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Sex differences in the peripheral and central immune responses following lipopolysaccharide treatment in pubertal and adult CD-1 mice

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ABSTRACT

Puberty is a critical developmental period that is characterized by significant brain development. Exposure to stress during this time can alter brain functioning setting the stage for long-lasting behavioural outcomes. The objective of this study was to investigate age and sex differences in the peripheral and central immune responses, along with sickness behaviour, following immune stress. The results showed that LPS treatment increased serum cytokine levels and sickness symptoms in all mice. Pubertal males displayed increased IL-1β concentrations at 2 h and increased IL-6 concentrations at 8 h post-treatment whereas increased concentrations of TNFα, IL-10, IL-12, IL-1β, IFNγ, and IL-6 persisted at 8 and 24 h in adult females. Consistent with peripheral cytokines, pubertal males displayed greater IL-1β, TNFα, and IL-6 mRNA expression in the prefrontal cortex at 2 h, whereas adult males expressed more of the aforementioned cytokines at 8 h compared to saline controls. Adult males also displayed greater IL-1β mRNA expression compared to their female counterparts, and adult females displayed greater TNFα mRNA expression compared to their male counterparts. These results not only provide a better understanding of the age and sex differences in acute immune response, but also show important region- and time-specific differences in the response to an immune challenge, and that the peripheral immune response differs from the central response. This highlights the need to examine immune markers in both the periphery and the central nervous system for an accurate depiction of acute immune response following an immune challenge.

1. Introduction

Puberty is defined as the period during which reproductive maturity is attained (Sisk and Foster, 2004). During this period, the influx of gonadal hormones leads to extensive brain remodeling (Schulz et al., 2009) and reorganizing (Levitt, 2003) that can permanently alter the brain circuitry and set the stage for functional differences between males and females (Wallen and Baum, 2002). The hormonal changes during puberty also make this period sensitive to stressors and other environmental influences, which can increase susceptibility to cognitive or neuropsychiatric disorders (Patton and Viner, 2007). As such, it is critical to examine the vulnerability of this pubertal period and understand the impact that stress exposure can have.

In mice, exposure to particular stressors during puberty can cause long-term changes in behavior (Blaustein and Ismail, 2013; Holder and Blaustein, 2014). For example, exposure to shipping stress during a pubertal stress sensitive period (six weeks of age) reduces sexual receptivity in adulthood despite female mice being hormonally primed with estradiol and progesterone and males primed with testosterone. Thus, shipping stress decreases the behavioural responsiveness to gonadal steroid hormones (Laroche et al., 2009a). Immune stress can also impact the pubertal period. Similar to shipping stress, pubertal female mice exposed to the bacterial endotoxin, lipopolysaccharide (LPS), at 6 weeks of age, display reduced sexual receptivity and behavioural responsiveness to estradiol and progesterone treatments in adulthood (Laroche et al., 2009b). The effects of pubertal LPS exposure also extend to non-reproductive behaviors such as decreased performance on object and social recognition tests (Ismail and Blaustein, 2013), reduced open-arm activity in the elevated plus maze (Olesen et al., 2011), and shorter latency to fall on the rotarod and reversed grid hang tests (Girard-Joyal

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and Ismail, 2017), which exemplify depression-, anxiety-, and Parkinson-like behaviours. The mechanisms underlying these long-term changes remain unknown, but the results suggest that the effects are limited to the stress-sensitive pubertal period (six weeks of age), as they are not seen in younger or older mice treated with LPS (Ismail et al., 2011; Laroche et al., 2009a,b). The findings suggest that there are important age and sex differences in stress reactivity and immune responses following LPS treatment.

LPS is derived from the cell wall of gram-negative bacteria (e.g., *Escherichia coli*) and, following administration, it triggers an immediate and measurable immune response at both the molecular and behavioral levels (Bilbo and Schwarz, 2009; Kentner and Pittman, 2010). When LPS enters the circulatory system of an animal, it binds to toll-like receptor-4 (TLR-4) on the membrane of immune cells. Then, it activates an intracellular signaling pathway leading to the activation of the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway (Conti et al., 2004), which is a major system involved in the transcription and translation of immune-modulating proteins called cytokines (Andreakos et al., 2004; Vitkovic et al., 2000). Cytokine levels increase in the periphery following LPS exposure (Beaty et al., 1994; de Bont et al., 1998), and these molecules activate the central nervous system and *de novo* synthesis of cytokines within the brain through activation of cytokine receptors at the circumventricular organs and the choroid plexus and through diffusion via the blood-brain-barrier (Galic et al., 2012; Gutierrez et al., 1993; Quan et al., 1994, 1998). These are only two of the many possible ways cytokines can enter the brain. It is not entirely known how peripheral immune activation leads to central inflammation, as these two systems are often not correlated in inflammatory situations (Mouihate et al., 2010). Cytokines can be classified as either pro-inflammatory (e.g. IL-1β, IFNγ, TNFα, and IL-12) or anti-inflammatory (e.g. IL-10) and interact to influence each other and induce sickness behaviour. For example, systemic IL-1β does not induce significant serum TNFα but does induce central TNFα mRNA expression. However, systemic TNFα induces both serum and central IL-1β (Skelly et al., 2013). Some cytokines, like IL-6, can also be classified as both pro- and anti-inflammatory. In its anti-inflammatory state, IL-6 initiates signaling transduction mechanisms to inhibit subsequent production and release of IL-1β and TNFα (Yasukawa et al., 2003). Pro- and anti-inflammatory cytokines also show opposite effects on sickness behaviour outcome. The activation of pro-inflammatory cytokines, such as IL-1β, TNFα, and IL-6, orchestrates sickness behaviours such as reduced socialization, sleep changes, lethargy, and performance disruptions on memory tasks, for example (Dantzer and Kelley, 2007; Maier et al., 1998; Vollmer-Conna et al., 2004). Anti-inflammatory cytokines, like IL-10, suppress sickness behaviour (Bluthé et al., 1999; Leon et al., 1999).

