The role of the neuroimmune system in the pathogenesis of fetal alcohol spectrum disorders

Maria Sopena Rios

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Supervised by Olga Valverde Granados

Pompeu Fabra University

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SUMMARY

Fetal alcohol spectrum disorders (FASD) results from maternal consumption of alcohol during pregnancy. Yet preventable, it still is very prevalent as it is estimated that 50% of the women of childbearing age consume alcohol and therefore it represents a major health concern worldwide. Alcohol can have detrimental effects that can persist throughout life on various organs and systems being the central nervous system the most damaged. Mechanisms underlying FASD impairments are not fully understood, however many factors have been established as possible candidates Recent reports have highlighted the importance of the alcohol-induced neuroimmune function in the developing brain impairments. This review discusses the information available about the neuroimmune molecular mechanisms responsible for some of the neuropathological and cognitive outcomes seen in FASD.

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KEYWORDS: FASD, alcohol, immune system, brain, pregnancy

ABBREVIATIONS

AP-1: Activator protein 1; **ASD:** Autism spectrum disorder; **BBB:** Brain-blood barrier; **CCR2:** C-C chemokine receptor type 2; **CNS:** Central nervous system; **COX-2:** Ciclooxigenase; **CP:** Cerebral palsy; **DAMPs:** Damage-associated molecular pathways; **ERK:** Extracellular signal-regulated kinase; **FAS:** Fetal alcohol syndrome; **FASD:** Foetal alcohol spectrum disorder; **GABA-A:** Gamma-aminobutyric acid A; **GFAP:** G-protein-coupled receptor; **GSH:** Glutathione; **GSK3-β:** Glycogen synthase beta; **HMGB1:** High mobility group box I; **HPC:** Hippocampus; **IL:** Interleukin; **iNOS:** Inducible nitric oxide synthase; **IκBα:** Inhibitory kappa Bα; **L1:** Neural cell adhesion molecule L1; **LPS:** lipopolysaccharide; **LTP:** Long term potentiation; **MAG:** Myelin-associated glycoprotein; **MBP:** Myelin basic protein; **MCP-1:** Monocyte chemoattractant protein 1; **MyD88:** Myeloid differentiation factor; **NCAMs:** Neural cellular adhesion molecule; **NF-κB:** Nuclear factor-kappa B; **NLR:** NOD-like receptors; **NMDA:** N-methyl-D-aspartate receptor; **PAE:** Prenatal alcohol exposure; **PAMPs:** Pathogen-associated molecular pathways; **PDK-1:** Phosphoinositide-dependent kinase-1; **PFC:** Prefrontal cortex; **ROS:** Reactive oxygen species; **TLR4:** Toll-like receptors 4; **TNF-α:** Tumour necrosis factor alpha; **TRIF:** Receptor-domain-containing adapter-inducing interferon-β

1. INTRODUCTION

Alcohol is a teratogen (Streissguth et al., 1980) hence its abuse during pregnancy has been established as a risk factor for the normal embryonic development (Drew and Kane 2014). Indeed alcohol exposure during gestation can cause a wide range of long-lasting physical, behavioral, cognitive and/or learning impairments in the offspring (Harper 2009; Petrelli et al., 2017) referred to as fetal alcohol spectrum disorders (FASD) (Drew et al., 2014; Popova et al., 2017). FASD encompasses numerous outcomes, from mild to severe, being fetal alcohol syndrome (FAS) the most severe and visible one (Denny et al., 2017; Jones and Smith 1973).

The global prevalence of alcohol consumption during pregnancy is around 9.8% in the general population (Popova et al., 2017) and despite the efforts of Public Health institutions to reduce these levels, FASD is recognized to be a major public health problem (Warren et al., 2011) and it still has an estimated prevalence of 2-5% worldwide (Denny et al., 2017; May et al., 2018). Prevalence of FASD is not equal among countries, and, in Europe, it has been reported that about 25% of women drink alcohol during pregnancy acquiring a prevalence of 2.6 times higher than the world average (Popova et al., 2017).

In utero alcohol triggers a wide range of consequences with a variable severity among individuals because there are many factors involved. These factors include the gestational timing, dose, and duration (May et al., 2013), the amount of alcohol consumed per session as well as the pattern of drinking (Kleiber et al., 2014; Mayer and West 2001). Even gender differences have been reported, being females more vulnerable to the effects of alcohol (Goodlett and Peterson, 2013; Sickmann et al., 2013). Besides, several maternal factors such as age, nutrition, stress, socioeconomic status, genetics, and alcohol metabolism also modulate the risk of FASD (May et al., 2011, 2013). Some of these factors are further discussed to comprehend better this review:

- **Timing of drinking**. Although drinking at any point in pregnancy can be harmful to the fetus, the extent of this damage is affected by the ontogenic period when the ingestion occurs (Guerri et al., 2009; Maier and West, 2001) (Figure 1). There are critical stages of the development when the effects are more severe, for example, exposure to the early postnatal period in rodents (equivalent to the third trimester of gestation in humans) induces apoptotic cell death, cerebellar abnormalities and microcephaly (For a review, to see Saito et al., 2016). Furthermore, different types of

cell populations are also affected during specific stages of embryogenesis (Sadrian et al., 2014).

- **Dose of drinking.** It is known there is a relationship dose-response (Chernoff et al., 1977) and, when alcohol levels in the blood reach 80 mg/dL is considered intoxication and, therefore, higher alcohol concentrations from this threshold the more probability of causing fetal damage. However, little consensus is found on whether the intake of lower levels of alcohol during pregnancy is considered safe (May et al., 2011; Reynolds et al., 2019). While some authors demonstrated that even low levels of alcohol during pregnancy affect fetal neuroimmune function thus causing long-term consequences (Terasaki and Schwarz 2016), a recent review of Reynolds et al. (2019) concludes there is no evidence that low doses may harm the baby and they suggest that alcohol abstinence may be impractical as one-third of the pregnancies of the study are unplanned and women did not avoid alcohol during the first moths.

