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Research report

Behavioral and monoamine perturbations in adult male mice with chronic inflammation induced by repeated peripheral lipopolysaccharide administration

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HIGHLIGHTS

- Chronic LPS produced biphasic effects on locomotion and anxiety in adult male mice.
- Chronic LPS induced persistent depressive-like behavior in adult male mice.
- Chronic LPS treatment caused persistent increase in splenic serotonin (5-HT) levels.

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ABSTRACT

Considering the limited information on the ability of chronic peripheral inflammation to induce behavioral alterations, including on their persistence after inflammatory stimuli termination and on associated neurochemical perturbations, this study assessed the effects of chronic (0.25 mg/kg; i.p.; twice weekly) lipopolysaccharide (LPS) treatment on selected behavioral, neurochemical and molecular measures at different time points in adult male C57BL/6 mice. Behaviorally, LPS-treated mice were hypoactive after 6 weeks, whereas significant hyperactivity was observed after 12 weeks of LPS and 11 weeks after 13 week LPS treatment termination. Similar biphasic responses, i.e., early decrease followed by a delayed increase were observed in the open field test center time, suggestive of, respectively, increased and decreased anxiety. In a forced swim test, mice exhibited increased immobility (depressive behavior) at all times they were tested. Chronic LPS also produced persistent increase in splenic serotonin (5-HT) and time-dependent, brain region-specific alterations in striatal and prefrontocortical dopamine and 5-HT homeostasis. Microglia, but not astrocytes, were activated by LPS early and late, but their activation did not persist after LPS treatment termination. Above findings demonstrate that chronic peripheral inflammation initially causes hypoactivity and increased anxiety, followed by persistent hyperactivity and decreased anxiety. Notably, chronic LPS-induced depressive behavior appears early, persists long after LPS termination, and is associated with increased splenic 5-HT. Collectively, our data highlight the need for a greater focus on the peripheral/central monoamine alterations and lasting behavioral deficits induced by chronic peripheral inflammation as there are many pathological conditions where inflammation of a chronic nature is a hallmark feature.

1. Introduction

Contrary to earlier views on the central nervous system (CNS) as an immunologically privileged site, accumulating evidence highlights the existence of an extensive dynamic bidirectional interaction between the immune system and the CNS [1–4]. From a neuropathological perspective, peripheral inflammation can aggravate an ongoing neurological damage and exaggerate motor and/or cognitive impairments in patients with neurodegenerative diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD) [5–7]. In animal models, peripherally-generated inflammatory

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mediators induced by systemic challenge with lipopolysaccharide (LPS) or double-stranded RNA (poly I:C), representative of, respectively, bacterial and viral infections, have been found to increase brain inflammatory cytokines, resulting in progressive neurotoxicity [8,9].

Peripheral LPS induced alterations in central monoamine homeostasis have been reported [10,11]. Specifically, single peripheral LPS administration increases the turnover of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) in several different brain regions [12,13]. Behaviorally, acute peripheral LPS administration causes sickness behavior in rodents [14,15] that is characterized with decreased exploratory activity, locomotion and social exploration [16–18]. Of note, if the immune system activation persists, sickness behavior can transition into a depressive-like behavior [19,20]. There is a partial symptoms-overlap between inflammation-induced sickness and depressive-like behaviors, but their course is quite different. Sickness behavior is an acute, quick, transient response to infections/inflammagens and it is reversible; depressive behavior is characterized by a delayed onset and a long-lasting nature, which results when activation of the innate immune response is exaggerated in intensity and/or duration and it is observed in the absence of concurrent increased circulating inflammatory cytokines [19,21,22].

Epidemiological investigations of the relationship between chronic inflammation and depression indicate increased rate of depressive disorders in patients suffering from chronic inflammatory conditions, as well as in patients undergoing interferon alpha (IFNα) therapy [23–25]. Few animal studies have reported behavioral deficits, including depressive-like behavior and impaired spatial memory following chronic central (intra-CNS) or peripheral LPS administration [26–29], but the laboratory studies that have investigated the impact of chronic peripheral inflammation on the induction of depression-like behavior are limited. Moreover, only limited information [30,31] exists on other motor and non-motor behavioral domains, including anxiety, and on associated neurochemical changes following long-term peripheral LPS administration. Also, data regarding the time-dependent changes in behavior, i.e., whether inflammagen (LPS)-induced initial behavioral impairments are persistent throughout the time course of chronic inflammatory stimulation or after treatment termination, are limited.

