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Neuroinflammation and cognitive function in aged mice following minor surgery

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ABSTRACT

Following surgery, elderly patients often suffer from postoperative cognitive dysfunction (POCD) which can persist long after physical recovery. It is known that surgery-induced tissue damage activates the peripheral innate immune system resulting in the release of inflammatory mediators. Compared to adults, aged animals demonstrate increased neuroinflammation and microglial priming that leads to an exaggerated proinflammatory cytokine response following activation of the peripheral immune system. Therefore, we sought to determine if the immune response to surgical trauma results in increased neuroinflammation and cognitive impairment in aged mice. Adult and aged mice underwent minor abdominal surgery and 24 h later hippocampal cytokines were measured and working memory was assessed in a reversal learning version of the Morris water maze. While adult mice showed no signs of neuroinflammation following surgery, aged mice had significantly increased levels of IL-1β mRNA in the hippocampus. Minor surgery did not result in severe cognitive impairment although aged mice that underwent surgery did tend to perseverate in the old target during reversal testing suggesting reduced cognitive flexibility. Overall these results suggest that minor surgery leads to an exaggerated neuroinflammatory response in aged mice but does not result in significantly impaired performance in the Morris water maze.

1. Introduction

Medical advances in techniques for anesthesia and surgery allow elderly patients to undergo surgical procedures later in life with a reduced risk in mortality. However, aged individuals are at a substantially increased risk for central nervous system (CNS) dysfunction and in particular, cognitive decline following surgery. Elderly patients often suffer from postoperative delirium following a surgical procedure and these cognitive deficits can linger for months and even years after physical recovery from the operation. Postoperative cognitive dysfunction (POCD) is characterized by a persistent decline of cognitive performance after surgery as assessed by preoperative and postoperative cognitive testing, and is defined as a "deterioration of intellectual function presenting as impaired memory or concentration" (Moller et al., 1998; Rasmussen, 2006). Estimated prevalence of postoperative cognitive dysfunction in patients over the age of 60 is 15–25% with approximately 10% exhibiting symptoms 3 months after surgery (Dodds and Allison, 1998; Olin et al., 2005; Rasmussen and Siersma, 2004). Patients who exhibit postoperative cognitive disorders after surgery often continue to deteriorate and are three times more likely to suffer further cognitive decline 1–2 years after surgery (Abildstrom et al., 2000; Lewis et al., 2007).

The mechanisms underlying the pathology of this cognitive disorder are not well understood and the possible contributions of anesthetics and/or analgesics versus the contribution of the surgical trauma itself are unclear. Human studies have identified advancing age, duration of anesthesia and multiple surgeries as risk factors for POCD but are unable to distinguish between the contribution of major surgery and anesthesia to the observance of POCD (Moller et al., 1998; Newman et al., 2001; Rohan et al., 2005). Other factors such as hypoxia–ischemia, hypotension and microembolism do not seem to influence the incidence of POCD suggesting it may be the surgical trauma itself that leads to changes in cognition (Cook et al., 2007; Koch et al., 2007; Moller et al., 1998). Several large-scale patient studies have consistently found increasing age to be the most relevant risk factor in the development of POCD (Moller et al., 1998; Monk et al., 2008; Rasmussen, 2006; Williams-Russo et al., 1995). Thus, it may be that the aging brain is more vulnerable to the additional insult of a surgical procedure resulting in long-lasting cognitive impairment.

Our laboratory has shown that normal aging is associated with increased neuroinflammation and that an exaggerated inflammatory response occurs in the healthy aged brain when lipopolysaccharide (LPS) activates the peripheral innate immune system

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(Godbout et al., 2005). In addition to increased expression of proinflammatory mediators such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) in the brain, aged animals suffer from exaggerated sickness behavior and disrupted cognitive processing following immune system activation (Abraham et al., 2008; Chen et al., 2008; Godbout et al., 2005). Normal aging is thought to "prime" the central cytokine compartment, therefore an amplified and prolonged inflammatory response in the brain occurs when the peripheral innate immune system is activated. This exaggerated neuroinflammatory reaction may underlie the neurobehavioral impairments observed in elderly patients with an infection and explain why infection can exacerbate neurodegenerative diseases such as Multiple Sclerosis and Alzheimer's (Perry et al., 2007).

