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Men and women differ in inflammatory and neuroendocrine responses to endotoxin but not in the severity of sickness symptoms

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ABSTRACT

Impaired mood and increased anxiety represent core symptoms of sickness behavior that are thought to be mediated by pro-inflammatory cytokines. Moreover, excessive inflammation seems to be implicated in the development of mood/affective disorders. Although women are known to mount stronger pro-inflammatory responses during infections and are at higher risk to develop depressive and anxiety disorders compared to men, experimental studies on sex differences in sickness symptoms are scarce. Thus, the present study aimed at comparing physiological and psychological responses to endotoxin administration between men and women. Twenty-eight healthy volunteers (14 men, 14 women) were intravenously injected with a low dose (0.4 ng/kg) of lipopolysaccharide (LPS) and plasma concentrations of cytokines and neuroendocrine factors as well as negative state emotions were measured before and until six hours after LPS administration. Women exhibited a more profound pro-inflammatory response with significantly higher increases in tumor necrosis factor (TNF)-α and interleukin (IL)-6. In contrast, the LPS-induced increase in anti-inflammatory IL-10 was significantly higher in men. The cytokine alterations were accompanied by changes in neuroendocrine factors known to be involved in inflammation regulation. Endotoxin injection induced a significant increase in noradrenaline, without evidence for sex differences. The LPS-induced increase in cortisol was significantly higher in woman, whereas changes in dehydroepiandrosterone were largely comparable. LPS administration also increased secretion of prolactin, but only in women. Despite these profound sex differences in inflammatory and neuroendocrine responses, men and women did not differ in endotoxin-induced alterations in mood and state anxiety or non-specific sickness symptoms. This suggests that compensatory mechanisms exist that counteract the more pronounced inflammatory response in women, preventing an exaggerated sickness response. Disturbance of these compensatory mechanisms by environmental factors such as stress may promote the development of affective disorders in women.

1. Introduction

Acute inflammation is a protective response of the body to infection or tissue injury, and is characterized by the production and release of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 from activated immune cells. These soluble mediators not only coordinate local and systemic immune responses by also act on the brain, inducing a complex set of behavioral, neuroendocrine, and metabolic changes aimed at battling the infection and restoring homeostasis (Dantzer et al., 2008; Larson and Dunn, 2001; Meisel et al., 2005). The cytokine-induced behavioral symptoms such as impaired mood, anxiety, cognitive disturbances, fatigue, anhedonia, social withdrawal, and hyperalgesia are highly conserved among mammals and are collectively termed 'sickness behavior' (Hart, 1988; Kent et al., 1992). Despite its negative impact on well-being, sickness behavior is considered an adaptive process as it conserves energy for the energy-demanding fever and immune responses (Shattuck and Muehlenbein, 2015). However, excessive or persistent inflammation can lead to an exacerbation of sickness behavior and is thought to be implicated in the development of mood/affective disorders, particularly depression (Dantzer et al., 2008; Haroon et al., 2012; Maes et al., 2012).

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Both clinical observations and experimental findings in humans demonstrate that humoral and cell-mediated immune responses markedly differ between sexes (Klein, 2012). For example, women mount stronger inflammatory and innate immune responses to bacterial and viral infections or to vaccination (Furman et al., 2014; Klein et al., 2010; Marriott and Huet-Hudson, 2006; Villacres et al., 2004). The more pronounced pro-inflammatory response pattern in women relative to men is generally thought to be beneficial by limiting pathogen spread and by accelerating pathogen clearance. Indeed, findings from large population-based studies indicate that female sex is associated with a better prognosis after sepsis or severe trauma (Angele et al., 2014; Choudhry et al., 2007; Schroder et al., 1998; Wohltmann et al., 2001). However, there seems to be a downside of this heightened pro-inflammatory responsiveness as inflammatory and autoimmune diseases are much more prevalent in women compared to men (Tam et al., 2011; Whitacre, 2001). In addition, females are two to three times more likely to develop mental disorders such as depression or anxiety disorders (Altemus et al., 2014; Bekker and van Mens-Verhulst, 2007; Kessler et al., 2005). However, it is not known whether the higher prevalence of affective disorders in women may be related to their greater inflammatory reactivity *per se*.