Recent findings suggest that differences in the acute stress and immune responses between pubertal and adult male and female mice can provide mechanistic insight into the enduring behavioural alterations following LPS exposure. While LPS treatment increases serum corticosterone concentration in all pubertal and adult male and female mice, adult females show the highest increase two hours after treatment (Girard-Joyal et al., 2015). Exposure to LPS also results in an increase in c-Fos expression in many brain regions in adult mice but not in pubertal mice two hours following LPS treatment (Girard-Joyal et al., 2015). Furthermore, senescent mice have been shown to be the most sensitive to LPS lethality and produce significantly elevated plasma TNFα and nitric oxide levels in comparison to young and mature mice (Chorinchath et al., 1996). Similarly, middle-aged mice display exaggerated peripheral and central immune responses for TNFα, IL-6, and IL-1β production in their microglia and spleens compared to young adults following LPS treatment (Nikodemova et al., 2016).

LPS treatment is known to initiate an immune response accompanied by sickness symptoms such as hypothermia, fever, anorexia (i.e., decreased food intake), and cachexia (i.e., decreased body weight) (Leon, 2002), and there are sex differences in these behavioral responses. For example, female mice recover significantly faster from an LPS-induced drop in body temperature and loss in body weight relative to their male counterparts (Tesfaigzi et al., 2001). With regards to another type of immune challenge (i.e., influenza), infected males mount a faster IL-1β mRNA expression in the lungs, as well as earlier and more pronounced anorexia and saccharin consumption and blunted corticosterone secretion, compared to females (Avitsur et al., 2011). Similarly, in clinical practice, female patients have a higher survival rate to the Ebola virus whereas males experience longer hospitalization periods (WHO Ebola Response Team, 2016). The sex difference in immune reactivity is likely attributed to the role of gonadal hormones, as these steroids influence stress and immune responses. Testosterone and estrogen receptors are present on various immune organs and cells, suggesting that they can directly influence the immune system. Testosterone is generally seen as an immune suppressor, while estrogens are known as immune enhancers (Kovats, 2015; Lai et al., 2012). There is also a sex difference in cytokine production following LPS challenge in both rodent models and human cell cultures. Higher levels of TNFα, IL-1β, and IL-6 have been seen in males compared to their female counterparts (Imahara et al., 2005; Marriott et al., 2006; Moxley et al., 2002; Naor et al., 2009; Queen et al., 2016). Overall, there are important age and sex differences following exposure to LPS and other immune challenges. Understanding these differences is critical to identifying the mechanism behind the long-term behavioural changes after a pubertal immune challenge.

A recent publication from our laboratory has shown age and sex differences in the peripheral immune response after LPS treatment. Male mice display greater and more prolonged hypothermic response and sickness behaviour symptoms compared to female mice. Pubertal mice also display fewer body temperature fluctuations and sickness behaviour symptoms compared to their adult counterparts after LPS treatment. Furthermore, LPS treatment induces more pro-inflammatory cytokines, like IL-1β, IFNγ, and TNFα, in adult mice and more anti-inflammatory cytokines, such as IL-10, in pubertal mice ten hours following exposure (Cai et al., 2016). Therefore, there are important age and sex differences in the acute immune response to LPS exposure suggesting that pubertal mice may be hypo-responsive to an immune challenge compared to their adult counterparts. However, it is still unclear whether these age and sex differences are present only at ten hours following LPS treatment or at other time points as well, and whether there are similar age and sex differences in cytokine mRNA expression in the brain, as presence of immune markers in the periphery are not always consistent with central changes (Mouihate et al., 2010). Moreover, it is unclear whether there are age and sex differences in the recovery of both peripheral and central cytokines following an immune challenge. As a result, the current paper adds to our recent publication depicting age and sex differences following pubertal and adult LPS treatment.

In the present study, we examined age and sex differences in immune response by examining sickness behaviour, concentration of pro- and anti-inflammatory cytokines (i.e., IL-1β, IFNγ, TNFα, IL-12, IL-10, and IL-6) in the blood, and central cytokine mRNA expression in the prefrontal cortex and hippocampus at various time points following LPS treatment. The cytokines examined in the current study were chosen from our recent publication that examined peripheral cytokine levels at one time point (10 h). The prefrontal cortex and hippocampus were selected for examination because they are particularly sensitive to brain dysfunction changes during immune stress and play important roles in the pathophysiology of stress- and mood-related disorders like depression (Wu et al., 2016; Zhao et al., 2017). This study allows us to clearly examine recovery of the immune system after LPS challenge and whether the brain responds similarly or differently to an immune challenge compared to the peripheral immune response. Since previous studies have shown that sex hormones modulate the immune system and that responsiveness to stressors varies with age, we hypothesized that LPS-treated adult mice would display more sickness symptoms and