Prenatal alcohol induces some cellular mechanisms that, in the last instance, cause cellular damage. However, these cellular and molecular mechanisms are not fully understood and many factors are established as possible candidates. They include oxidative stress and free radical formation, growth factor dysfunctions, neuronal cell death, DNA damage, interactions to neurotrophic factors and cell adhesion molecules, and excitotoxicity (Alfonso-Loeches and Guerri, 2011; Harper and Matsumoto, 2005; Kruman et al., 2012). In addition to these mechanisms, in the recent years, many studies have reported the implication of the immune system on the neurotoxic effects of alcohol (For a review, to see Guerri and Pascual 2019). These results have been supported by *in vitro* studies and also experimental studies using animal models and clinical human studies, showing that alcohol activates the innate immune response and cause neuroinflammation and damage to the developing nervous system (Alfonso-Loeches et al., 2011; Crews and Vetreno, 2014; Montesinos et al., 2016a).

The aim of this review is therefore to discuss the current information available about the role of the neuroimmune system in the pathogenesis of fetal alcohol spectrum disorders.

2. THE ROLE OF IMMUNE SYSTEM

The immune system plays a key role in many events of normal brain development, such as neuronal migration and synaptogenesis (Marc et al., 2013). Indeed, dysregulation of the immune system during prenatal and early postnatal stages is demonstrated to be involved in the development of a wide range of disorders such as autism spectrum disorders (ASD) (Ashwood et al., 2011), schizophrenia (Penner et al., 2007), cerebral palsy (CP) (Dammann et al., 1997) and others (Knuesel et al., 2014). Moreover, there is a lot of evidence pointing that an imbalance between the expression of pro- and anti-inflammatory cytokines, at both central and peripheral levels, is related to the brain alterations occurring on these disorders (Hagberg et al., 2015; Sowell et al., 2018). These evidences are supported by the crucial role that cytokines play on brain development and function (Deverman and Patterson 2009). Under normal developmental conditions, proinflammatory cytokines have cytotoxic effects through the activation of microglia and macrophages that can induce cell loss and tissue damage, whereas anti-inflammatory cytokines are neuroprotective and initiate cellular repair by inhibiting the expression of proinflammatory cytokines (Boschen et al., 2016).

Moreover, while during normal pregnancy the maternal immune system is dampened to protect the fetus, it has been reported that alcohol induces an immune response, in both the mother and the fetus (Ahluwalia et al., 2000; Bodnar et al., 2018). Maternal immune system activation leads to an increase of cytokine production that can eventually cross the placenta (Aaltonen et al., 2005; Zaretsky et al., 2004). Then, cytokines can activate downstream molecules on the fetal compartment causing long-lasting consequences on the offspring (Bodnar et al., 2020; Terasaki et al., 2016).

Different neuroimmune mechanisms have been proposed as the cause of possible abnormalities observed in FASD including microglial activation (Saito et al., 2016) up/downregulation of cellular pathways (Blanco et al., 2010; Ren et al., 2019; Zhang et al., 2019), alterations on neuroinflammatory molecules such as cytokines and chemokines (Cantacorps et al., 2017; Mukherjee et al., 2019), oxidative stress and free radical formation (Ren et al., 2019) and cell adhesion molecules alteration (Noor et al., 2017; Sanchez et al., 2019), among others. These mechanisms are explained in the following paragraphs.

2.1.Glial activation

Several studies of animal models of FASD have shown prenatal alcohol exposure causes persistent microglial activation (Kane et al., 2012; Topper et al., 2015; Ren et al., 2017) that triggers acute or/and long-lasting neural damage (For a review, to see Saito et al., 2016) (Figure 2).

The persistent microglial activation is noticed by the change of its shape from a resting state to a partially active state, with a bigger cell body and thicker branches (amoeba-like shape) (Saito et al., 2015). Besides, markers of microglial activation, such as Iba-1, CD68, and PX7, have been found in the developing central nervous system (CNS) (Ren et al., 2019; Saito et al., 2015).

Microglial activation is accompanied by neuronal loss causing both necrosis and apoptosis (Ren et al., 2017; Topper et al., 2015). It is not entirely known, however, whether this neuronal loss is caused by microglial activation alone or this activation needs to be accompanied by other molecular events (Topper et al., 2015).

In addition, microglial activation has been linked to some of the long-term alcohol-related dysfunctions (Guerri et al., 2019; Noor et al., 2018). Indeed, some other studies reveal that the adverse effects of glial activation due to alcohol are reduced by anti-inflammatory molecules that block this activation (Drew et al., 2015; Kane et al., 2012). Other reports, however, have found opposite roles of alcohol-induced microglia on the prenatal brain because it seems that it may be protective because it phagocytes apoptotic neurons (Boyadjieva et al., 2010; Saito et al., 2016; Topper et al., 2015) especially in the cerebral cortex, hippocampus, and cerebellum (Boschen et al., 2016).

In addition to microglia, astrocytes are also stimulated by alcohol in the developing nervous system (Figure 2). They are thought to be activated by the release of molecules from the active microglia (TNF-α, IL-1β, and HMGB1) (Gadani et al., 2010) and they are involved in the synthesis of toxic mediators such as NO and ROS, chemokines, excitotoxic glutamate that compromise the brain-blood-barrier (BBB) (Karve et al., 2016). In this sense, Blanco et al. (2005) demonstrated that neurons incubated with astrocytes form rats prenatally exposed to alcohol showed a reduction of survival and synaptic plasticity. Moreover, the increased expression of active astrocytes, evaluated using the glial fibrillary acidic protein (GFAP) antibody (Sofroniew et al., 2010), have been found on specific areas of the neonatal brain, such as cerebellum and hippocampus of alcohol-exposed mice (Topper et al., 2015). The

sustained activation of astrocytes, as well as microglia, may result in neuronal cell loss (Lehnardt et al., 2010).

Although glial activation is clearly a consequence of alcohol exposure during developmental periods, it differs depending on the brain region assessed, the developmental period, and concentration and frequency of alcohol exposure. Further studies are required in order to understand these specific responses (Lacagnina et al., 2017; Topper et al., 2015).

2.2. Toll-like receptors

Innate immune signaling receptors, such as Toll-like receptors (TLR), are seen to be involved in the early perinatal brain damage (Hagberg et al., 2015). TLR activation can be through extracellular signals, such as pathogens (PAMPs) or intracellular signals, such as damaged cells (DAMPs) and it can stimulate several molecular pathways like nuclear factor NF-κB which triggers the release of cytokines and inflammatory mediators (Alfonso-Loeches et al., 2011; Mallard et al., 2012) (Figure 3). TLR4 is the most studied one and evidence points it causes brain injury during mid to late pregnancy and the early postnatal period (Hagberg et al., 2015).