Sickness symptoms can be experimentally elicited in humans by peripheral administration of bacterial endotoxin (lipopolysaccharide [LPS]), a major component of the outer membrane of Gram-negative bacteria (Eisenberger et al., 2009; Grigoleit et al., 2013; Reichenberg et al., 2001). LPS is a prototypical pathogen-associated molecular pattern which is specifically recognized by Toll-like receptor (TLR) 4, a pattern recognition receptor expressed on the surface of innate immune cells. Engagement of TLR4 triggers intracellular signaling cascades ultimately leading to the production and release of pro-inflammatory cytokines and type-I interferons (Lu et al., 2008). Despite clinical evidence for a higher inflammatory reactivity in women, the vast majority of studies investigating endotoxin-induced sickness behavior have been conducted in male-only samples (reviewed in Schedlowski et al., 2014). So far, only few studies have addressed sex-related differences in inflammatory and behavioral responses to endotoxin, with inconsistent results (Eisenberger et al., 2009; Karshikoff et al., 2015; Moieni et al., 2015). In addition, important aspects of the anti-inflammatory response including cytokines and neuroendocrine mediators have not been considered in these studies. Thus, using the administration of low-dose endotoxin as experimental model of acute systemic inflammation, the present study aimed at investigating whether differences in inflammatory responses between men and women lead to sex-related differences in cytokine-mediated psychological and physiological responses. We hypothesized that women would mount a stronger pro-inflammatory and a weaker anti-inflammatory cytokine response compared to men, and, due to their greater inflammatory reactivity, would develop more severe sickness symptoms and stronger activation of neuroendocrine systems involved in inflammation regulation.

2. Methods

2.1. Participants

The study has been conducted within a larger project on sex differences in endotoxin-induced sickness responses (Wegner et al., 2015). From this cohort, a subset of 28 healthy men and women, matched by age and body mass index, was selected for in-depth analysis of inflammatory, neuroendocrine, and mood parameters. Participants were recruited by public advertisement and underwent an extensive screening and safety procedure consisting of a physical examination, a personal interview conducted by a physician, and assessment of blood and clinical chemistry parameters (i.e., complete blood cell count, C-reactive protein, coagulation factors, liver enzymes, and renal parameters). Laboratory screening was conducted before, 24 h after endotoxin administration, and up to one week after completion of the study. General exclusion criteria were pre-existing or current medical or psychiatric conditions, body mass index (BMI) <18 or $\geqslant$29 kg/m^2, current medications, smoking, regular high alcohol use (>four drinks per week), or anxiety and/or depression scores exceeding published cut-offs of the Hospital Anxiety and Depression Scale (HADS; (Zigmond and Snaith, 1983). To exclude pregnancy, only women using hormonal contraceptives were included and a pregnancy test was conducted on the day of the experiment. Participants were instructed to refrain from strenuous exercise 48 h before and 24 h after the study day. The study protocol was approved by the Ethics Review Board of the University Hospital Essen (permit number 09-4271). All subjects provided written informed consent and received financial compensation for their participation in the study.

2.2. Study protocol

The experiments were conducted in a medically equipped room and were supervised by an emergency physician. Upon arrival of the participants, an intravenous catheter was inserted into an antecubital forearm vein for repeated blood collection and endotoxin application. After a rest period of 30 min, body temperature as well as heart rate and blood pressure were measured with an auricular thermometer and a sphygmomanometer, respectively, and a first blood sample (baseline) was obtained. Fifteen minutes later, subjects received an intravenous injection of 0.4 ng LPS/kg of body weight (reference standard endotoxin from *Escherichia coli*, lot H0K354; United States Pharmacopeia, Rockville, MD). The LPS had been subjected to a microbial safety testing routine by the German Federal Agency for Sera and Vaccines (Paul-Ehrlich Institute, Langen, Germany) and was stored in endotoxin-free borosilicate tubes (Pyroquant Diagnostik, Mörfelden-Waldorf, Germany) at −20 °C until use. Additional blood samples for WBC counts, cytokine analyses, and endocrine measures were collected in EDTA-coated tubes at 1, 2, 3, 4, and 6 h after endotoxin injection. Plasma was separated by centrifugation and was stored at −80 °C until analysis. Following each blood draw, body temperature, blood pressure, and heart rate were assessed.

2.3. White blood cell counts

Complete blood counts including white blood cell (WBC) differential were obtained using an automated hematology analyzer (KX-N21, Sysmex, Horgen, Switzerland).

2.4. LBP and sCD14

Enzyme-linked immunosorbent assays (ELISA) were used to measure plasma concentrations of lipopolysaccharide binding protein (Human LBP ELISA, Abnova, Taipei City, Taiwan) and soluble CD14 (Human sCD14 Quantikine ELISA, R&D Systems, Minneapolis, MN, USA). The sensitivity of the assays was 1.5 ng/ml for LBP and 125 pg/ml for sCD14.

2.5. Cytokine analyses

Plasma concentrations of TNF-α, IL-6, IL-10, and IL-1ra were measured by ELISA (Human Quantikine ELISA, R&D Systems). The sensitivity of the assays was 0.11 pg/ml for TNF-α, 0.70 pg/ml for

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