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Research report

Tumor necrosis factor-alpha during neonatal brain development affects anxiety- and depression-related behaviors in adult male and female mice

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HIGHLIGHTS

- Neonatal TNF-α exposure decreases body weight in neonate mice.
- Neonatal TNF-α exposure increases anxiety in adult male and female mice.
- Neonatal TNF-α exposure increases depression in adult males, but not in females.

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ABSTRACT

A nascent literature suggests that neonatal infection is a risk factor for the development of brain, behavior and hypothalamic–pituitary–adrenal axis which can affect anxiety- and depression-related behaviors in later life. It has been documented that neonatal infection raises the concentrations of tumor necrosis factor-alpha (TNF-α) in neonate rodents and such infections may result in neonatal brain injury, at least in part, through pro-inflammatory cytokines. In addition, previous studies have shown that TNF-α is involved in cellular differentiation, neurogenesis and programmed cell death during the development of the central nervous system. We investigated for the first time whether neonatal exposure to TNF-α can affect body weight, stress-induced corticosterone (COR), anxiety- and depression-related behaviors in adult mice. In the present study, neonatal mice were treated to recombinant mouse TNF-α (0.2, 0.4, 0.7 and 1 μg/kg) or saline on postnatal days 3 and 5, then adult male and female mice were exposed to different behavioral tests. The results indicated that neonatal TNF-α treatment reduced body weight in neonatal period in both sexes. In addition, this study obtained some experimental findings indicating the high doses of TNF-α increase stress-induced COR levels, anxiety- and depression-related behaviors in adult males, while decrease the levels of anxiety without any significant effect on depression in adult female mice. Our findings suggest that TNF-α exposure during neonatal period can alter brain and behavior development in a dose and sex-dependent manner in mice.

1. Introduction

The prenatal and neonatal periods are two fundamental phases in normal brain development in humans and experimental animals. Some adverse events such as stress and infection during these neurodevelopmental stages can increase the risk of occurrence of neuropsychiatric disorders like anxiety and depression in adulthood [1,2]. For instance, neonatal immune challenge during a sensitive period of brain development causes the long-lasting changes in immunological [3], behavioral [4], physiological [5] and neurochemical [3] parameters in adult animals. In addition, a large spectrum of studies have shown that neonatal infection leads to behavioral abnormalities such as anxiety and depression as well as abnormal hypothalamic–pituitary–adrenal (HPA) axis activity in response to stress in adulthood [4–13]. For example, we and others have indicated that neonatal immune activation on postnatal days 3 and 5 results in increased anxiety- and depression-like behaviors in adult rats and mice [4–9]. The aforementioned studies have utilized the systemic administration of lipopolysaccharide (LPS) or polyinosinic–polycytidylic acid (Poly I:C) to mimic bacterial

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and viral infections in neonate animals. It was found that peripheral inflammation leads to increased levels of pro-inflammatory cytokines (PICs) including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6 and IL-1β within the brain [14–16]. In this regard, previous studies have shown that neonatal immune activation with LPS raises the concentrations of TNF-α in neonate rats [17,18] and such infections may result in neonatal brain injury, at least in part, through PICs that cause the alteration of gene expression in the target cells. These cytokines are mainly released in body in response to the stimulation of immune system, cellular injury, hypoxia or hyperoxia [19]. Furthermore, maternal–fetal infection and hypoxia–ischemia are proposed as major pathogenetic mechanisms for perinatal brain damage, so that a relationship between PICs and perinatal brain damage has been demonstrated [20–22].