The role of TLR4 on alcohol-induced neuroinflammation is widely studied in the adult and adolescent brain (Blanco et al., 2008; Yu et al., 2010). However, the information regarding Prenatal alcohol exposure (PAE)-induced neurotoxicity and TLR4 is limited as it is very recent (Pascual et al., 2017). In this sense, TLR4 is upregulated in embryonic, fetal and postnatal stages (Pascual et al., 2017) and their *in utero* activation through its ligand lipopolysaccharide (LPS) is linked with alterations of white matter (Duncan et al., 2002), increased microglial and macrophage activation, enhanced TNF-α expression (Lin et al., 2009) and alterations on pyramidal neurons of the hippocampus and prefrontal cortex in rats (Baharnoori et al., 2009). TLR4-KO mice are protected against alcohol-induced neuroinflammation (Pascual et al., 2017; Shukla et al., 2018). When TLR4 is activated, through alcohol or alarmins (e. g. HMGB1) (Alfonso-Loeches et al., 2011) it can induce the expression of many downstream molecules such as receptor-domain-containing adapter-inducing interferon-β (TRIF), myeloid differentiation factor 88 (MyD88), JNK, GSK3β, ERK causing neuroinflammation and neurotoxicity (Akira et al., 2004; Zhang et al., 2019).

Moreover, TLR4 dysregulation can lead to alterations of cortical myelin and synaptic proteins, such as HMGB1 (Topper et al., 2015) which has been shown to alter social

interaction in male mice through the activation of the candidate autistic genes (Ube3a, Gabrb3, Mecp2) (Shukla et al., 2018).

As happens with microglia, there are also opposite reports about the role of TLR4. Although there is strong evidence exposing the key role of TLR4 in the activation of the immune system after alcohol exposure, some recent studies are pointing out that prenatal alcohol dampens TLR4 response suggesting that there is a suppression of innate immune response instead (Muralidharan et al., 2018; Wang et al., 2019). These contradictory results, as it has been repetitively mentioned, could be due to the different experimental conditions, the animal model used, the pattern of drinking, etc. (Zhang et al., 2019).

2.3. NOD-like receptors

NOD-like receptors (NLR) are another group of receptors of the innate immune system and they have recently emerged as potential contributors to the alcohol-induced neuroimmune damage (Montesinos et al., 2016). As TLRs, NLR activation can be triggered by PAMPs and DAMPs (Jo et al., 2016) and there is a link between these two types of receptors. TLRs induce pro-IL1β that enables the recruitment of a complex called inflammasome. Among all the NLR-inflammasome complexes, NLRP3 is the most studied and its activation is related to oxidative stress and apoptosis (Guo et al., 2015) (Figure 3).

2.4.Cellular pathways

TLR4 activation results in the activation of two distinct signaling pathways, MyD88-dependant and MyD-independent. In MyD88-dependant, TLR4 by interacting with MyD88 triggers the activation of TRAF6 that leads to the induction of transcription factors NF-κB and AP-1. MyD88-independent or TRIF pathway, on the other hand, ultimately results in the activation of IRF-3 and NF-κB (Akira et al., 2004; Hagberg et al., 2015; Kawasaki et al., 2014). NF-κB and AP-1 have been linked to the expression of Inducible nitric oxide synthase (iNOS), ciclooxigenase (COX-2), IL-1β, IL-6, and tumor necrosis factor alpha (TNF-α) (Alfonso-Loeches et al., 2010) (Figure 3).

The induction of TLR4 is also related to the activation of other pathways such as glycogen synthase beta (GSK-β) that is dysregulated in many neurological disorders and plays a crucial role in neuroinflammation and microglial activation (Maixner et al., 2013). This protein is inactivated by the phosphorylation at Ser 9 (Golpich et al., 2015; Maixner et al., 2013) and alcohol exposure increases the concentration of its active form by decreasing its phosphorylation in a TLR4-dependent manner (Ren et al., 2019). This way, GSK-β

substrates, β-catenin, cAMP response element-binding protein CREB, and c-Jun, are also dysregulated (Golpich et al., 2015). CREB, for instance, is a neuroprotective component involved in the expression of plasticity-related genes that is inactivated by GSK-β. Therefore, if alcohol overactivates GSK-β, CREB activity decrease and it may cause some neurodevelopmental alterations (Qiao et al., 2018). c-Jun, on the other side, is involved in the synthesis of AP-1, a pro-inflammatory transcription factor of molecules like TNF-α and IL-6 (Zhao et al., 2016). In addition, GSK3-β creates a positive feedback loop with TLR4, since they promote each other, eventually causing an amplification of neuroinflammation and neurotoxicity (Zhang et al., 2019).

Alcohol is also related to the disruption of pro-survival signaling molecules, such as PDK1/AKT and Erk1/2 (Ren et al., 2019). PDK1/AKT is involved in cell growth and proliferation, differentiation, motility, and survival (Porta et al., 2014). In normal conditions, AKT, which is an upstream molecule of GSK3-β, phosphorylates GSK3-β thus inhibiting inflammatory pathways (Xu et al., 2017). However, alcohol inhibits AKT by reducing its phosphorylation, so there is no protection against neuroinflammation on the developing brain of PAE individuals (Ren et al., 2019).

Finally, some studies show the role of the chemokine system in alcohol neurotoxicity, particularly C-C chemokine receptor type 2 (CCR2) that participates in microglial activation. In this sense, animal models showed that the inactivation of its ligand, the monocyte chemoattractant protein-1 (MCP-1/CCL2) alleviates neurotoxic effects of prenatal alcohol exposure, such as microglial activation, alcohol-induced apoptosis and inflammation (Ren et al., 2017; Wang et al., 2019; Zhang et al., 2018).

Zhang et al. (2018) proposed a mechanism underlying the relation between MCP-1/CCR2, glial activation and neuroinflammation upon alcohol administration. There is an interaction between this pathway and some other key regulators of glial activation, such as GSK3-β, TLR4, or MAPKs (Jope et al., 2007). Indeed, it has been reported that blocking MCP-1/CCR2 signaling pathway attenuated the alcohol-mediated activation of MCP-1, GSK3-β and JNK (Ren et al., 2017; Wang et al., 2019; Zhang et al., 2018) therefore, they seem to be involved in alcohol-induced neurotoxicity (Zhang et al., 2018).

