Chapter 7
Neuroimmune Effects of Developmental TCE Exposure

Sarah J. Blossom

Abstract Exposure to certain chemical, biological or physiological risk factors prior to adulthood can alter developmental processes and may in some instances enhance disease risk. This chapter will concentrate on the known effects of exposure to trichloroethylene (TCE) during gestation, lactation, and/or early life on the brain and immune system and discuss how this persistent environmental pollutant may impede immunologic and neurologic development to promote developmental pathology. Possible neuroimmune mechanisms and therapeutic interventions to circumvent the neurotoxic and adverse neurobehavioral effects of developmental TCE exposure are proposed.

Keywords Trichloroethylene • Neurotoxicity • Immunotoxicity • Oxidative stress • Developmental exposure • Locomotor behavior • $CD4^+$ T cells • Cerebellum • Hippocampus • Neuroimmune • Autoimmune-prone mice

7.1 Neurologic and Immunologic Sensitivity to Environmental Exposures During Developmental Periods

The effects of environmental toxicant exposures occurring during fetal development and early life has become an important research focus based on a fetus/child's unique exposure patterns. There is strong evidence to suggest that humans at early stages of development may be more susceptible to environmental exposures than

S.J. Blossom, PhD
Department of Pediatrics, University of Arkansas for Medical Sciences,
College of Medicine, Arkansas Children's Hospital Research Institute,
13 Children's Way, Little Rock, AR 72202, USA
e-mail: blossomsarah@uams.edu

K.M. Gilbert, S.J. Blossom (eds.), *Trichloroethylene: Toxicity and Health Risks*, Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6311-4_7,

adults. This differential sensitivity is due, in part, to the fact that key developmental processes (e.g., cellular maturation, differentiation and organ development) occur primarily during gestation and postnatally rather than during adulthood.

Humans develop in various stages spanning throughout gestation and postnatally. Human gestational development includes three general stages; peri-conception (2 weeks post-fertilization), embryogenesis (3–7 weeks post conception), and the fetal growth period (8–38 weeks gestation) (Fetal Growth and Development 2010). Postnatally, the neonatal period extends from birth to 1 month. Infancy begins at 1 month and continues to approximately 2 years of age. Childhood begins at 2 years of age and lasts until adolescence. The onset of the adolescent age is extremely variable but typically begins at around 12–13 years of age and ends with the beginning of adulthood (~18 years of age). Aging or senescence is characterized by changes in immunologic and neurological processes over time including a generalized decline in function and activity (McEwen and Morrison 2013; Wong and Goldstein 2013). Generally speaking, most major organ systems fully develop during embryogenesis. The heart, for example, is fully formed by 8 weeks gestation in humans (Bogin 1999). In contrast, the brain and immune system have extensive developmental growth periods that begin during gestation and continue postnatally well into childhood (Bayer et al. 1993; Dietert 2008). Therefore, this extended period of immunologic and neurologic development may increase the likelihood of negative effects due to toxic environmental exposures.

7.2 Developmental TCE Exposure

Most epidemiological studies of TCE toxicity have focused on adult occupational exposure since it is relatively easy to document and often involves relatively high level exposure. In humans the occupational 8 h exposure limit for TCE is 100 ppm or approximately 80 mg/kg/day (A.T.S.D.R.U.S 1995). Human exposure to TCE can occur at low levels in instances of environmental contamination. Aside from occupational exposure, the most common source of human exposure includes ingestion of contaminated drinking water (A.T.S.D.R.U.S 1995). Although TCE levels in water systems are generally monitored, TCE levels in private wells that comprise 10 % of US drinking water supply are often unknown. In addition, exposure to TCE may be elevated for people living near waste facilities where TCE is released, residents of urban or industrialized areas, or individuals using TCE-containing products.

Although adult exposure to TCE has received the most attention, human contact with TCE can occur at all stages of life. TCE and its metabolites can cross the placenta and reach the developing fetus. The United States Environmental Protection Agency has identified quantification of TCE in breast milk as a high priority need for risk assessment. Due to its lipophilic nature, TCE can accumulate in the breast milk (Pellizzari et al. 1982). It is possible that a nursing infant whose mother is exposed to the occupational exposure limit for TCE could receive greater than 80 %

of the daily limit advisable for lifetime exposure for adults (Fisher et al. 1997). In a recent study conducted in a TCE-contaminated area in Nogales, Arizona, TCE was detected in 35 % of the mothers' breast milk samples with the maximum concentration of 6 ng/ml (Beamer et al. 2012). Because TCE concentration in the breast milk was significantly correlated with the concentration in household water, TCE exposure is also a potential concern for bottle-fed infants who also ingest more water on a bodyweight basis than adults. In addition to infants, TCE exposure has been documented in school-aged children. The School Health Initiative: Environment, Learning, and Disease (SHIELD) study, studied school-age children from two inner-city schools in Minneapolis, MN. Samples obtained from the home as well as personal samples using organic vapor monitors attached to the clothes in the breathing zone of the child to detect TCE vapors reached the level of detection in approximately 7 % of subjects 6–10 years of age (Adgate et al. 2004; Sexton et al. 2005). Together these studies confirm that children are exposed to TCE at multiple levels during development.

In terms of functional consequences, studies of mothers exposed to TCE occupationally or in instances of industrial spills have documented increased adverse birth outcomes including low birth weight and cardiac defects (Forand et al. 2012). Although epidemiologic studies have typically focused on birth outcomes, other health effects not studied as extensively may manifest from maternal or early-life exposure.

7.3 Developmental Neurotoxicity of TCE

One system known to be vulnerable to environmental exposures during developmental periods is the central nervous system (CNS). While outside of the focus of this chapter, the development of the brain and its cellular components is a complex process that that extends across the lifespan. The CNS begins to develop during the early embryonic period and continues well into postnatal life. During the third trimester in humans the hippocampal region of the brain involved in learning and memory undergoes a dramatic increase in size and synaptic plasticity by the end of the second postnatal week (Dumas and Foster 1998; Dumas 2005). In the hippocampus, neuronal migration, cell proliferation, and synapse formation continue postnatally from birth through 3 years of age. The process of myelination that involves the development of cellular insulation around nerve fibers continues well into childhood (Rice and Barone 2000). Neurogenesis continues to occur throughout adulthood, albeit to a lesser degree as compared to early development (Semple et al. 2013). In humans, microglia, which are a group of monocyte-derived cells associated with immune and macrophage-like properties, colonize the brain as early as the mid-late trimester (Harry and Kraft 2012). This event corresponds to vascularization, neuronal migration, and myelination. Postnatally, microglia, as well as neuronal and glial cells, continue to disseminate and mature into all regions of the brain including cerebellum and hippocampus (Ponti et al. 2008). Taken together, the

dynamic nature and cellular plasticity of the brain throughout gestational and postnatal development and beyond is well established. This unique feature undoubtedly enhances its susceptibility to environmental influences to toxicants like TCE.

TCE was once used as an anesthetic at doses of around 2,000 ppm. Consequently, significant information is available on the acute neurotoxicity of high-level TCE exposure and its metabolites on the brain. A comprehensive assessment of adult neurotoxicity with occupational exposure to TCE in humans and acute, high-level doses in rodents was reviewed in the National Academy of Sciences document and will not be repeated here (Chiu et al. 2006). As far as human populations exposed to lower levels of TCE, one study reported that environmental TCE exposure through consumption of contaminated drinking water by residents living near the TCE-contaminated Rocky Mountain Arsenal Superfund site was associated with higher mean scores for depression, lower intelligence scores, and impaired memory recall, as compared to individuals who did not ingest contaminated water (Reif et al. 2003). Overall, less is known about chronic and/or lower dose exposures on the developing neurologic system (Laslo-Baker et al. 2004; Till et al. 2001a, b). One study found that subjects who were children at the time of TCE exposure by contaminated well water had enhanced cognitive deficits over subjects exposed as adults (White et al. 1997) More recently, studies have shown that children of mothers working with TCE who were exposed both gestationally and postnatally through lactational exposure had poorer visual acuity, as well as impaired motor coordination and behaviors characterized by inattention and hyperactivity (Laslo-Baker et al. 2004; Till et al. 2001a, b).

