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Lipopolysaccharide-induced sepsis induces long-lasting affective changes in the mouse

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ABSTRACT

Post-septic encephalopathy is a poorly understood condition in survivors of sepsis that is characterised by cognitive and affective impairments. In this study we have sought to better understand this condition by undertaking a comprehensive behavioural and cognitive assessment of mice who had previously survived sepsis. Mice were treated with lipopolysaccharide (LPS; 5 mg/kg) and one month after this assessed on a battery of tests. Post-septic animals were found to display significantly more immobility in the tail suspension test and show a significantly decreased sucrose preference. Acute fluoxetine treatment reversed the increase in immobility in the tail suspension test in post-septic animals. Post-septic animals also showed less overall exploratory behaviour in the novel object recognition task and also showed increased anxiety-like behaviour in the elevated plus maze. Post-septic mice did not show signs of cognitive impairment, as assessed in the Morris watermaze, the 8-arm radial maze or on preference for the novel object in the novel object recognition task. Immunohistochemical analysis revealed significant upregulation of the microglial marker CD-11b, F4/80 and IBA-1 in the hippocampus of post-septic animals, as well as significant downregulation of the plasticity-related immediate early gene products ARC and EGR1. We also observed a decrease in neural stem cell proliferation in the dentate gyrus of post-septic animals as judged by BrdU incorporation. Co-treatment with the NF-κB pathway inhibitor PDTC attenuated the long-lasting effects of LPS on most of the affected parameters, but not on neural stem cell proliferation. These results show that LPS-induced sepsis in the mouse is followed by long-lasting increases in depressive- and anxiety-like behaviours, as well as by changes in neuroinflammatory- and neural plasticity-associated factors, and that attenuation of the severity of sepsis by PDTC attenuates many of these effects.

1. Introduction

Sepsis refers to a severe inflammatory response to infection co-occurring with alterations in at least two of the following parameters – body temperature, heart rate, respiratory rate and white blood cell count (Robertson and Coopersmith, 2006). Septic encephalopathy is a common complication of sepsis, occurring in up to 70% of patients (Papadopoulous et al., 2000) involving a deleterious mental state ranging from confusion to coma (Jacob et al., 2011). Structural and neuroinflammatory alterations persist in survivors, including reduced hippocampal volume and altered EEG activity, leading to cognitive and psychological impairments for years following recovery, and this condition is termed post-septic encephalopathy (Lazosky et al., 2010; Iwashyna et al., 2010; Davydow et al., 2012; Semmler et al., 2013; Winters et al., 2010; Ziaja, 2013). The aetiology of post-septic encephalopathy is not well understood and there are currently no successful treatments available.

While sepsis in humans is a heterogeneous condition, it is often modelled in rodents using the intraperitoneal injection of a high dose of the bacterial endotoxin lipopolysaccharide (LPS; Buras et al., 2005; Doi et al., 2009). Peripheral administration of high-dose LPS leads to a potent inflammatory response, via TLR-4 binding and subsequent NF-κB pathway activation. These LPS treatments mimic many of the clinical features of sepsis and results in a hyper-inflammatory response, accompanied by depressed EEG rhythmicity, low blood pressure, oxidative stress, multiple organ failure and a significant mortality rate (Chang et al., 2013; Lin et al., 2010; Okazaki et al., 2014). Following recovery from the acute effects of LPS-induced sepsis, an increased level microglial activation persists in the CNS, accompanied by

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prolonged over-expression of pro-inflammatory cytokines as well as time and dose dependent neuronal degeneration (Bossù et al., 2012; Weberpals et al., 2009; Qin et al., 2007).

In addition to persistent neuroimmunological changes, there have been a number of studies examining cognitive and affective parameters following recovery from both LPS-induced, and cecal ligation and puncture (CLP)-induced sepsis. There is considerable variance in the behavioural changes present in post-septic animals depending on both the method of sepsis induction and the latency to test behaviour following recovery from sepsis. Within the domain of learning and memory, post-septic animals have shown cognitive impairments across tasks such as the step-down passive avoidance task, the radial arm maze, and novel object recognition tasks (Barichello et al., 2007; Cassol-Jr et al., 2010, 2011; Semmler et al., 2007; Weberpals et al., 2009). Ten months after LPS-induced sepsis rats show no differences in the elevated plus maze or Morris watermaze, although they exhibit reduced exploration and recognition of a novel object in the open field test (Bossù et al., 2012). Sepsis survivor mice also show alterations in their circadian rhythms, and in their circadian responses to subsequent LPS challenge (O'Callaghan et al., 2012; Anderson et al., 2013). Post-septic animals have also been shown to display increased levels of depressive-like behaviour including both anhedonia and behavioural despair (Comim et al., 2010; Tuon et al., 2008) as well as anxiety-like behaviour in the elevated plus maze ten days after CLP (Calsavara et al., 2013). These affective changes may be linked to the growing evidence for a neuroimmune basis to mood disorders such as major depressive disorder (Berk et al., 2013; Miller et al., 2009).

