changes that occur throughout the CNS as well as neuropsychological and behavioral changes that contribute to the clinical diagnosis of FASD. Future studies will undoubtedly elaborate on the correlation between specific neuropathological changes in the brain and behavioral alterations. Animal models have been instrumental in defining the relationship between temporal exposure to alcohol during early stages of development and the sequelae of events that follow as the animal ages. The neuroimmune system is emerging as central player in alcohol pathology although the extent of our knowledge is still in its infancy. Microglia appear to be one of the most important players in this regard. These cells normally function in maintaining homeostasis within the CNS, however, if chronically activated they can cause damage to resident cells and tissues. The microglial response to alcohol is complex and involves the TLR signaling pathway. A more complete understanding of the signaling pathways involved in alcohol modulation of neuroimmune mechanisms in the CNS as well as the role of these molecules in modulating addiction to alcohol must be a focus of future research studies.

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