Experimental studies of developmental TCE-induced neurotoxicity in rodents have focused on adverse effects in the hippocampal region of the brain. In two reports, selective hippocampal damage was documented in rodents exposed developmentally to ~16–32 mg/kg/day of TCE via the drinking water. Both combined prenatal and neonatal, as well as neonatal-only exposure was associated with a decrease in myelinated fibers in the CA1 region of the hippocampus at weaning age (Isaacson et al. 1990). Other studies have reported significant changes in neuronal plasticity in hippocampal slices in vitro with TCE exposure (Altmann et al. 2002; Ohta et al. 2001). Although the exact nature of TCE's mode of action in the brain is not understood, studies in our lab found that TCE-induced alterations in metabolic pathways important in the control of oxidative stress and cellular methylation represent an important feature of developmental TCE-induced neurotoxicity (Blossom et al. 2008, 2012, 2013).

7.4 TCE and Neurologic Redox Imbalance and Oxidative Stress During Development

The cellular maturational processes that occur in the brain during gestation throughout early life increase the need for cellular oxygen, which can result in enhanced free radical and reactive oxygen species (ROS) production leading to an increased sensitivity to cellular damage and oxidative stress. To compensate for this

vulnerability, the brain utilizes mechanisms involving the glutathione system to restore redox balance and combat oxidative stress. The tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine) derived from the transsulfuration pathway functions as the major intracellular antioxidant against oxidative stress and plays an important role in the detoxification of reactive oxygen species (ROS) in the brain (Biswas et al. 2006; Jain et al. 1991). Additional insults such as pro-oxidant environmental exposures have the potential to enhance an already sensitive redox imbalance by decreasing the active form of glutathione (GSH) and increasing the inactive oxidized disulfide form (GSSG) leaving the cell vulnerable to oxidative damage.

Alterations in glutathione redox potential have been shown to modulate the fate of oligodendrocyte precursor cells and maturing cortical neurons in the fetus (Maffi et al. 2008; McLean et al. 2005). This suggests that altered brain redox status and increased oxidative stress resulting from pro-oxidant environmental exposures, including toxicant exposures, could hinder neural development and promote behavioral pathology. Therefore, maintenance of redox status by restorative glutathione levels in the brain is a critical protective mechanism during developmental periods where the brain is more vulnerable to oxidative stress. The clinical significance of these studies is underscored by the presence of altered redox regulation and oxidative stress biomarkers in patients with neurologic disorders including Parkinson's disease (Mythri et al. 2011) Alzheimer's disease (Butterfield et al. 2006) and autism (James et al. 2004; Sajdel-Sulkowska et al. 2011).

In an effort to determine whether TCE impairs glutathione redox imbalance and promotes oxidative stress during developmental periods, our laboratory conducted studies with the MRL+/+ strain of mice. MRL+/+ mice are "autoimmune-prone" but also develop several behavioral deficits and neuropathological changes with age and are considered to be a model of idiopathic neurological lupus (Sakic 2012; Kapadia et al. 2012; Marcinko et al. 2012). In addition, the MRL+/+ strain has been recently identified as a novel model to study hippocampal neurogenesis. MRL+/+ mice apparently display an enhanced response to pharmacologic agents that target neuroplasticity in the hippocampus over the response observed in non-autoimmune C57BL/6 mice (Balu et al. 2009; Hodes et al. 2010). Therefore, this strain of mice may represent a unique and relevant mouse model to examine the neurological impact of TCE exposure.

In the MRL+/+ mouse model, our lab demonstrated that exposure to TCE in the drinking water from birth (postnatal day 0) through early adulthood (postnatal day 42) caused decreased levels of glutathione and an increase in the reduced glutathione (GSH) to oxidized glutathione (GSSG) ratio in both hippocampus and cerebellum indicating cellular redox imbalance (Blossom et al. 2012, 2013). These metabolic changes were accompanied by alterations in the inter-related transmethylation pathway metabolites in the plasma. Figure 7.1 shows the folate-dependent interrelated methionine transmethylation and transsulfuration pathways involved in redox potential cellular methylation. Arrows in the figure demonstrate the effect of TCE (increased or decreased) on key pathway metabolites in plasma, hippocampus, and cerebellum. Also observed in cerebellum, but not hippocampus, was a global decrease in DNA methylation. This finding may implicate potential epigenetic

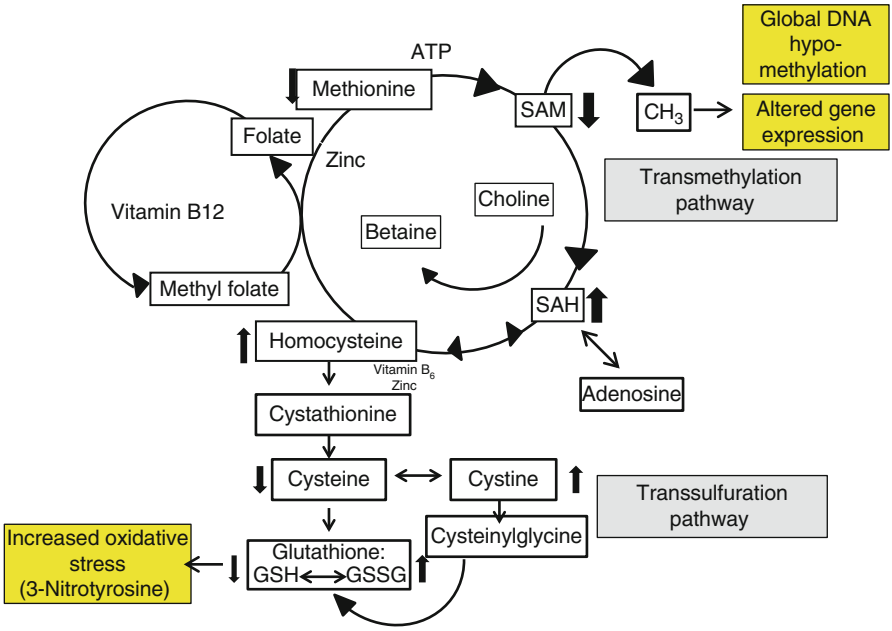

Fig. 7.1 Folate-dependent methionine transmethylation and transsulfuration pathways involved in redox potential and cellular methylation. Block arrows show the impact of postnatal exposure on the metabolite

mechanisms in TCE neurotoxicity. The decreased methionine observed with TCE exposure could indicate a decrease in methyl donors available for cellular methylation events which may have wide-ranging and long-term impacts on behavior.

7.5 Behavioral Changes Associated with Developmental TCE Neurotoxicity

Due to the observed TCE-related effects in cerebellum, a brain region functionally important for coordinating motor activity, including exploratory and social approach behaviors, we examined behavioral parameters using the EthoVision™ video tracking system from Noldus Information Technology (Leesburg, VA). MRL+/+ mice exposed to 28 mg/kg/day postnatally until 6 weeks of age showed significantly increased locomotor activity in the open-field test, as well as increased novelty/exploratory behavior in the novel object/novel mouse testing paradigm (Blossom et al. 2013). Studies by others found that unlike MRL+/+ mice, CD-1 mice exposed to much higher levels of TCE (2,000–8,000 ppm via inhalation) for 6 days in utero did not demonstrate decreased motor activity (Jones et al. 1996). However, TCE levels at this range reach doses that are associated with its anesthetic properties even though the authors did not report a decline in motor function as would be expected.

The discrepancy between the results of these studies and ours could be explained by a number of factors including route of exposure, developmental exposure period, duration of exposure, and strain differences. Thus, the presence of attention deficits and increased hyperactivity with gestational TCE exposure that has been reported in humans points to the relevance of the MRL+/+ model for studying TCE-induced developmental neurotoxicity (Laslo-Baker et al. 2004; Till et al. 2001a, b).