2.5. Cytokine networks

The disturbance of the immune system under prenatal alcohol exposure triggers, in the last instance, the release of cytokines. Normal concentrations of cytokines on the developing

brain are related to the myelination (Jakovcevski et al., 2009), synaptogenesis (Marc et al., 2013), and neurogenesis (Smith et al., 2007). However, a deviation in normal levels of cytokines has been shown to endanger normal brain development leading to lifelong consequences (Bodnar et al., 2016; Cai et al., 2000). This dysregulated neuronal immune response is associated with neuronal death and later-life cognitive impairment, nevertheless, the exact mechanism of the altered regulation and expression of these cytokines remains elusive (Feltham et al., 2020).

Many studies have been performed in order to elucidate the pattern of cytokine expression caused by prenatal alcohol exposure (Table 1). However, there is still no consensus regarding all the cytokines involved or whether they have a transplacental transfer (Bodnar et al., 2019; Dahlgren et al., 2006; Zaretsky et al., 2004). These discrepancies may likely depend on different factors: whether cytokines are found on the mother plasma or fetus (Aaltonen et al., 2005), the brain region assessed (Cantacorps et al., 2017) and gestational time of exposure (Jonakait, 2007), but also between animal models, given the case.

TNF-α, IL-1β, and IL-6 are the proinflammatory cytokines for which there are more evidence to be increased after prenatal alcohol exposure (Ahluwalia et al., 2000; Bodnar et al., 2016; Boschen et al., 2016; Cantacorps et al., 2017; Dahlgren et al., 2006; Holbrook et al., 2019; Sowell et al., 2018; Terasaki et al., 2006, 2016, 2017; Tiwari et al., 2011; Vink et al., 2006; Wang et al., 2019) (Table 1), the three of them are downstream molecules of the transcriptional factors NF-kB and AP-1 and they act directly on the induction of neuronal cell death (McCoy and Tansey, 2008; Simi et al., 2007). High levels of TNF-α are neurotoxic to the developing brain, disrupting the blood-brain barrier (Megyeri et al., 1992), and inducing apoptosis of oligodendrocytes and impairments in myelination (Cammer and Zhang, 1999; Pang et al. 2005). IL-1β, on the other side, seems to be necessary for the maintenance of long-term potentiation, whereas, its overexpression may also impair myelination (Pang et al., 2005) as well as learning and memory processes (Bilbo et al., 2012). IL-6 is secreted in response to both IL-1β and TNF-α and its increase *in vitro* was related to cell cycle withdrawal and the premature maturation of oligodendrocytes that contribute to the dysmyelination process (Hagberg et al., 2015).

Anti-inflammatory cytokines, mainly IL-10, are found to be downregulated during the third trimester of pregnancy instead. This imbalance in cytokine homeostasis increases the risk of having FASD pathologies (Sowell et al., 2018).

As previously mentioned, there are brain regions more affected for alcohol toxicity than others (e.g. PFC and hippocampus), therefore cytokine dysregulation is not homogeneous among all brain and overexpressed proinflammatory as well decreased anti-inflammatory cytokines are found on these regions mentioned (Bodnar et al., 2016; Boschen et al., 2016; Cantacorps et al., 2017; Terasaki et al., 2017).

Since there is not a consensus about cytokine expression in FASD, recent reports highlight the importance to evaluate cytokines as a network rather than the effects of individual cytokines (Bodnar et al., 2018, 2020). Cytokines operate in concert with each other to create a coordinated action rather than independently (Becher et al., 2017). Besides, establishing these cytokine patterns will allow us to elucidate biomarkers for the detection of neurodevelopmental disorders, not only FASD but also other pathologies such as schizophrenia or ASD.

Bodnar et al., (2018, 2020) have recently identified for the first time the cytokine milieu indicative of alcohol-related neurodevelopmental delay. They analyzed plasma samples of Ukrainian children with mothers reporting low/no alcohol consumption during pregnancy or moderate-to-heavy alcohol consumption. They also assessed neurodevelopmental impairments. Different cytokine clusters were found based on maternal consumption and/or child neurodevelopmental outcomes. The highest levels were found in alcohol-consuming mothers with children of neurodevelopmental delay and they vary between the second and third trimester. So these findings are a very important step for establishing cytokine patterns for alcohol-related and alcohol-independent neurodevelopmental delay (Bodnar et al., 2018, 2020).

2.7. Oxidative stress and apoptosis

Gestational or early post-natal alcohol exposure may cause oxidative stress and mitochondrial dysfunction that leads to the apoptosis of SN cells (Reddy et al., 2013; Watts et al., 2005; Ren et al., 2017).

Oxidative stress, in the form of superoxide and hydroperoxyl free radicals (ROS), is produced by enzymes such as COX-2, NADPH and nitric oxide synthase which have an increased activity (Guizzetti et al., 2014; Ren et al., 2019). ROS production results in protein and lipid peroxidation causing membrane disruption (Haorah et al., 2008; Tiwari and Chopra et al., 2011; Wang et al., 2009) and endoplasmic reticulum stress (ER) (Haorah et al., 2008; Ren et al., 2017).

Free radicals are usually neutralized by glutathione (GSH) and antioxidant enzymes, such as glutathione peroxidase, superoxide dismutase, and glutathione reductase (Haorah et al., 2008; Siler-Marsiglio et al. 2004). However, alcohol exposure induces GSH depletion and reduces antioxidant enzymatic activity (Ojeda et al., 2018; Guizzetti et al., 2014) thus causing an increased oxidative environment that ultimately leads to neuronal loss (Feltham et al., 2020). Besides, the developing brain is even more vulnerable to the toxic effects of oxidative stress since it has less antioxidant activity than the mature brain (Henderson et al., 1999).

Furthermore, ROS producing enzymes induce the activation of NF-κB (Kono et al., 2000) and it is suggested that this activation may be related to the induction of inflammatory molecules, such as IL-1β in a TLR4 dependent manner (Crew et al., 2006). Along with the activation of NADP oxidase, mROS also induces the activation of the complex NLRP3-inflammasome (Figure 3) that triggers the production of cytokines, such as IL-1β, by inducing caspase-1. This causes pyroptosis as well as an enhanced neuroimmune response (Alfonso-Loeches et al., 2014; Guerri et al., 2019).