Besides inducing depression and anxiety, peripheral inflammatory events can influence the etiology and progression of many ongoing degenerative diseases, including AD and PD [7,32]. Chronic central LPS challenge models have successfully replicated key components of the characteristic neurodegenerative pattern seen in PD and have demonstrated significant early microglial activation followed by delayed and time-dependent nigral dopaminergic neuron degeneration [33,34] and a long-term disruption of AD-relevant hippocampal network activity [35,36]. Peripheral administration of a single high dose (5 mg/kg) of LPS resulted in long-lasting neuroinflammation and nigral dopaminergic neurodegeneration [37]. Data that chronic peripheral LPS administration can cause both neuroinflammation and progressive dopaminergic neuron loss similar to PD are very limited [31,38] and indicate that a genetic susceptibility might be necessary for the full pathology to occur when the inflammatory stimulus is at a lower level and of chronic nature [31].

Besides causing neuroinflammation, acute peripheral LPS administration also induces inflammatory cytokines expression in peripheral tissues, such as spleen and liver. Interestingly, chronic mild stress induced dysregulation of the anti/inflammatory cytokine balance in different brain regions and spleen was implicated in the induction of depressive-like behavior in a rat study [39]. Spleen, the secondary lymphoid organ, is largely innervated by noradrenergic fibers of the sympathetic nervous system and functions as a platelet storage site [40]. The neural signals transmitted by NE (major neurotransmitter of the sympathetic nervous system) are received by platelets where this neural input is converted to immunomodulatory signals by platelet-released 5-HT [40]. Importantly, acute central administration of immune mediators, such as interleukin-1 beta (IL-1β) or prostaglandins, causes activation of sympathetic nerves, resulting in altered splenic tissue levels of NE and/or 5-HT [41,42]. However, limited information exist on splenic monoamine alterations (if any) induced by chronic peripheral inflammation and their potential contribution in the induction of depressive-like behavior.

Considering all of the aforementioned data gaps and the high prevalence of chronic inflammatory conditions of bacterial origin [43,44], the main objectives of the current study were to investigate the effect of chronic peripheral LPS treatment in adult male C57BL/6 mice on selected behavioral, neurochemical, and molecular parameters. To assess the potential neurobehavioral consequences of chronic LPS, we employed a battery of behavioral tests that could effectively assess both the locomotor (open field, grip strength and pole tests) as well as depressive-like (forced swim test) alterations. Additionally, to gain an insight into the persistent effects of repeated peripheral LPS administration, the behavioral, neurochemical and molecular analyses were performed on separate set of mice chronically treated with LPS, followed by a substantial wait period during which treatment was discontinued.

2. Materials and methods

2.1. Reagents

Unless otherwise stated, all chemicals including lipopolysaccharide *Escherichia coli*, serotype 0111:B4 (LPS) were purchased from Sigma (St. Louis, MO).

2.2. Animals

Animals were male C57BL/6 mice (4–5 months old; Taconic, Hudson, NY) weighing 30.10 ± 0.25 g (mean ± SEM) prior to treatment and were housed (n = 5/cage) with food and water available *ad libitum* on a 12-h light/dark cycle in an AAALAC accredited facility throughout the study. All procedures involving animal handling were in accordance with the latest NIH guidelines and were approved in advance by the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia.

2.3. Animal treatment

Mice (n = 15 per group) were injected intraperitoneally (i.p.) with sterile normal saline (vehicle) or LPS at a dose of 0.25 mg/kg body weight (BW) twice weekly for up to 25 weeks. LPS/saline administration occurred at midmorning on the same two days (consistently spaced apart) of each week throughout the entire treatment duration. The dose and regimen of LPS treatment for this study was based on recently published chronic peripheral inflammatory models that have shown neuroinflammation, behavioral alterations, and nigrostriatal dysfunction (genotype-dependent) in mice [31,38,45]. Behavioral tests (described in detail below) were carried out (24 h after the last injection) at 6 and 12 weeks (n = 5 per group) post LPS/saline treatment on the same sets of mice. Randomly selected subsets (n = 5 per group) from each of the saline or LPS groups were sacrificed (24 h after the last injection) after 13 or 25 weeks of the treatment. Additionally, another subset of mice (n = 5 per group) were given LPS/vehicle for 13 weeks followed by a 12 week period without any treatment; these mice were sacrificed after the 12 week wait period (13 week on + 12 week off). Behavioral tests of these mice, which were 6- and 12-week behaviorally-experienced, were done 1 week prior to the sacrifice,