7.6 Effects of Early Postnatal TCE Exposure on Gene Expression in the Brain: Possible Role of Neuroprotective in the Control of Oxidative Stress

From a functional standpoint, redox imbalance, impaired methyl metabolism and epigenetic mechanisms could impact key cellular processes including gene expression in the brain. In particular, epigenetic mechanisms are important for the functional expression of neurotrophic genes (Branchi et al. 2011; Fuchikami et al. 2011; Roth et al. 2011). Changes in the expression of these genes can lead to impaired behavior (Chestnut et al. 2011; Lubin et al. 2008; Numata et al. 2012). Neurotrophic factors including Brain Derived Neurotrophic Factor (BDNF), Nerve Growth Factor (NGF), and Neurotrophin-3 (NT-3) are classically recognized as important mediators of neural growth and plasticity promoting neuronal survival and differentiation (Reichardt 2006). Emerging evidence suggests that neurotrophic factors can maintain control of inflammation in the brain by regulating glutathione redox status (Kapczinski et al. 2008; Sable et al. 2011; Wu et al. 2004). Developmental exposure to the solvent, toluene increased biomarkers of oxidative stress and decreased neurotrophic factors leading to neuroinflammation (Win-Shwe et al. 2010). Similar findings in offspring with mouse models of maternal infection have been demonstrated (Pang et al. 2010). Along this line, antioxidant therapy increased BDNF levels in hippocampus (Xu et al. 2011) and in neurodevelopmental disorders of the CNS, including autism, oxidative stress appear to be linked to the loss of neurotrophic support (Sajdel-Sulkowska et al. 2009, 2011). Thus, normal functioning of the brain appears to involve a positive feedback loop between anti-oxidant processes and neurotrophic expression and function in response to pro-oxidant exposures.

Our lab reported that hippocampal tissue from mice exposed to TCE postnatally expressed lower levels of key neurotrophic factors (e.g., BDNF, NGF, and NT-3) relative to controls confirming the experimental link between impaired redox status, increased oxidative stress with a decrease in neurotrophins observed in our model (Blossom et al. 2012). Based on these intriguing results, we extended our study to include an analysis of gene expression in cerebellum from TCE-treated MRL+/+ mice. We expanded the study to include functionally important gene families that might be impacted by TCE including chemokines/receptors, cytokines/receptors, astrocyte/microglial specific markers, and neurotrophins and receptors. Fluorescence-based quantitative real-time PCR (qRT-PCR) was conducted using methods previously described (Blossom et al. 2012). Gene expression changes in

Table 7.1 qRT-PCR was performed using hippocampus and cerebellum samples from 6 mice per treatment group collected at postnatal day 42

Gene family	Gene name	Hippocampus	Cerebellum
Chemokines	Chemokine (C-C motif) receptor 2 (*CCR2*)	NC	NC
	Chemokine (C-C motif) ligand 2 (*MCP-1*)	NC	NC
	Chemokine (C-C motif) ligand 3 (*MIP1-α)*	NC	NC
	Chemokine (C-C motif) ligand 4 (*MIP1-β)*	NC	2.7[b]
Astrocyte marker	Glial fibrillary acidic protein (*GFAP*)	−1.4[a] −1.7[b]	−2.1[b]
Microglial activation	Allograft inflammatory factor 1 (*Iba-1*)	−1.4[b]	−1.6[b]
Neurotrophins/receptors	Brain derived neurotrophic factor (*BDNF*)	−2.5[b,c]	−2.5[b]
	Neurotrophin 3 (*NTF3*)	−1.8[b,c]	NC
	Nerve growth factor (*NGF*)	−1.9[b,c]	NC
	Neurotrophic tyrosine kinase, receptor type I (*TrkA*)	NC	NC
	Neurotrophic tyrosine kinase receptor, type 2 (*TrkB*)	NC	NC
	Neurotrophic tyrosine kinase receptor, type 2 (*TrkC*)	−2.0[b]	−4.2[a] −3.5[b]

Numbers in the table represent fold change (increase or decrease) difference in gene expression
NC indicates no difference in gene expression between TCE and control mice. N compared with a standardized control sample
[a]Statistically different (0.01 mg/ml TCE vs. control)
[b]Statistically different (0.1 mg/ml TCE vs. Control)
[c]Some results have been published previously

both hippocampal and cerebellar tissues from individual mice (n = 6/treatment group) were compared among mice exposed to TCE (0, 2, or 28 mg/kg/day) from postnatal day 1–42. Interestingly, ~50 % of the genes evaluated in the hippocampus were significantly down regulated, as compared with no significant change, with the highest dose of TCE treatment relative to controls (Table 7.1). Expression of glial fibrillary acidic protein (GFAP), a marker associated with astrocyte differentiation was significantly decreased in hippocampal tissue isolated from TCE exposed mice (1.4-fold and 1.7-fold; 2 and 28 mg/kg/day, respectively). It is noteworthy to mention that neurotrophins play an important role in maturation of neurons and glial cells (Abe et al. 2010). Thus, the decrease in neurotrophic factors may represent a plausible explanation for the decreased expression GFAP. Further study to address this question is necessary in order to fully understand developmental neurotoxicity of TCE.

Similarly, in the cerebellum, ~41 % of the genes examined were significantly down regulated in TCE-treated mice (higher dose) relative to control as compared with no change relative to control values. TrkC, the receptor for the neurotrophin,

NGF, was also down regulated in the low TCE exposure groups. Collectively, our data supports an inverse association between increased oxidative stress and altered methyl metabolism with decreased expression of neurotrophic genes and their receptors. A positive correlation between increased oxidative stress and expression of proinflammatory markers would be expected. Studies to explore the proinflammatory cytokines expressed by cultured and activated microglial cells in mice developmentally exposed to TCE are currently underway in our laboratory, and could provide insight and possible mechanisms concerning the role of proinflammatory cytokines in TCE-induced neurotoxicity.

Opposed to all other genes tested, the highest dose of TCE significantly *increased* expression of MIP-1β, but not other important chemokines, relative to controls in the cerebellum. MIP-1β is a chemokine that is expressed in epithelial cells important in regulating traffic of recently activated peripheral T cells across the blood brain barrier (BBB) during inflammation. The functional implication of this finding is not known, but methylmercury exposure has been shown to selectively increase expression of MIP-1β, but not other chemokines, in the cerebellum of mice (Lee et al. 2012). It is possible that TCE and methylmercury alter a common pathway that increases the production of this chemokine in the cerebellum possibly leading to impaired blood brain barrier permeability and enhanced neuroinflammation and/or oxidative stress. This mechanism has not yet been tested in our model, but may represent a plausible mechanism, together with the decrease in neuroprotective factors, leading to effects observed following developmental TCE exposure. Collectively, based on our evidence, many of these neurologic events could represent an effect downstream of TCE's ability to promote immune hyperactivity following developmental exposure as demonstrated by our lab.

7.7 Increased Susceptibility of Developing Immune System to Toxicity

The role of developmental immunotoxicity in the etiology of childhood disease is becoming an important public health concern. The immune system has several well-characterized age-specific developmental stages. The major maturational events occurring during immune system development in humans includes (1) hematopoiesis (gestational week 8–10), (2) stem cell migration and cellular expansion (gestational week 10–16), (3) colonization of the bone marrow and thymus (gestational week 16-birth), (4) maturation to imunocompetence (birth to 1 year), and (5) establishment of immunologic memory (1–13 years) (Dietert 2008).

