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Effects of early-life adversity on immune function are mediated by prenatal environment: Role of prenatal alcohol exposure

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ABSTRACT

The contribution of the early postnatal environment to the pervasive effects of prenatal alcohol exposure (PAE) is poorly understood. Moreover, PAE often carries increased risk of exposure to adversity/stress during early life. Dysregulation of immune function may play a role in how pre- and/or postnatal adversity/stress alters brain development. Here, we combine two animal models to examine whether PAE differentially increases vulnerability to immune dysregulation in response to early-life adversity. PAE and control litters were exposed to either limited bedding (postnatal day [PN] 8-12) to model early-life adversity or normal bedding, and maternal behavior and pup vocalizations were recorded. Peripheral (serum) and central (amygdala) immune (cytokines and C-reactive protein – CRP) responses of PAE animals to early-life adversity were evaluated at PN12. Insufficient bedding increased negative maternal behavior in both groups. Early-life adversity increased vocalization in all animals; however, PAE pups vocalized less than controls. Early-life adversity reduced serum TNF-α, KC/GRO, and IL-10 levels in control but not PAE animals. PAE increased serum CRP, and levels were even higher in pups exposed to adversity. Finally, PAE reduced KC/GRO and increased IL-10 levels in the amygdala. Our results indicate that PAE alters immune system development and both behavioral and immune responses to early-life adversity, which could have subsequent consequences for brain development and later life health.

1. Introduction

Brain development is a dynamic and continuous process that starts very early in prenatal life and extends through adolescence (Andersen, 2003; O'Mahony et al., 2017). Exposure to adversity and/or stress in any of these life stages can negatively alter the neurodevelopmental trajectory, putting the individual on a pathway to pathology. Among many potential adverse environmental factors, alcohol, in addition to its teratogenic effects, can program developing neurobiological systems, altering brain development and increasing vulnerability to cognitive and behavioral deficits, as well as physical and mental health problems (Hellemans et al., 2010a; Riley et al., 2011; Schneider et al., 2011; Valenzuela et al., 2012; Weinberg et al., 2008). Importantly, exposure to alcohol during gestation carries with it an increased risk of being exposed to adverse and/or stressful environments during postnatal life (O'Connor and Kasari, 2000; O'Connor and Paley, 2006; Streissguth et al., 2004). Moreover, consistent findings indicate that early postnatal adversity such as neglect, abuse, and/or maltreatment – especially from the caregiver – can also change brain development and have long-lasting consequences for the physical and mental health of the offspring (Chen and Baram, 2016; Cirulli et al., 2009; Danese and McEwen, 2012; Drury et al., 2016; Heim et al., 2010; McEwen, 2008; Raineki et al., 2012; Teicher et al., 2003). Nevertheless, relatively few studies have investigated how exposure to adverse and/or stressful environments early in postnatal life contributes to the pervasive and long-lasting negative effects of PAE (Alberry and Singh, 2016; Price et al., 2017).

A leading mechanistic hypothesis about how pre- and/or postnatal adversity can affect brain development suggests that dysregulation of the normal cytokine balance may play a role (Ganguly and Brenhouse, 2015; Hennessy et al., 2010; Miller et al., 2011; Nusslock and Miller, 2016). Cytokines are potent neuromodulators of brain development, affecting neurogenesis, neuronal migration, synaptogenesis, and synaptic pruning (Bajetto et al., 2001; Bessis et al., 2005; Deverman and Patterson, 2009; Smith et al., 2007; Stephan et al., 2012). As a result, altered cytokine balance may affect many important neuronal processes, resulting in abnormal brain development and increased vulnerability to adverse adaptive, functional and health outcomes in later life (Babri et al., 2014; Bauman et al., 1997; Bilbo and Schwarz, 2009; Ganguly

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and Brenhouse, 2015; Meyer et al., 2009). Importantly, it has been shown that exposure to adverse environmental factors during sensitive early-life periods can result in a long-lasting, enhanced proinflammatory phenotype, which is embedded in the functioning of critical immune system cells in both the periphery and the brain (Hennessy et al., 2010; Nusslock and Miller, 2016). This, in turn, could underlie the increased prevalence of physical and mental health problems observed in individuals exposed to early-life adversity (Danese and McEwen, 2012; Hennessy et al., 2010; Miller et al., 2011; Raison et al., 2006). We have demonstrated that PAE alters both central and peripheral cytokine levels during early development (Bodnar et al., 2016). Specifically, at postnatal day (PN) 8, PAE animals show increased basal levels of cytokines in serum and in the hippocampus and prefrontal cortex and decreased levels in the hypothalamus and spleen. This PAE-induced alteration in cytokine balance early in life is hypothesized to underlie some of the pervasive alterations in neurobehavioral and immune function associated with PAE (for review see Bodnar and Weinberg, 2013; Drew and Kane, 2014). However, it remains to be determined if exposure to early-life adversity has a greater impact on the immune function of animals that have an altered cytokine balance early in life, such as those exposed to alcohol during gestation. Additionally, clinical research has consistently shown increased levels of plasma C-reactive protein (CRP), an acute-phase protein, in children and adults with a history of abuse and/or maltreatment (Coelho et al., 2014; Danese et al., 2008, 2011; Slopen et al., 2013). Interestingly, the CRP increase in response to early-life adversity is more prominent in individuals with current depression (Danese et al., 2008, 2011), suggesting that increased levels of CRP may be indicative of adversity-related mental health problems or may even mediate, at least in part, the negative outcomes.