Oxidative stress effects are not homogeneous through all CNS, but certain regions are more sensitive (Alfonso-Loeches et al., 2010; Henderson et al., 1999). Numerous reports showed increased levels of apoptotic markers after alcohol exposure, mainly caspase-3, in particular brain regions including the hippocampus, PFC (Cantacorps et al., 2017; 2018; Tiwari and Chopra 2011) and developing spinal cord (Ren et al., 2019).

2.8. Adhesion molecules

Cell adhesion molecules of the immunoglobulin superfamily play a crucial role in cell-cell interactions. In the brain, neural cell adhesion molecules, NCAM and L1, are basic for brain organization during development by participating in synaptic plasticity, cell migration, and morphogenesis (Miñana et al. 2000). Since alcohol is involved in the impairment of some of these developmental processes, cell adhesion molecules have also been studied as possible targets for alcohol neurotoxicity.

In utero expression of NCAM, on the one side, is found reduced under alcohol exposure. This is because, in order to properly fulfill its function, NCAM needs to be transferred to the plasma membrane. To do so, it first needs to be sialyzed in the Golgi apparatus by sialyltransferase enzymes (ST). Prenatal alcohol inhibits ST thus resulting in a reduction in the cell surface expression of PSA-NCAMs (Miñana et al., 2000).

L1, on the other side, is responsible for regulating cell-cell but also cell-matrix interaction. Alcohol interacts with L1 to inhibit the phosphorylation and dephosphorylation of a tyrosine (Ramanathan et al., 1996) and therefore it disrupts the L1-mediated cell-cell adhesion.

Finally, considering that NCAM and L1 molecules are involved in the plasticity of the CNS by regulating interactions, the impairment of its function by alcohol will lead to defects in brain developmental processes (For a review, to see Alfonso-Loeches et al., 2011).

3. NEURODEVELOPMENTAL AND NEUROBEHAVIOURAL EFFECTS OF PAE

FASD is an umbrella term used to describe a wide range of consequences of PAE, not only the more severe expressions of FAS but also some mild manifestations that occur in absence of physical malformations (e.g. decreased brain volumes and defects in the development of certain brain structures) but have cognitive and structural dysfunctions. Some of these include deficits across several domains, such as general intelligence, memory, language, attention, learning, visuospatial abilities, executive functioning, fine and gross motor skills, and social and adaptive functioning (Mattson et al., 1998).

As was previously explained, each brain region has critical sensitive periods to alcohol toxicity depending on the stage of pregnancy their development and maturation occurs (Sandrian et al., 2014). Consequently, the timing of ingestion is particularly influential toward the type of neurobehavioral pathology observed in both human FASD (O'Leary et al., 2010) and animal-modeled FASD (Maier et al., 1997).

In severe cases of FASD disruptions do not only occur on the developing brain but also in other systems, such as cardiovascular (Parkington et al., 2010; Cook et al., 2019), respiratory (Bodnar et al., 2020) and immune (Reid et al., 2019). Nevertheless, in this review, it will just be exposed the main outcomes related to neurodevelopmental, cognitive, and behavioral impairments, summarized in Table 2.

Some of these outcomes are related to some neuropathological mechanisms that are caused by alcohol-induced neuroimmune system activation. These mechanisms include myelin and BBB disruption as well as alterations in synaptic plasticity and early neurodegeneration (Cantacorps et al., 2017) and they will be discussed in the following questions. However, not all the alterations mentioned above have been related to neuroimmune activation (Cantacorps et al., 2017), since more studies need to be carried out in order to elucidate the whole process of neuroimmune damage.

3.1. Myelin impairments

Abnormalities in myelin structure and function have been reported during brain development since exposure in the neonatal stage, when myelination takes place, reduces the number of mature oligodendrocytes and the proliferating oligodendrocyte progenitor cells (Newville et al., 2017) (Figure 2). In fact, myelin impairments have been reported to be involved in some of the FASD outcomes, including cognitive impairments and deficient neuronal transmission (For a review, to see Guizzetti et al., 2014).

Myelin and oligodendrocytes are targets of proinflammatory cytokines induced by alcohol-exposure (Cammer and Zhang, 1999; Pang et al., 2005; Sowell et al., 2008). Moreover, TLR4-induced neuroinflammation has been linked to myelin disruption in the adult and adolescent brain (Alfonso-Loeches et al., 2012; Montesinos et al., 2015) suggesting this mechanism can also be involved on FASD (Cantacorps et al., 2017). The protein composition of myelin is also modified because of the persistent neuroimmune activation. Myelin basic protein (MBP) or myelin-associated glycoprotein (MAG), critical elements of the myelin sheath, are delayed and reduced in their expression following postnatal alcohol exposure in the cerebellum (Zoeller et al., 1994), PFC and HPC (Cantacorps et al., 2017).

3.2. Blood-brain barrier disruption

BBB is sensitive to circulating cytokines, released by active microglia, and oxidative stress occurring under neuroinflammatory conditions (Alvarez et al., 2013) and therefore, its integrity can be shaped allowing leukocyte migration into CNS (Alfonso-Loeches et al., 2011). Although there are some reports pointing in this direction (DeVito et al., 2001), there is no clear evidence confirming that PAE alone leads to BBB disruption or other factors are needed (Noor et al. 2018). Noor et al. (2018) propose that PAE can lead to a low-level glial reactivity that allow the BBB to reach a threshold of permeability easier, thus facilitating leukocyte trafficking. Nevertheless, it is important to point that astrocytes and pericytes are heterogeneous in different regions, hence the structure and function of the neurovascular unit of the barrier may be affected differently in each region of CNS (Kane et al., 2014, Topper et al., 2015).