There is increasing evidence that the developing immune system is more sensitive to toxicant exposure than the adult immune system. More severe effects tend to occur at lower doses and often persist into adult life (Dietert and Piepenbrink 2006). Examples of the more commonly studied developmental suppressive immunotoxicants that induce more severe or persistent immune effects in offspring include the heavy metals (e.g., lead), polycyclic hydrocarbons (e.g., benzo [a] pyrene) and

polyhalogenated hydrocarbons (dioxin). A recent review compared early life vs. adult exposure to several immunosuppressive chemicals including lead and tributylin in animal models (Luebke et al. 2006). In all cases, sensitivity was greater if exposure occurred during development. In fact, immune suppression in developmentally exposed offspring often occurred at doses that did not alter adult immune responses.

The immune system's extended period of maturation may leave it especially vulnerable to environmental influences. Thus, in this way, the immune system is similar to the developing brain in terms of vulnerability to environmental insults. Developmental sensitivity to toxicants has also been demonstrated in humans. For example, prenatal exposure to polychlorinated biphenyls decreased the immune response to standard immunizations (Heilmann et al. 2010). Prenatal exposure to polybrominated diphenyl ethers produced a persistent decrease in lymphocyte numbers (Leijs et al. 2009). These studies focused on the ability of toxicants to promote immunologic hyporesponsiveness. Aside from immune suppression, there is increasing evidence that adult onset autoimmune disease can be triggered by pre- and early post-natal toxicant exposure (Colebatch and Edwards 2011; Langer 2010). Children continuously exposed for 3–19 years beginning *in utero* to a water supply contaminated with solvents (including TCE at levels reaching 267 ppb) had altered ratios of T cell subsets and early signs of tissue inflammation (Gist and Burg 1995). Human TCE exposure was associated with a proinflammatory IFN-γ $CD4^+$ T cell response in cord blood isolated from neonates (Lehmann et al. 2002). Thus, unlike the majority of immunotoxicants which tend to suppress the immune system, TCE promotes T cell hyperactivity and proinflammatory responses.

7.8 Immunotoxicity with Developmental TCE Exposure in MRL+/+ Mice

Our lab and others have conducted several studies concerning the immunostimulatory effects of TCE in MRL+/+ mice (Griffin et al. 2000a, b; Khan et al. 1995). Adult female MRL+/+ mice exposed to TCE (0.5 mg/ml) developed autoimmune hepatitis. This pathology was accompanied by expansion of activated ($CD62L^{lo}$) $CD4^+$ T cells that secreted increased levels of the proinflammatory cytokine, IFN-g. Based on the increased sensitivity to toxicants by the developing immune system, our lab used the MRL+/+ mouse model to examine the effects of continuous developmental and early life exposure (gestation through ~6–8 weeks of age) to a substantially lower dose of TCE.

Studying the impact of developmental exposure to different concentrations of TCE is a lengthy and complex process involving multiple breeding pairs. As a first step most likely to demonstrate efficacy, the effects of continuous (gestational throughout adulthood) TCE exposure was examined. This developmental exposure to TCE (126 mg/kg/day) calculated from maternal and direct water consumption increased the production of IFN-γ by $CD4^+$ T cells from the pups as early as

4 weeks of age (Blossom and Doss 2007). TCE exposure also impacted the thymus, the site of T cell development, as early as postnatal day 20, causing an increase in thymus cell numbers as well as an increase in the percentage of mature ($CD24^{lo}$) single-positive $CD4^+$ T cells indicating increased maturational events in the thymus. In a subsequent study, mice continuously exposed to a 5–25-fold lower, more environmentally-relevant dose of TCE showed similar thymus and $CD4^+$ T cell IFN-γ responses in 6 week old mice (Blossom et al. 2008). In addition, TCE enhanced $CD4^+$ T cell TNF-α production in these mice. TNF-α is an inflammatory cytokine secreted by activated T cells and macrophages that plays an important role in many pathological conditions including neurologic disorders. Together these findings suggest that a continuous developmental exposure alters the threshold (decreases the concentration or exposure-time) for TCE-induced T cell hyperactivity.

Other investigators reported that a continuous gestational and early-life exposure to 14,000 ppb TCE in the drinking water of *non-autoimmune* mice induced significantly increased T lymphocyte-mediated delayed-type hypersensitivity (DTH) responses, decreased antibody-mediated responses, and enhanced thymus cellularity in 8 week old mice (Peden-Adams et al. 2006). This group also reported that "life-time" exposure to TCE did not increase the level of anti-dsDNA antibodies in female MRL+/+ mice (Peden-Adams et al. 2008). Their assessment did not start until the mice were 4 months of age, however; a time point at which constitutive production of autoantibodies in untreated MRL+/+ mice can obscure a TCE-induced effect. In addition, since that study was confined to lupus-associated autoantibodies, the effects of lifetime TCE exposure on other types of disease (e.g. autoimmune hepatitis), are unknown.

7.9 Increased Susceptibility of Developing Brain to Neurotoxicity by Peripheral Immune Activation as a Mechanism for TCE's Effects in the Brain

There is emerging evidence that altered neuroimmune mechanisms might play a role in the development of certain neurologic disorders. The brain, once thought to be an immune privileged site, allows small molecules (e.g. cytokines) and lymphocyte trafficking in healthy individuals for immune surveillance during infection or immune responses to a CNS injury (Schwartz et al. 1999). This passage is tightly controlled and regulated by the blood brain barrier (BBB). The BBB provides diffusion restraint in order to control ionic gradients between blood and cerebrospinal fluid (Bito 1969). This restraint is provided by tight junctions located in the BBB interface. The BBB in the embryo, fetus, and newborn is believed to be immature and has been described as poorly formed, "leaky." or even absent (Siegenthaler et al. 2013). Thus a certain level of "cross talk" between the brain and the peripheral immune system occurs during both developmental periods and during adulthood.

During development, an emerging role for peripheral T cells in regulating normal neuronal differentiation and synaptic plasticity has been described (Ziv et al. 2006). In contrast to the positive effect of low level immune interaction in the brain, inflammatory conditions at sites outside of the CNS can lead to neurologic disorders. One of the best characterized peripheral inflammatory insults in this context is maternal and early-life infection. In humans, maternal infection has been linked to autism (Atladottir et al. 2012) attention deficit hyperactivity disorder (ADHD) (Mann and McDermott 2011) and adult-onset schizophrenia in the offspring (Anderson and Maes 2013; Khandaker et al. 2013). Several pieces of evidence in rodent models of linking maternal infection using live virus, viral mimics, the bacterial endotoxin, lipopolysaccharide, and selected inflammatory cytokines with adverse neurologic outcome in the offspring occurring later in life support this human evidence (reviewed in Meyer 2013). Mechanisms for these effects are currently being explored. However, recent evidence suggests that developmental LPS exposure alters neurotrophic factors leading neurobehavioral alterations similar to what is observed in our model (Xu et al. 2013a, b). Whether or not developmental exposure to environmental toxicants, like TCE, that promote immune hyperactivity mediate neurologic effects in a similar manner have not been examined.