Here, we combine a rodent model of PAE, which results in an early-life altered cytokine balance and increased predisposition to later life adverse health problems (Bodnar et al., 2016; Hellemans et al., 2010a; Weinberg et al., 2008; Zhang et al., 2012), with a naturalistic model of early-life adversity that replicates several aspects of the psychopathologies related to early life abuse and/or adversity in humans (Raineki et al., 2010, 2012, 2015) to examine the immune response (cytokines and CRP) of PAE animals to early-life adversity. To model early-life adversity, we provided rat mothers with insufficient bedding from PN8-12. This limited bedding environment decreases the mother's ability to construct a nest, leading to increased negative maternal behaviors (Raineki et al., 2010, 2012, 2015). Exposure to this type of early-life adversity has been shown to induce dysfunctional social behavior with the mother and peers, as well as depressive-like behavior later in life (Raineki et al., 2010, 2012, 2015; Rincón-Cortés and Sullivan, 2016; Yan et al., 2017). These behavioral deficits are, at least in part, supported by amygdala dysregulation (Raineki et al., 2010, 2012; Rincón-Cortés and Sullivan, 2016; Yan et al., 2017), data that corroborate the clinical literature demonstrating that adversity during infancy is associated with greater amygdala reactivity when exposed to emotional stimuli later in life (McCrory et al., 2013; Tottenham et al., 2011).

In rats, development of the amygdala begins prenatally, with a peak in development during the first two postnatal weeks, when there is a marked increase in neurogenesis, nuclei subdivision and neuron maturation (Bayer, 1980; Berdel et al., 1997; Berdel and Morys, 2000; Bouwmeester et al., 2002; Cunningham et al., 2002; Ryan et al., 2016; Thompson et al., 2008). As the immune system plays an important role in brain development, including development of the amygdala, and in later-life physical and mental health problems, we compared immune responses of PAE and control offspring to early-life adversity. Specifically, we evaluated the peripheral (serum) and central (amygdala) immune system responses (cytokines and CRP) of PAE animals in response to early-life adversity at PN12. Moreover, the insufficient bedding environment significantly alters how the mothers behave (Raineki et al., 2010, 2012, 2015). Because the quality of care received from the caregiver has long-lasting consequences for both physical and mental health (Champagne et al., 2003; Hofer, 1994; Rincón-Cortés and Sullivan, 2014; Weaver et al., 2004), in the present study, we evaluated the impact of the insufficient bedding environment on maternal behavior. We hypothesized that early-life adversity will differentially impact the behavior of PAE and control dams as well as the immune function of PAE and control offspring.

2. Methods

2.1. Animals and breeding

Male and female Sprague-Dawley rats were obtained from Charles River Laboratories (St. Constant, Quebec, Canada). Rats were housed with a same-sex cage mate and maintained at a constant temperature (21 ± 1 °C) and on a 12:12 light-dark cycle (lights on at 0700 h) with *ad libitum* access to water and standard laboratory chow (Harlan, Canada). After a 10-day acclimation period, males and females were paired for breeding. Vaginal smears were taken each morning, and the presence of sperm indicated gestation day 1 (G1). All experiments were performed in accordance with the National Institutes of Health (NIH) Guidelines For The Care And Use Of Laboratory Animals and the Canadian Council on Animal Care guidelines and were approved by the University of British Columbia Animal Care Committee.

2.2. Prenatal alcohol exposure

On G1, females were single-housed and randomly assigned to one of three treatment groups: alcohol, pair-fed or *ad libitum*-fed control. Dams in the alcohol group were offered *ad libitum* liquid ethanol diet with 36% ethanol-derived calories (Dyets Inc; Bethlehem, PA). The liquid ethanol diet was introduced gradually over the first 3 days with bottles containing: G1 – 66% control diet, 34% ethanol diet; G2 – 34% control diet, 66% ethanol diet; G3-21-100% ethanol diet. This diet is formulated to provide adequate nutrition to pregnant rats regardless of ethanol intake (Lan et al., 2006). Pair-fed dams were offered a liquid control diet with maltose-dextrin isocalorically substituted for ethanol, in an amount matched to the consumption of an alcohol-fed partner (g/Kg body weight/day of gestation). The control dams were offered *ad libitum* access to a pelleted form of the liquid control diet. All animals had *ad libitum* access to water, and were provided with fresh diet daily within 1 h of lights off to prevent a shift in corticosterone circadian rhythms, which occurs in animals that are on a restricted feeding schedule, such as the pair-fed dams (Gallo and Weinberg, 1981; Krieger, 1974). Experimental diets were continued through G21. Beginning on G22, all animals were offered *ad libitum* access to standard laboratory chow and water, which they received throughout lactation. Pregnant dams were left undisturbed except for cage changing on G1, G7, and G14. On the day of birth (postnatal day 1 – PN1), litters were culled to 12 pups with an attempt to preserve an equal number of males and females per litter, and transferred to a clean cage.

While a pair-fed group was included in the initial study design, specific pair-feeding effects are analyzed and presented separately in the Supplementary section. Historically, the pair-fed group was introduced to account for the decreased food intake associated with chronic alcohol consumption, with the goal of separating alcohol effects from those of undernutrition. However, adverse

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