The aim of this study was to comprehensively characterize the cognitive and affective state of sepsis-survivor mice one month after LPS induced sepsis, as well as assessing changes in neuroimmune markers and neurogenesis in post-septic animals.

2. Methods

2.1. Animals

For the purpose of all experiments male C57BL/6 mice (Charles River, Kent, UK) aged between 8 and 16 weeks were used. Animals were group housed in a 12:12 light:dark cycle for 2 weeks prior to LPS administration. Food and water were available *ad libitum* unless animals were on a food restriction protocol, temperature was 21 ± 1 °C and humidity was 50 ± 10%. Animals remained housed in groups of 2–4 in polypropylene cages (33 cm long × 15 cm wide × 13 cm high) with wood chip bedding and environmental enrichment (shredded paper and cardboard tubes). All procedures were approved by the Research Ethics Committee, National University of Ireland Maynooth, and were licensed by the Department of Health and Children, Ireland under statutory instrument (S.I.) No. 543 of 2012 and the European directive 2010/63/EU.

2.2. Drug treatments

All injections were prepared fresh on the treatment day, and given intraperitoneally in a final injection volume of 0.1–0.15 ml. 0.9% sterile saline was made up fresh for control injections, while lipopolysaccharide (serotype 0111.B4, Sigma Ireland; Qin et al., 2007) was made up to a 5 mg/kg dose in sterile saline. PDTC (Sigma Ireland) was administered in a 200 mg/kg dose (Liu et al., 1999) 10 min before LPS treatment while 20 mg/kg fluoxetine (Tocris Bioscience, Bristol, UK) was administered 90 min before behavioural testing. Mortality and significant moribundity requiring euthanasia occurred in approximately 10% of animals following the induction of sepsis. Following sepsis induction animals were individually assessed in terms of weight, activity in the homecage, general appearance, behaviour and altered breathing at 1, 4, 24 and 48 h as well as at one week post LPS-administration. Animals were scored between 0 and 5 on each parameter with scores then totalled. To assess the number of neural precursor cells in the hippocampus mice which had one month previously undergone sepsis-induction were injected i.p. with 50 mg/kg BrdU (Sigma Ireland) and transcardially perfused 24 h later.

2.3. Behavioural experiments

Behavioural testing began one month after sepsis induction. Each animal underwent no more than four tests, with less stressful tests (open field, sucrose preference, etc.) preceding more stressful tests (elevated plus maze, forced swim test, tail suspension test). All animals only underwent one aversive test.

2.3.1. Marble burying task

As mice may bury novel objects which they find anxiety inducing, the number of marbles buried during the test served as the dependent measure of anxiety. Animals were placed in an isolated testing arena (26 cm length × 20 cm width × 27 cm high) filled with roughly 6 cm of wood chip bedding for 30 min with marbles arranged in four rows of three, evenly spaced from each other and the sides of the test cage (Deacon, 2006). After 30 min mice were removed and marbles were considered buried where at least 2/3 of their surface was covered with woodchip. The dependent variable from this test was the number of marbles buried.

2.3.2. Open field test

Open field testing occurred in an arena of diameter 100 cm. Trial length was 300 s during which distance moved, velocity in cm/s and time spent moving as well as time in the inner and outer 50% were automatically tracked with Ethovision 3.1 software (Ethovision 3.1; Noldus Information Technology, Leesburg, VA). Time spent in the inner corridor versus the outer corridor of the arena is a measure of anxiety-like behaviour while distance, velocity and time spent moving are indexes of general locomotor behaviour.

2.3.3. Hyponeophagia test

Measuring the time it takes for rodents to sample a novel food in a novel situation is taken as a measure of anxiety-like behaviour. Testing occurred in a large transparent plexiglass container (26 length × 20 width × 13.5 cm high) placed on a white surface using existing methodology with chocolate as the novel food (Deacon, 2011). Animals were given up to 3 trials of 4 min, after which testing for that animal stopped and they were given a score of 720 s if they had not yet eaten.

2.3.4. Sucrose preference test

Preference for a sucrose solution over water functions as a dependent measure of anhedonia Following a method set out in Strekalova et al. (2004) bottles were filled with either animals' standard drinking water or 1% sucrose in the animals standard drinking water, lids securely taped in place to minimize spillage and weighed. Cages were modified to accommodate two bottles, and food was placed in both sides. Mice were then given a free choice between either tap water, or a 1% sucrose in tap water solution for 24 h. After 12 h had passed, the position of the two bottles was switched, in order to control for a side preference in drinking behaviour. After 24 h had passed, the bottles were then weighed to measure how much liquid was consumed, and sucrose preference was calculated as [sucrose consumed/(water consumed + sucrose