7.10 Neuroimmune Impact of TCE and Implications for Neurodevelopmental Disorders

A mechanism involving the pro-inflammatory effect of TCE on the peripheral immune system during developmental periods may be an important consideration in the etiology for some neurologic disorders including autism and ADHD. One specific set of initiating or triggering events in these disorders may involve the immune system. Onore, et.al., indicated in a recent review that sufficient evidence was available to implicate altered immune responses in autism (Onore et al. 2012). There is plenty of supportive evidence of neuroinflammation and oxidative stress in the brains of autistic children involving a marked increase of the inflammatory chemokines together with reduced neurotrophic support (James et al. 2004; Sajdel-Sulkowska et al. 2009, 2011; Ashwood and Wakefield 2006). Thus, many of the characteristics observed in our mouse model of TCE exposure mirror what is observed in autism. One additional compelling link between our model and autism is the association of this disorder with autoimmunity with more than 40 % of autistic children having two or more first-degree family members with an autoimmune disease (Sweeten et al. 2003). The association between autism and parental autoimmunity was recently confirmed in a case-control study (Money et al. 1971). Serological evidence of autoimmunity in the form of anti-brain antibodies have been detected in both mothers of autistic children as well as in the children themselves (Braunschweig et al. 2013; Nordahl et al. 2013; Bauman et al. 2013; Fox et al. 2012). In terms of TCE and neurodevelopmental disorders, one epidemiologic study highlighted the possibility that maternal TCE exposure may be an

environmental risk factor for autism (Windham et al. 2006). This study reported increased incidence of autism in children living in areas with the highest quartile (25 %) of TCE in air using EPA HAPS data. Although this linkage needs to be confirmed by a larger more quantitative study, it raises an intriguing possibility that developmental TCE exposure may be a risk factor for the development of autism. We reported and increased exploratory and motor activity in developmentally exposed offspring (Blossom et al. 2013). At this time, studies to address the linkage between TCE exposure and ADHD in humans have not been conducted. The possibility of this association is underscored by reports in the literature showing hyperlocomotor and increased exploratory effects with perinatal alcohol exposure (Brady et al. 2012; Schneider et al. 2011). Both alcohol and TCE share an important metabolite, acetaldehyde. The system that transforms ethanol to acetaldehyde is even more robust in the perinatal rodent, and acetaldehyde itself is capable of enhancing motor activity (March et al. 2013). Thus, the presence of attention deficits and hyperactivity in association with developmental exposure to TCE needs to be studied further.

7.11 Neuroimmune Mechanisms and Future Directions

Collectively our findings demonstrate that developmental exposure to TCE promoted increased maturation of T cells in the thymus, T cell hyperactivity, and increased production of proinflammatory cytokines in association with neurobehavioral alterations. We observed increased locomotor activity and increased novelty/exploratory behavior with TCE exposure. These effects were associated with neural alterations in metabolites in the transsulfuration and transmethylation pathways indicating redox imbalance and altered methylation capacity (Blossom et al. 2008, 2012, 2013; Blossom and Doss 2007).

The neurologic effects of TCE could be a result of a direct effect of TCE and its metabolites in the brain. One potential mechanism may involve the activity of TCE's reactive metabolite trichloroacetaldehyde hydrate (TCAH). TCE is metabolized primarily by the cytochrome P-450 s isoform CYP2E1 to a trichloroethylene oxide intermediate, which spontaneously rearranges to form TCAH. TCAH is a highly reactive aldehyde that has been proposed to spontaneously condense with the biogenic amine tryptamine to produce an alkaloid-type neurotoxin (Bringmann and Hille 1990). Our lab has extensively studied the ability of TCAH to form adducts with T cells and promote their activation in vitro and in vivo (Blossom et al. 2004, 2007). The ability of reactive aldehydes (i.e., from ethanol metabolism) to inhibit methionine synthase activity and subsequently lower glutathione has been documented (Waly et al. 2004, 2011). Decreased methionine synthase activity would therefore result in an accumulation of SAH and inhibition of SAM, and a depletion of GSH similar to what is observed in our model. Therefore it is plausible to hypothesize that TCE, via TCAH, acts in a similar manner.

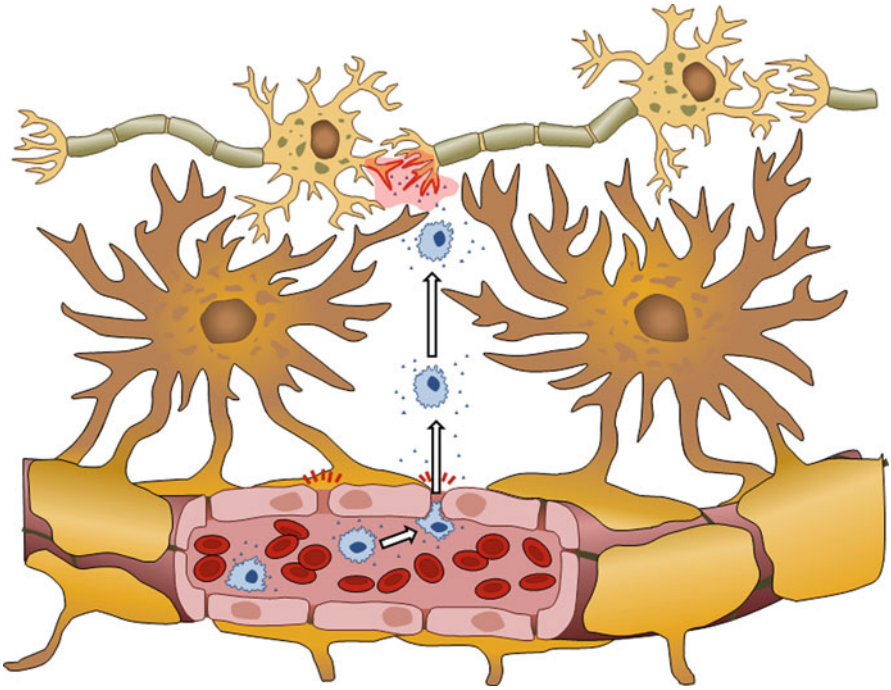

Fig. 7.2 Proposed mechanism. Activated CD4+ T cells from TCE-treated mice may cross a compromised blood brain barrier and promote inflammatory/oxidative stress which could dysregulate neuronal cells or astrocytes leading to adverse behavior. (Illustration courtesy of Mr. Dustyn A. Barnette)

One other attractive hypothesis that will be investigated further is that adverse neurologic and neurobehavioral effects may be secondary to the *early* effects of TCE on CD4+ T cells in early life following developmental exposure. We reported that TCE enhances thymic T cell maturation and $CD4^+$ T cell -oxidant activation (at postnatal day 20–28) in MRL+/+ mice. In contrast, the neurologic effects were only evident 6 weeks of age. It is therefore plausible that activated peripheral $CD4^+$ T cells and/or the cytokines they produce cross the BBB that may already be in a fragile state due to direct effects of TCE or metabolites or possibly by increased cerebellar MIP1β. The cytokines/cells cross the BBB to promote generalized inflammation and decrease the production of neurotrophins which leads to impaired redox status and methylation potential and increased oxidative stress resulting in abnormal behavior. The decrease in neurotrophic factors may also be a consequence of impaired DNA methylation based on our metabolic profile The role of peripheral T cells in adverse neurobehavior could be easily tested in $CD4^+$ T cell depleted mice. This possible scenario is depicted in Fig. 7.2.

Evidence to support our hypothesis is strengthened by emerging evidence that altered neuroimmune mechanisms might play a role in the development of certain neurologic disorders. The brain, once thought to be an immune privileged site, allows small molecules (e.g., cytokines) and $CD4^+$ T cell trafficking in healthy

individuals for immune surveillance during infection or immune responses to a CNS injury (Schwartz et al. 1999). This passage is tightly controlled and regulated by the blood brain barrier (BBB). The BBB provides diffusion restraint in order to control ionic gradients between blood and cerebrospinal fluid (Bito 1969). This restraint is provided by tight junctions located in the BBB interface. The BBB in the embryo, fetus, and newborn is immature and has been described as poorly formed, leaky, or even absent (Siegenthaler et al. 2013). Thus a certain level of so-called "cross-talk" between the brain and the peripheral immune system occurs during developmental periods in particular.

Pivotal work has shown that mice deprived of mature $CD4^+$ T cells (but not B cells or $CD8^+$ T cells) manifested hippocampal-dependent cognitive defects and behavioral abnormalities that were reversed by replenishing T cells (Kipnis et al. 2012; Marin and Kipnis 2013). A later study found that at the interface between the BBB, the epithelial layers of the choroid plexus are populated with $CD4^+$ T cell effector memory cells with a T cell receptor repertoire specific to CNS antigens (Baruch and Schwartz 2013; Baruch et al. 2013). This type of immunological control may be lost as a normal part of aging/senescence leading to cognitive decline. As far as development, an emerging role for peripheral $CD4^+$ T cells in regulating normal neuronal differentiation and synaptic plasticity has been described (Ziv et al. 2006). Despite these intriguing findings, the interactions between T cells and microglia and/or neurons in the brain and what this may mean in neurodevelopmental disorders where immunological function is abnormal remains a mystery.