3.3. Long-term potentiation alterations

LTP is one of the most studied models to understand synaptic plasticity (Prieto and Cotman 2017) and, apart from the classic neuromodulators (e. g. norepinephrine, dopamine, and acetylcholine), cytokines are also involved on this process (Vezzani and Viviani, 2015),

hence a dysregulation of their expression may be related to changes on synaptic plasticity (Yirmiya and Goshen 2011; Prieto and Cotman 2017). For instance, TNF-α induces the insertion of NMDA receptors in the hippocampus (Wheeler et al., 2009) and promotes the endocytosis of GABA-A receptors by decreasing the inhibitory strength and reinforcing excitability (Pascual et al., 2017; Stellwagen et al., 2005). IL-1β is also involved in LTP since it modulates the exocytosis of several neurotransmitters (Zhu et al., 2006) and enhances NMDA-mediated calcium influx enhancing the excitotoxicity (Viviani et al., 2003). However, excessive levels of TNF-α, IL-8, and IL-6 results in synaptic plasticity dysfunction (Huang et al., 2013).

Microglia have also been related to LTP since its transient loss resulted in synaptic pruning impairments related to weak synaptic transmission in animal models (Rogers et al., 2011; Zhan et al., 2014). In addition, the deletion of chemokine receptor CXCR1 demonstrated impaired hippocampal synaptic plasticity (Kane et al., 2014; Rogers et al. 2011).

Finally, as was previously mentioned, a lack of activation of CREB leads to the reduced expression of the plasticity-related genes that produce disruption of the normal process of synaptic plasticity (Pandey et al., 2001). This has been supported by several animal models showing less expression of genes such as c-Jun and c-Fos in the hippocampus or PFC after ethanol exposure (Clements et al. 2005).

3.4 Neurodegeneration

Alcohol-induced neurodegeneration is another neuropathological mechanism related to alcohol exposure. Crews et al., (2006, 2009) suggest that alcohol-induced neurodegeneration is related to oxidative stress through the activation of ROS-producing enzymes, microglial activation and pro-inflammatory molecules (e.g. NF-κB) that eventually cause apoptosis and even necrosis in the brain (Crews and Nixon, 2009). In addition, active glia affects de cytoarchitecture in the mice developing brain with the induction of deficits in GABAergic neurons (Creeley et al., 2013) as well as a reduction of Purkinje cells in the cerebellum (Li et al., 2015; Topper et al., 2015). In addition, blocking TLR4-MyD88 pathway in mice have been shown to mitigate the cortical neuronal loss and motor impairments (Wu et al., 2012) .

4. CONCLUSION AND FUTURE PERSPECTIVE

Despite being completely preventable, prenatal alcohol exposure still represents one of the main causes of birth neurodevelopmental defects. For instance, due to the diversity of social and personal factors involved, maternal alcohol intake during pregnancy is difficult to solve. The high prevalence altogether with the limited diagnostic and therapeutic approaches make FASD a worldwide Public Health problem with an important necessity to find new action strategies for management.

Alcohol exposure can affect almost all body tissues and organs, nevertheless, the brain is considered the most significant target showing structural and functional alterations. Although brain impairments have been reported during all periods of life, the developing brain is particularly vulnerable. The cognitive and behavioral consequences, however, can vary among the ontogeny period of the brain being the early alcohol exposure more permanent and important. Besides, there are also brain regions apparently more vulnerable to alcohol including PFC, hippocampus, and cerebellum.

Dysregulation of the immune system is emerging as one of the central mechanisms of alcohol pathogenesis, however, gaps still exist in our current knowledge. Glial cells seem to be one of the important factors in this regard with a complex response which involved several cellular pathways such as TLR signaling. Through these signaling pathways, in the last instance, a huge amount of proinflammatory molecules will be released creating an inflammatory environment that will disrupt the physiological developmental processes of the CNS.

There is evidence to demonstrate that this inflammatory environment induced by ethanol in the brain is associated with alterations to myelin, BBB, synaptic dysfunctions, and neurodegeneration. Nonetheless, there is yet a need to completely understand the whole mechanisms by which prenatal alcohol induces its neuroimmune damage to the CNS and also link them with specific cognitive and neurophysiological outcomes.

In the search for answers there is the need to carry out more studies with human and/or nonhuman primates to validate some of the hypotheses because with rodent models, although many studies have been conducted, interspecies differences may be found.

Finally, being able to elucidate all this lacking knowledge will allow us to provide potential new treatments and intervention strategies in addition to more specific diagnostic techniques. For example, sex differences are emerging as a factor involved in FASD

pathogenicity. So it is important to keep researching in this direction to establish differential diagnostic parameters between females and males.

In addition, finding FASD biomarkers is also necessary for a proper diagnostic. Recent studies establishing the FASD cytokine profile are the first step in the search for these biomarkers. They could be used for early identification of at-risk children that do not show early FASD outcomes and, otherwise may be misdiagnosed. Besides, a premature diagnosis would bring an early intervention and treatment avoiding the risk of FASD impairments that may appear later on in life as well as restoring alcohol-induced brain damage.

Figure 1. Effects of prenatal alcohol exposure in the central nervous system in different developmental stages. *Adapted from Guerri and Alfonso-Loeches 2011.*

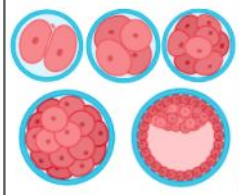
OVUM STAGE	EMBRYONIC STAGE	FETAL STAGE - 2nd YEAR
1-2 weeks	3-8 weeks	9-108 weeks
	 4 weeks	10 weeks 25 weeks  18 months
	Major abnormalities	**Functional and minor abnormalities**
Neural tube formation NSCs proliferation	Differentiation specific brain areas NSCs proliferation & differentiation Neuronal migration	Brain growth Massive neural cell death (pruning) Astrogliogenesis and myelin development Functional neural connections (synaptogenesis)
FAS dismorphia Reduced NSCs proliferation Neural tube defects	Abnormal radial glia: neuronal and astroglial deficits Abnormal cell migration Neuronal loss Corpus callosum malformations	Prominent microcephaly Abnormal glial development Increase in necrosis and apoptosis Alterations in neural connections Alterations in the cerebellum

Figure 2. Main **effects produced by alcohol in microglia, astrocytes, and oligodendrocytes.** *Adapted from Guizzetti et al, 2014.*