Moreover, clinical studies have shown a significant relationship between TNF-α levels and depression in humans. In this regard, a meta-analysis study indicated that there is a considerable increase in the serum TNF-α concentrations in depressed patients [23]. It is also well established that TNF-α is involved in inflammation, cellular differentiation and programmed cell death in the central nervous system (CNS) [24,25]. In addition, TNF-α can be synthesized and released by glial and neuronal cells within CNS and its normal and abnormal effects on neuronal transmission are now accepted within the brain [26]. In this context, it has been shown that TNF-α can regulate the blood-brain barrier permeability and affect neuronal and glial activity in the brain [27,28]. This cytokine plays a central role in a number of neurodegenerative diseases such as Alzheimer, stroke and multiple sclerosis [27]. TNF-α has also a key role during the development of the CNS, so that TNF-α-mediated apoptosis during this period occurs when neurons do not receive enough trophic factors [29]. Moreover, it has been reported that TNF-α affects embryonic and adult neurogenesis [30] and causes neuronal death in the hippocampus [31]. Golan et al. showed that there is an association between the lack of TNF-α expression and enhanced levels of nerve growth factor (NGF) to accelerate dentate gyrus development in the hippocampus [32]. In line with this evidence, Bernardino et al., have shown that exposure of neural precursor cells derived from the subventricular zone of neonatal mice to a low dose of TNF-α resulted in cell proliferation while exposure to a higher dose led to apoptotic cell death [33]. In another study Barker et al., reported that TNF-α-induced cell death was blocked through the treatment with anti-TNF-α on embryonic neurons [29]. Thus, because the early neonatal brain produces TNF-α in response to a peripheral immune challenge and given that TNF-α is involved in the death and dysfunction of neurons, it is possible that TNF-α be considered as a mediating agent for linking neonatal infection and neurodevelopmental damage. We therefore tested the hypothesis that TNF-α may affect brain development in neonatal phase and alter anxiety- and depression-related behaviors in adulthood.

2. Materials and methods

2.1. Ethics

All procedures of the study were performed in accordance with the ethical guidelines set by Research and Ethics Committee of the Tabriz University of Medical Sciences (GN-90-71) which completely coincides with the "National Institutes of Health NIH Guide for the Care and Use of Laboratory Animals" (NIH; Publication No. 85-23, revised 1985).

2.2. Animals

Male and female NMRI mice (70–80 days) were obtained from the animal house of Pasteur Institute (Tehran, Iran). Animals were housed in standard polycarbonate cages in a room with a 12:12 h light/dark cycle (lights on 08:00–20:00 h), controlled temperature (23 ± 1 °C) and had free access to food and water. These conditions were kept as a standard housing condition in all stages of experiments.

2.3. Newborn mice

Following a 2-week period of acclimatization to the new animal housing room, to facilitate the mating, male and female mice were kept together one-by-one in a cage. Female mice were visually monitored daily for confirmation of pregnancy, when it was confirmed the female mice were removed from the breeding cages and housed individually in standard cages. All pregnant animals were allowed to have normal delivery and the first day of birth was considered PND 0 [5]. One day after the birth, all litters were culled to 6 pups per mother (3 male and 3 female). On the day 23, litters were weaned by removal of the mother and then were housed with the same sex litter-mates (3 animals per cage). A total of thirty-six litters were used during this study in three stages, each of which included 12 litters. Only one mouse per litter was used for each of the experiment to avoid the litter-effect.

2.4. Experimental design

A summary of the experimental design is shown in Fig. 1. The dams were removed from their pups for approximately 5 min and the pups were weighed and received subcutaneously (in the interscapular region) injection of recombinant mouse TNF-α (0.2, 0.4, 0.7 and 1 μg/kg; carrier-free; BioLegend Inc., San Diego, USA) or vehicle solution (1 ml/kg) on the PNDs 3 and 5. The timing of TNF-α treatment was chosen based on our previous studies [4,5]. TNF-α was dissolved in saline (0.9% NaCl) and injections were performed between 10:00 and 11:00 A.M. Each injection was performed through a needle (30-gauge) connected by polyethylene tubing to a 10-μl Hamilton syringe. Newborn mice were returned to their housing immediately after saline or TNF-α administration. In order to perform the behavioral tests, in each sex, mice from both neonatal treatment conditions were divided into 2 clusters (each cluster only used for two behavioral tests, with a 10-day interval between each test; $N = 10$/group).

2.5. Body weight

The body weights of the male and female mice (0.01 g) were regularly monitored at 11:00 A.M. every 10 days from PNDs 3 to 40.

2.6. Behavioral tests

Behavioral assessments began at PND 70. The following behavioral tests are widely accepted models for measuring anxiety and depression-related behaviors in mice. All behavioral parameters were recorded by observers blind to the treatment. In addition, all behavioral tests were conducted in a quiet room during the light period (between 13:00 and 18:00 h) under bright and moderate illumination and the mice were kept in the room for at least 1 h before the assessment. At the end of each test session, the arena and the objects were carefully cleaned with 70% ethanol. In all experiments, each male or female mouse was tested only once in the one test. Furthermore, the female mice were not monitored for estrus cycle since it is an invasive and stressful procedure that can impose dramatic changes in the animal response to other stressors. In addition, it was not possible to provide the adult male mice with