In contrast to the positive benefit of low-level immune interaction in the brain, peripheral inflammation has been shown to contribute to the development of neurologic disorders. One of the best characterized peripheral inflammatory insults in this context is infection. In humans, maternal infection has been linked to ASD, ADHD, and adult onset schizophrenia in the offspring (Atladottir et al. 2012; Mann and McDermott 2011; Anderson and Maes 2013). Mechanisms for these effects are currently being explored in animal models (Meyer 2013). In humans, increased peripheral T cells in the brain of Alzheimer's patients have been detected (Liu et al. 2010). Whether developmental exposure to toxicants like TCE that promote $CD4^+$ T cell hyperactivity and mediate neurologic and adverse behavioral effects in a similar manner have not been examined.

Additional future experiments to address therapeutic strategies could involve experiments designed to implement a dietary intervention to circumvent the neurologic effects we observe in our model. Methyl-supplemented diets are designed to provide increased amounts of cofactors and methyl donors to support methyl metabolism. The diet will most likely include B12 and folic acid; essential nutrients and cofactors for the production of methyl groups, betaine; a methyl donor to regenerate methionine, choline; an essential nutrient and precursor of betaine, zinc; a cofactor for the mouse DNA methyltransferase and other key enzymes involved in DNA methylation. The diet will provide more methionine which can (via cysteine) increase glutathione production and through effects on SAM and SAH levels affecting DNA methylation (Melnyk et al. 2011; Mosharov et al. 2000; Vitvitsky et al. 2006). Because available data do not indicate that increasing methionine levels will

enhance glutathione levels sufficiently (Powell et al. 2010), N-Acetylcysteine (NAC) will be added to the special diet at previously described levels (Filosto et al. 2011; Conaway et al. 1998; Parachikova et al. 2010). NAC is a thiol anti-oxidant form of the amino acid cysteine and is used as a precursor of glutathione. These sets of experiments could potentially lead to novel therapies with real clinical value.

The literature reporting enhanced risk of neurodevelopmental disease after early-life insult to inflammatory insults is still evolving. We have used MRL+/+ mice to model these associations in the context of TCE exposure, and have demonstrated that these mice are sensitive to TCE's neuroimmune effects. Expanding this work to other strains of mice, including knockout mice, and other toxicants that may promote inflammation would truly further our understanding of how toxicant exposure and inflammation increases the risk of neurodevelopmental brain disorders.

References

A.T.S.D.R.U.S. Department of Health and Human Services: Center for Disease Control, Atlanta, Georgia. Agency for Toxic Substances and Disease Registry (1995) Toxicological profile for trichloroethylene. Update Draft for Public Comments. Ref Type: Report.

Abe M, Kimoto H, Eto R et al (2010) Postnatal development of neurons, interneurons and glial cells in the substantia nigra of mice. Cell Mol Neurobiol 30:917–928

Adgate JL, Eberly LE, Stroebel C et al (2004) Personal, indoor, and outdoor VOC exposures in a probability sample of children. J Expo Anal Environ Epidemiol 14(Suppl 1):S4–S13

Altmann L, Welge P, Mensing T et al (2002) Chronic exposure to trichloroethylene affects neuronal plasticity in rat hippocampal slices. Environ Toxicol Pharmacol 12:157–167

Anderson G, Maes M (2013) Schizophrenia: linking prenatal infection to cytokines, the tryptophan catabolite (TRYCAT) pathway, NMDA receptor hypofunction, neurodevelopment and neuroprogression. Prog Neuropsychopharmacol Biol Psychiatry 42:5–19

Ashwood P, Wakefield AJ (2006) Immune activation of peripheral blood and mucosal CD3+ lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms. J Neuroimmunol 173:126–134

Atladottir HO, Henriksen TB, Schendel DE et al (2012) Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. Pediatrics 130:e1447–e1454

Balu DT, Hodes GE, Anderson BT et al (2009) Enhanced sensitivity of the MRL/MpJ mouse to the neuroplastic and behavioral effects of chronic antidepressant treatments. Neuropsychopharmacology 34:1764–1773

Baruch K, Schwartz M (2013) CNS-specific T cells shape brain function via the choroid plexus. Brain Behav Immun

Baruch K, Ron-Harel N, Gal H et al (2013) CNS-specific immunity at the choroid plexus shifts toward destructive Th2 inflammation in brain aging. Proc Natl Acad Sci U S A 110:2264–2269

Bauman MD, Iosif AM, Ashwood P et al (2013) Maternal antibodies from mothers of children with autism alter brain growth and social behavior development in the rhesus monkey. Transl Psychiatry 3:e278

Bayer SA, Altman J, Russo RJ et al (1993) Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. Neurotoxicology 14:83–144

Beamer PI, Luik CE, Abrell L et al (2012) Correction to concentration of trichloroethylene in breast milk and household water from Nogales. Arizona Environ Sci Technol 46:11483

Biswas S, Chida AS, Rahman I (2006) Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem Pharmacol 71:551–564

Bito LZ (1969) Blood-brain barrier: evidence for active cation transport between blood and the extraceliular fluid of brain. Science 165:81–83
Blossom SJ, Doss JC (2007) Trichloroethylene alters central and peripheral immune function in autoimmune-prone MRL++ mice following continuous developmental and early life exposure. J Immunotoxicol 4:129–141
Blossom SJ, Pumford NR, Gilbert KM (2004) Activation and attenuation of apoptosis of CD4(+) T cells following in vivo exposure to two common environmental toxicants, trichloroacetaldehyde hydrate and trichloroacetic acid. J Autoimmun 23:211–220
Blossom SJ, Doss JC, Gilbert KM (2007) Chronic exposure to a trichloroethylene metabolite in autoimmune-prone MRL+/+ mice promotes immune modulation and alopecia. Toxicol Sci 95:401–411
Blossom SJ, Doss JC, Hennings LJ et al (2008) Developmental exposure to trichloroethylene promotes CD4(+) T cell differentiation and hyperactivity in association with oxidative stress and neurobehavioral deficits in MRL+/+ mice. Toxicol Appl Pharmacol
Blossom SJ, Melnyk S, Cooney CA et al (2012) Postnatal exposure to trichloroethylene alters glutathione redox homeostasis, methylation potential, and neurotrophin expression in the mouse hippocampus. Neurotoxicology 33:1518–1527
Blossom SJ, Cooney CA, Melnyk SB et al (2013) Metabolic changes and DNA hypomethylation in cerebellum are associated with behavioral alterations in mice exposed to trichloroethylene postnatally. Toxicol Appl Pharmacol 269:263–269
Bogin B (ed) (1999) Patterns of human growth, 2nd edn. Cambridge University Press, Cambridge
Brady ML, Allan AM, Caldwell KK (2012) A limited access mouse model of prenatal alcohol exposure that produces long-lasting deficits in hippocampal-dependent learning and memory. Alcohol Clin Exp Res 36:457–466
Branchi I, Karpova NN, D'Andrea I et al (2011) Epigenetic modifications induced by early enrichment are associated with changes in timing of induction of BDNF expression. Neurosci Lett 495:168–172
Braunschweig D, Krakowiak P, Duncanson P et al (2013) Autism-specific maternal autoantibodies recognize critical proteins in developing brain. Transl Psychiatry 3:e277
Bringmann G, Hille A (1990) Endogenous alkaloids in man, VII: 1-trichloromethyl-1,2,3,4-tetrahydro-beta-carboline–a potential chloral-derived indol alkaloid in man. Arch Pharm (Weinheim) 323:567–569
Butterfield DA, Perluigi M, Sultana R (2006) Oxidative stress in Alzheimer's disease brain: new insights from redox proteomics. Eur J Pharmacol 545:39–50
Chestnut BA, Chang Q, Price A et al (2011) Epigenetic regulation of motor neuron cell death through DNA methylation. J Neurosci 31:16619–16636
Chiu WA, Caldwell JC, Keshava N et al (2006) Key scientific issues in the health risk assessment of trichloroethylene. Environ Health Perspect 114:1445–1449
Colebatch AN, Edwards CJ (2011) The influence of early life factors on the risk of developing rheumatoid arthritis. Clin Exp Immunol 163:11–16
Conaway CC, Jiao D, Kelloff GJ et al (1998) Chemopreventive potential of fumaric acid, N-acetylcysteine, N-(4-hydroxyphenyl) retinamide and beta-carotene for tobacco-nitrosamine-induced lung tumors in A/J mice. Cancer Lett 124:85–93
Dietert RR (2008) Developmental immunotoxicology (DIT): windows of vulnerability, immune dysfunction and safety assessment. J Immunotoxicol 5:401–412
Dietert RR, Piepenbrink MS (2006) Perinatal immunotoxicity: why adult exposure assessment fails to predict risk. Environ Health Perspect 114:477–483
Dumas TC (2005) Late postnatal maturation of excitatory synaptic transmission permits adult-like expression of hippocampal-dependent behaviors. Hippocampus 15:562–578
Dumas TC, Foster TC (1998) GABA(b) receptors differentially regulate hippocampal CA1 excitatory synaptic transmission across postnatal development in the rat. Neurosci Lett 248: 138–140
Fetal Growth and Development (2010) Williams obstetrics, vol 23. McGraw-Hill, New York