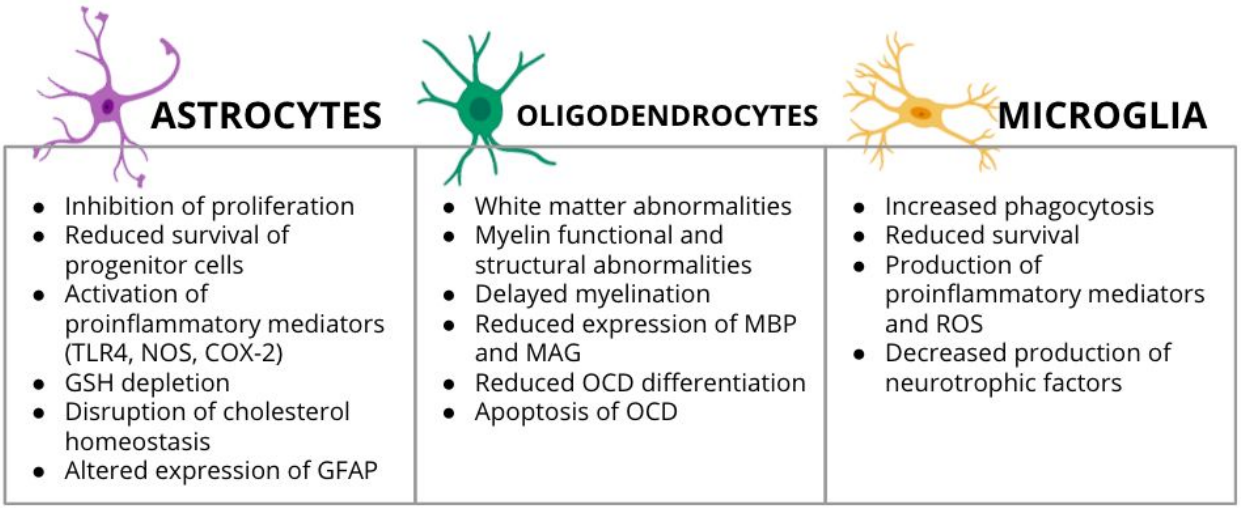


Figure 3. Neuroimmune pathways induced by PAE. Alcohol induction of neuroinflammation through the activation of TLR4. Ethanol, PAPS, or DAMPS activate the receptor that leads to the recruitment of two proteins MyD88 and TRIF to trigger downstream signaling pathways (MyD88-dependant and MyD88-independent). This terminates with the phosphorylation of transcription factors (AP-1, NF-κB, and IRF-3) that enable the traduction of cytokines (e.g. IL-1β, TNF-α, and IL-6), inflammatory mediators (e.g. iNOS, COX-2) and chemokines (e. g. MCP-1) that eventually cause neuroinflammation. In addition, NLR3-inflammasome is also activated by alcohol and ROS and triggers the conversion of pro-IL1β into IL-1β that contributes to the amplification of the neuroinflammation.

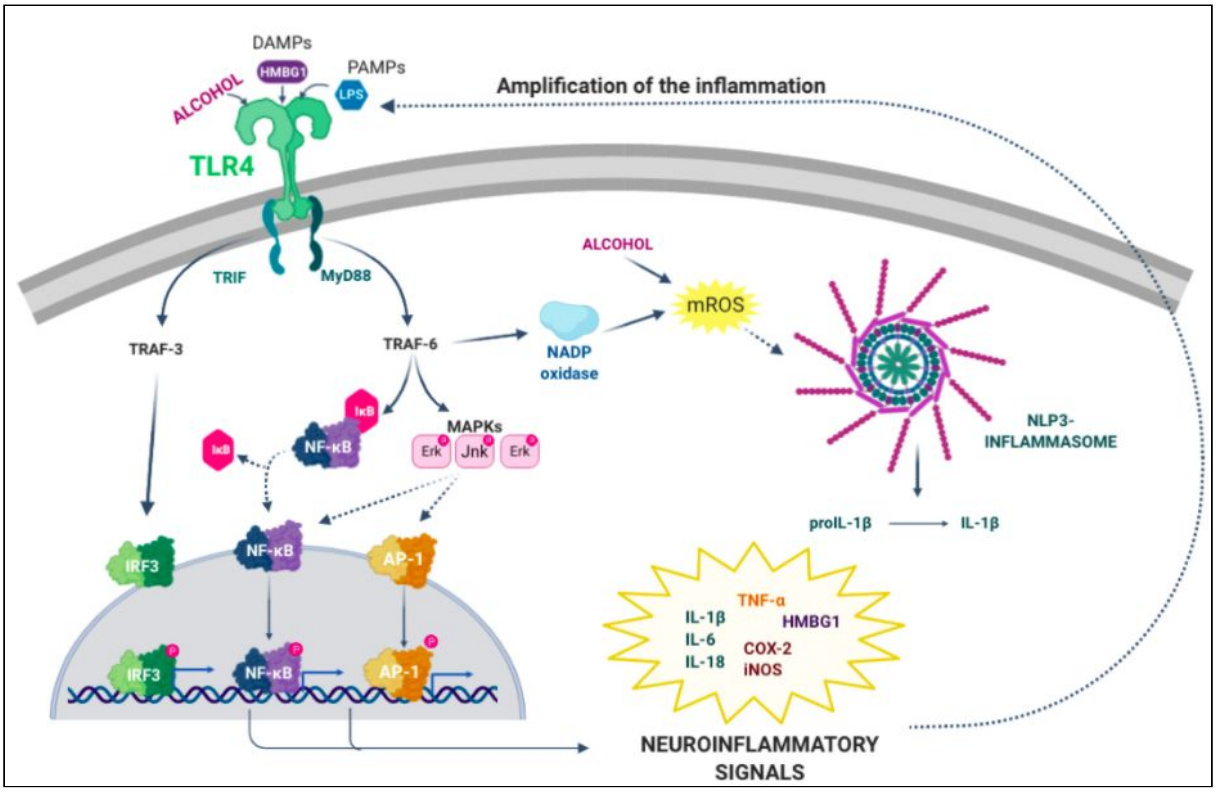


Table 1. Summary of the evidence showing an increase of alcohol-induced expression of cytokine in samples from human and animal models.

Authors	Cytokines increased	Sample	Subject of study
Ahluwalia et al., 2000	TNF-α, IL-1β, and IL-6	Mother's blood and cord blood at delivery	Humans
Vink et al., 2005	TNF-α and IL-6	Embryo/decidua	Mouse model
Dahlgren et al., 2006	IL-6	Fetus sample and amniotic fluids	Rat model
Terasaki et al. 2006	mRNA IL-6	Placenta	Human
	mRNA of IL-10, IL-5 and TNF-α	Brain	Human
Tiwari and Chopra 2011	IL - 1β, TNF - α, and TGF - β	Hippocampus and cortex	Rat model
Tiwari and Chopra 2012	IL - 1β, TNF - α, TGF - β, NF-κβ and caspase 3	Cortex and hippocampus	Rat model
Lippai et al. 2013	TNFα, MCP 1, IL-1β	Cerebellum	Mice model
Li et al., 2015	IL-1β	cerebellum	Mice model
Drew et al., 2015	mRNA of IL-1β, TNF-α	PFC, Hippocampus, cerebellum	Mice model
	CCL2	Hippocampus, cerebellum	
Bodnar et al., 2016	TNF-α, IFN-ɣ, IL-1β, IL-2, IL-4, and IL-5	Hippocampal	Human
	IL-5, and IL-6	PFC	Human
Terasaki et al., 2016	IL-6 and TNFSF4	Placenta	Human
Boschen et al., 2016	IL-1β, TNF-α, CD11b, and CCL4;	Hippocampus	Rat model
Pascual et al., 2017	IL-1β, IL-17, MIP-1α	Maternal sera, amniotic fluid, and brains of fetuses	Mice model
Terasaki et al., 2017	TNF-α, IL-5, IL-21 and chemokines CCL3, CCL6, CCL9,	PFC and hippocampus	Rats
Cantacorps et al., 2017	TLR4, NF-κB/p65, NLRP3, caspase-1 and IL-1β	Hippocampus	Mouse
Sowell et al., 2018	Increasing in the ratio of TNFα: IL-10 or IL-6: IL-10 IL-1β, IL-2, IL-4, IL-6, and IL-10 in early-mid pregnancy and IL-1β and IL-10 during late pregnancy.	Plasma samples	Human

Table 2. Summary of the main cognitive outcomes seen in individuals with FASD and their relation with the dysregulation of the immune system. *Adapted from Mattson et al., 2019*

IMPAIRMENTS	CHARACTERISTICS	NEUROIMMUNE EFFECTS
Executive functions	Deficits in verbal fluency (Schonfeld et al., 2001) Deficiencies in concept formation (McGee et al., 2008) Impairments in problem-solving and planning (Mattson and Riley, 1998)	*No available bibliography*[1]
Learning and memory	Slower learning, impaired recall and impaired discrimination (Lewis et al., 2015; Mattson and Riley 1998) Deficits in spatial learning and memory have been seen in animal models (Berman and Hannigan, 2000). Lower academic achievement than expected for the intellectual level (see rev. Hagan et al., 2016)	Dysregulation of the neuroimmune response in offspring is related to impairments in learning and memory (Cantacorps et al., 2017) The hippocampus and cortex, critical regions for learning and recognition memory, are vulnerable to glial immune activation after PAE (Goodfellow and Linquist 2014) Increased IL-6, TNF-a affect long term potentiation (Huang et al., 2013) Changes in dendritic spines, synaptic ultrastructure, and/or synaptic plasticity are altered by prenatal alcohol exposure (Cui et al., 2010).
Visual-spatial abilities	Impairments in visual perception and construction tasks (Mattson et al., 1996) Delay in motor reaction time to visual stimuli (Mattson and Riley, 2011)	*No available bibliography*
Emotional alterations	Increased risk of ADHD, anxiety, depression PTSD, conduct disorder, language disorder (Cullen et al., 2013; O'Connor and Paley, 2009; Pei et al., 2011; Popova et al., 2017; Weyrauch et al., 2017) Difficulties maintaining emotional stability and self-monitoring (Fryer et al., 2007)	Behavioral alterations are associated with impairments in neurotransmitter systems, neuromodulators, and synaptic plasticity in several brain regions (For a review, to see Valenzuela et al., 2012). Alterations on myelin structure and synaptic dysfunctions due to IS dysregulation lead to behavioral dysfunctions (For a review, to see Guerri and Pascual 2019).
Attention	Deficits in establishing, organizing and sustaining attention (Nanson and Hiscock, 1990)	*No available bibliography*
Social interactions	Difficulties in social interactions and overall social skills, hyperactive behaviors,	*No available bibliography*

[1] There is currently no available evidence of the implication of the immune system in these alterations

	communication difficulties and problems assuming responsibilities (Carr et al., 2010; Rasmussen et al., 2011) Increased social impairments have been seen in females compared to males (Rasmussen et al., 2011)	
Language	Deficits language skills in both expressive and receptive language (Wyper and Rasmussen, 2011;) Impaired articulation and grammatical ability (Thorne, 2017)	*No available bibliography*
Motor Skills	Deficits in balance, fine motor control (Connor et al., 2006) and hand-eye coordination (Adnams et al., 2001) and motor reaction time (Mattson et al., 2011) Alterations in spontaneous locomotor activity depending on age and alcohol dosage: adolescent mice acquired hyperactivity-like behavior (Cantacorps et al., 2018; Nunes et al., 2011) whereas adult mice presented hypoactivity (Cantacorps et al., 2018)	It has been related to neurodegeneration, since the blocking TLR4-MyD88 pathway in mice mitigates neuronal loss as well as motor impairments (Wu et al., 2012)
Neuropathic pain	High immune reactivity in the brain and spinal cord related to sensory touch impairments (Noor et al., 2017; Sanchez et al., 2017; Topper et al., 2015)	Release of proinflammatory cytokines (IL-1β, TNF-α) (Sanchez et al., 2017), in addition to reduced IL-10 levels in the dorsal root ganglia (DRG) and CNS in animal models of PAE (Noor et al., 2017; Sanchez et al., 2019) Aberrant increase of CCL2 expression (Ren et al., 2017; Wang et al., 2019; Zhang et al., 2018) and dysregulation of adhesion molecules (LFA-1) lead to the induction of allodynia.
Vulnerability to substance consuming	PAE leads to an increase drug-seeking and drug-taking behaviour (Cantacorps et al., 2020) Prenatal alcohol leads to a higher sensitivity to the reinforcing effects of cocaine (Barbier et al., 2009; Cantacorps et al., 2020), amphetamine (Barbier et al., 2009; Wang et al., 2019)	*No available bibliography*

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