Filosto S, Castillo S, Danielson A et al (2011) Neutral sphingomyelinase 2: a novel target in cigarette smoke-induced apoptosis and lung injury. Am J Respir Cell Mol Biol 44:350–360
Fisher J, Mahle D, Bankston L et al (1997) Lactational transfer of volatile chemicals in breast milk. Am Ind Hyg Assoc J 58:425–431
Forand SP, Lewis-Michl EL, Gomez MI (2012) Adverse birth outcomes and maternal exposure to trichloroethylene and tetrachloroethylene through soil vapor intrusion in New York State. Environ Health Perspect 120:616–621
Fox E, Amaral D, Van de Water J (2012) Maternal and fetal antibrain antibodies in development and disease. Dev Neurobiol 72:1327–1334
Fuchikami M, Morinobu S, Segawa M et al (2011) DNA methylation profiles of the brain-derived neurotrophic factor (BDNF) gene as a potent diagnostic biomarker in major depression. PLoS One 6:e23881
Gist GL, Burg JR (1995) Trichloroethylene–a review of the literature from a health effects perspective. Toxicol Ind Health 11:253–307
Griffin JM, Blossom SJ, Jackson SK et al (2000a) Trichloroethylene accelerates an autoimmune response by Th1 T cell activation in MRL +/+ mice. Immunopharmacology 46:123–137
Griffin JM, Gilbert KM, Lamps LW et al (2000b) CD4(+) T-cell activation and induction of autoimmune hepatitis following trichloroethylene treatment in MRL+/+ mice. Toxicol Sci 57:345–352
Harry GJ, Kraft AD (2012) Microglia in the developing brain: a potential target with lifetime effects. Neurotoxicology 33:191–206
Heilmann C, Budtz-Jorgensen E, Nielsen F et al (2010) Serum concentrations of antibodies against vaccine toxoids in children exposed perinatally to immunotoxicants. Environ Health Perspect 118:1434–1438
Hodes GE, Hill-Smith TE, Lucki I (2010) Fluoxetine treatment induces dose dependent alterations in depression associated behavior and neural plasticity in female mice. Neurosci Lett 484:12–16
Isaacson LG, Spohler SA, Taylor DH (1990) Trichloroethylene affects learning and decreases myelin in the rat hippocampus. Neurotoxicol Teratol 12:375–381
Jain A, Martensson J, Stole E et al (1991) Glutathione deficiency leads to mitochondrial damage in brain. Proc Natl Acad Sci U S A 88:1913–1917
James SJ, Cutler P, Melnyk S et al (2004) Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. Am J Clin Nutr 80:1611–1617
Jones HE, Kunko PM, Robinson SE et al (1996) Developmental consequences of intermittent and continuous prenatal exposure to 1,1,1-trichloroethane in mice. Pharmacol Biochem Behav 55:635–646
Kapadia M, Stanojcic M, Earls AM et al (2012) Altered olfactory function in the MRL model of CNS lupus. Behav Brain Res 234:303–311
Kapczinski F, Frey BN, Andreazza AC et al (2008) Increased oxidative stress as a mechanism for decreased BDNF levels in acute manic episodes. Rev Bras Psiquiatr 30:243–245
Khan MF, Kaphalia BS, Prabhakar BS et al (1995) Trichloroethene-induced autoimmune response in female MRL +/+ mice. Toxicol Appl Pharmacol 134:155–160
Khandaker GM, Zimbron J, Lewis G et al (2013) Prenatal maternal infection, neurodevelopment and adult schizophrenia: a systematic review of population-based studies. Psychol Med 43:239–257
Kipnis J, Gadani S, Derecki NC (2012) Pro-cognitive properties of T cells. Nat Rev Immunol 12:663–669
Langer P (2010) The impacts of organochlorines and other persistent pollutants on thyroid and metabolic health. Front Neuroendocrinol 31:497–518
Laslo-Baker D, Barrera M, Knittel-Keren D et al (2004) Child neurodevelopmental outcome and maternal occupational exposure to solvents. Arch Pediatr Adolesc Med 158:956–961
Lee JY, Hwang GW, Kim MS et al (2012) Methylmercury induces a brain-specific increase in chemokine CCL4 expression in mice. J Toxicol Sci 37:1279–1282
Lehmann I, Thoelke A, Rehwagen M et al (2002) The influence of maternal exposure to volatile organic compounds on the cytokine secretion profile of neonatal T cells. Environ Toxicol 17:203–210

Leijs MM, Koppe JG, Olie K et al (2009) Effects of dioxins, PCBs, and PBDEs on immunology and hematology in adolescents. Environ Sci Technol 43:7946–7951
Liu YJ, Guo DW, Tian L et al (2010) Peripheral T cells derived from Alzheimer's disease patients overexpress CXCR2 contributing to its transendothelial migration, which is microglial TNF-alpha-dependent. Neurobiol Aging 31:175–188
Lubin FD, Roth TL, Sweatt JD (2008) Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. J Neurosci 28:10576–10586
Luebke RW, Chen DH, Dietert R et al (2006) The comparative immunotoxicity of five selected compounds following developmental or adult exposure. J Toxicol Environ Health B Crit Rev 9:1–26
Maffi SK, Rathinam ML, Cherian PP et al (2008) Glutathione content as a potential mediator of the vulnerability of cultured fetal cortical neurons to ethanol-induced apoptosis. J Neurosci Res 86:1064–1076
Mann JR, McDermott S (2011) Are maternal genitourinary infection and pre-eclampsia associated with ADHD in school-aged children? J Atten Disord 15:667–673
March SM, Cullere ME, Abate P et al (2013) Acetaldehyde reinforcement and motor reactivity in newborns with or without a prenatal history of alcohol exposure. Front Behav Neurosci 7:69
Marcinko K, Parsons T, Lerch JP et al (2012) Effects of prolonged treatment with memantine in the MRL model of CNS lupus. Clin Exp Neuroimmunol 3:116–128
Marin I, Kipnis J (2013) Learning and memory … and the immune system. Learn Mem 20: 601–606
McEwen BS, Morrison JH (2013) The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course. Neuron 79:16–29
McLean CW, Mirochnitchenko O, Claus CP et al (2005) Overexpression of glutathione peroxidase protects immature murine neurons from oxidative stress. Dev Neurosci 27:169–175
Melnyk S, Fuchs GJ, Schulz E et al (2011) Metabolic imbalance associated with methylation dysregulation and oxidative damage in children with autism. J Autism Dev Disord.
Meyer U (2013) Prenatal Poly(I:C) exposure and other developmental immune activation models in rodent systems. Biol Psychiatry
Money J, Bobrow NA, Clarke FC (1971) Autism and autoimmune disease: a family study. J Autism Child Schizophr 1:146–160
Mosharov E, Cranford MR, Banerjee R (2000) The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes. Biochemistry 39:13005–13011
Mythri RB, Harish G, Dubey SK et al (2011) Glutamoyl diester of the dietary polyphenol curcumin offers improved protection against peroxynitrite-mediated nitrosative stress and damage of brain mitochondria in vitro: implications for Parkinson's disease. Mol Cell Biochem 347:135–143
Nordahl CW, Braunschweig D, Iosif AM et al (2013) Maternal autoantibodies are associated with abnormal brain enlargement in a subgroup of children with autism spectrum disorder. Brain Behav Immun 30:61–65
Numata S, Ye T, Hyde TM et al (2012) DNA methylation signatures in development and aging of the human prefrontal cortex. Am J Hum Genet 90:260–272
Ohta M, Saito T, Saito K et al (2001) Effect of trichloroethylene on spatiotemporal pattern of LTP in mouse hippocampal slices. Int J Neurosci 111:257–271
Onore C, Careaga M, Ashwood P (2012) The role of immune dysfunction in the pathophysiology of autism. Brain Behav Immun 26:383–392
Pang Y, Campbell L, Zheng B et al (2010) Lipopolysaccharide-activated microglia induce death of oligodendrocyte progenitor cells and impede their development. Neuroscience 166:464–475
Parachikova A, Green KN, Hendrix C et al (2010) Formulation of a medical food cocktail for Alzheimer's disease: beneficial effects on cognition and neuropathology in a mouse model of the disease. PLoS One 5:e14015
Peden-Adams MM, Eudaly JG, Heesemann LM et al (2006) Developmental immunotoxicity of trichloroethylene (TCE): studies in B6C3F1 mice. J Environ Sci Health A Tox Hazard Subst Environ Eng 41:249–271

Peden-Adams MM, Eudaly JG, Lee AM et al (2008) Lifetime exposure to trichloroethylene (TCE) does not accelerate autoimmune disease in MRL +/+ mice. J Environ Sci Health A Tox Hazard Subst Environ Eng 43:1402–1409

Pellizzari ED, Hartwell TD, Harris BS III et al (1982) Purgeable organic compounds in mother's milk. Bull Environ Contam Toxicol 28:322–328

Ponti G, Peretto P, Bonfanti L (2008) Genesis of neuronal and glial progenitors in the cerebellar cortex of peripuberal and adult rabbits. PLoS One 3:e2366

Powell CL, Bradford BU, Craig CP et al (2010) Mechanism for prevention of alcohol-induced liver injury by dietary methyl donors. Toxicol Sci 115:131–139

Reichardt LF (2006) Neurotrophin-regulated signalling pathways. Philos Trans R Soc Lond B Biol Sci 361:1545–1564

Reif JS, Burch JB, Nuckols JR et al (2003) Neurobehavioral effects of exposure to trichloroethylene through a municipal water supply. Environ Res 93:248–258

Rice D, Barone S Jr (2000) Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 108(Suppl 3):511–533

Roth TL, Zoladz PR, Sweatt JD et al (2011) Epigenetic modification of hippocampal Bdnf DNA in adult rats in an animal model of post-traumatic stress disorder. J Psychiatr Res 45:919–926

Sable P, Dangat K, Kale A et al (2011) Altered brain neurotrophins at birth: consequence of imbalance in maternal folic acid and vitamin B metabolism. Neuroscience 190:127–134

Sajdel-Sulkowska EM, Xu M, Koibuchi N (2009) Increase in cerebellar neurotrophin-3 and oxidative stress markers in Autism. Cerebellum

Sajdel-Sulkowska EM, Xu M, McGinnis W et al (2011) Brain region-specific changes in oxidative stress and neurotrophin levels in autism spectrum disorders (ASD). Cerebellum 10:43–48

Sakic B (2012) The MRL, model: an invaluable tool in studies of autoimmunity-brain interactions. Methods Mol Biol 934:277–299

Schneider ML, Moore CF, Adkins MM (2011) The effects of prenatal alcohol exposure on behavior: rodent and primate studies. Neuropsychol Rev 21:186–203

Schwartz M, Cohen I, Lazarov-Spiegler O et al (1999) The remedy may lie in ourselves: prospects for immune cell therapy in central nervous system protection and repair. J Mol Med 77: 713–717

Semple BD, Blomgren K, Gimlin K et al (2013) Brain development in rodents and humans: identifying benchmarks of maturation and vulnerability to injury across species. Prog Neurobiol 106–107:1–16

Sexton K, Adgate JL, Church TR et al (2005) Children's exposure to volatile organic compounds as determined by longitudinal measurements in blood. Environ Health Perspect 113:342–349

Siegenthaler JA, Sohet F, Daneman R (2013) 'Sealing off the CNS': cellular and molecular regulation of blood-brain barriergenesis. Curr Opin Neurobiol.

Sweeten TL, Bowyer SL, Posey DJ et al (2003) Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. Pediatrics 112:e420

Till C, Westall CA, Rovet JF et al (2001a) Effects of maternal occupational exposure to organic solvents on offspring visual functioning: a prospective controlled study. Teratology 64: 134–141

Till C, Koren G, Rovet JF (2001b) Prenatal exposure to organic solvents and child neurobehavioral performance. Neurotoxicol Teratol 23:235–245

Vitvitsky V, Thomas M, Ghorpade A et al (2006) A functional transsulfuration pathway in the brain links to glutathione homeostasis. J Biol Chem 281:35785–35793

Waly M, Olteanu H, Banerjee R et al (2004) Activation of methionine synthase by insulin-like growth factor-1 and dopamine: a target for neurodevelopmental toxins and thimerosal. Mol Psychiatry 9:358–370

Waly MI, Kharbanda KK, Deth RC (2011) Ethanol lowers glutathione in rat liver and brain and inhibits methionine synthase in a cobalamin-dependent manner. Alcohol Clin Exp Res 35:277–283

White RF, Feldman RG, Eviator II et al (1997) Hazardous waste and neurobehavioral effects: a developmental perspective. Environ Res 73:113–124

Windham GC, Zhang L, Gunier R et al (2006) Autism spectrum disorders in relation to distribution of hazardous air pollutants in the san Francisco bay area. Environ Health Perspect 114: 1438–1444

Win-Shwe TT, Tsukahara S, Yamamoto S et al (2010) Up-regulation of neurotrophin-related gene expression in mouse hippocampus following low-level toluene exposure. Neurotoxicology 31:85–93

Wong C, Goldstein DR (2013) Impact of aging on antigen presentation cell function of dendritic cells. Curr Opin Immunol 25(4):535–541

Wu A, Ying Z, Gomez-Pinilla F (2004) The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. Eur J Neurosci 19:1699–1707

Xu JX, Yang M, Deng KJ et al (2011) Antioxidant activities of Dracocephalum tanguticum maxim extract and its up-regulation on the expression of neurotrophic factors in a rat model of permanent focal cerebral ischemia. Am J Chin Med 39:65–81

Xu M, Sulkowski ZL, Parekh P et al (2013a) Effects of perinatal lipopolysaccharide (LPS) exposure on the developing rat brain; modeling the effect of maternal infection on the developing human CNS. Cerebellum 12:572–586

Xu M, Sajdel-Sulkowska EM, Iwasaki T et al (2013b) Aberrant cerebellar neurotrophin-3 expression induced by lipopolysaccharide exposure during brain development. Cerebellum 12:316–318

Ziv Y, Ron N, Butovsky O et al (2006) Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nat Neurosci 9:268–275