During aging, increased inflammation and microglial priming set the stage for an amplified central cytokine reaction to a secondary stimulus of the innate immune system. It is known that tissue damage resulting from surgery activates the peripheral innate immune system leading to activation of the cytokine cascade and the release of many inflammatory mediators including oxygen free radicals, arachadonic acid metabolites, cytokines, nitric oxide, and endothelins (Giannoudis et al., 2006; Karlidag et al., 2006; Levy and Tanaka, 2003; Lin et al., 2000). However, it is not yet known if a peripheral immune response to surgical trauma results in an exaggerated neuroinflammatory response in the aged brain. Therefore, the present study investigated whether aged mice demonstrate an increased neuroinflammatory response and prolonged deficits in cognitive function following surgery-induced activation of the peripheral innate immune system. An initial study determined that anesthetics and analgesics alone did not alter proinflammatory cytokines levels in the hippocampus of adult or aged mice. Furthermore, while adult mice showed no signs of neuroinflammation following surgery, aged mice had significantly increased levels of IL-1β mRNA in the hippocampus. Although elevation in IL-1β did not result in gross learning and memory impairment, aged mice that underwent surgery tended to perseverate in the old target quadrant during reversal testing suggesting alterations in cognitive flexibility. These results suggest that minor surgery significantly increases IL-1β production in the brain of aged animals but does not result in significant behavioral deficits in a reversal learning version of the Morris water maze.

2. Materials and methods

2.1. Animals

Adult (4–6 months old) and aged (23–25 months old) male BALB/c mice from our in-house specific pathogen free colony were used. Mice were housed in polypropylene cages and maintained at 23 °C under reverse phase 12-h light:12-h dark cycle with ad libitum access to water and rodent chow. At the end of each study mice were examined postmortem for gross signs of disease (e.g., splenomeglia and tumors). Data from mice determined to be unhealthy were excluded from analysis. All procedures were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and approved by the University of Illinois Institutional Animal Care and Use Committee.

2.2. Surgical procedure

A crucial asset in an animal model of surgery is the ability to control for a variety of confounding factors that make data from human studies of POCD difficult to interpret. Using an animal model, we were able to control both the length and severity of the abdominal surgery. Mice were deeply anesthetized using ketamine and xylazine (1.0 mg and 0.1 mg/10 g BW intraperitoneally (i.p.), respectively). After the surgical site was shaved and sterilized, a 1.5 cm incision was made in the upper left quadrant through the skin and muscle wall. A sterile probe was then inserted into the body cavity to gently manipulate the internal organs for 1 min. Three dissolvable sutures were used to close the muscle wall and four silk thread sutures were used to close the skin. To limit variability all surgeries were performed by one person and lasted ~10 min. Animals receiving postoperative analgesics were injected subcutaneously with buprenorphine (1.0 mg/10 g BW) upon recovery from anesthesia.

2.3. Brain cytokine mRNA measurement by quantitative real-time PCR

Total RNA was isolated from homogenized brain regions using the Tri Reagent protocol (Sigma, St. Louis, MO). A QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA) was used for cDNA synthesis with integrated removal of genomic DNA contamination according to the manufacturer protocol. In brief, RNA samples were mixed with gDNA Wipeout Buffer and RNase-free water and incubated at 42 °C for 2 min. Quantiscript Reverse Transcriptase, Quantiscript RT Buffer and RT Primer mix were added to samples and incubated at 42 °C for 15 min, followed by incubation at 95 °C for 3 min to inactivate Quantiscript Reverse Transcriptase. Quantitative real-time PCR was performed using the Applied Biosystems (Foster, CA) Assay-on Demand Gene Expression protocol. In short, cDNA was amplified by PCR where a target cDNA (IL-1β, Mm00434228_ml; TNF-α, m00443258_ml; IL-6, Mm00446190_ml) and a reference cDNA (glucose-3 phosphate dehydrogenase, Mn99999915_gl) were amplified simultaneously using an oligonucleotide probe with a 5′ fluorescent reporter dye (6-FAM) and a 3′ quencher dye (NFQ). PCR reactions were performed at the following conditions: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Fluorescence was determined on an ABI PRISM 7900HT-sequence detection system (Perkin Elmer, Forest City, CA). Data were analyzed using the comparative threshold cycle (C_t) method, and results are expressed as fold change.

2.4. Behavioral tests

2.4.1. Locomotor activity

To estimate locomotor activity, mice were kept in their home cage and video recorded during 3 min tests using a camera mounted directly above the cage. On video records, cages were divided into six identical rectangles and a trained observer who was blind to experimental treatments determined the frequency of line crossing. A subject was considered to have crossed a line only if its fore and hind limbs entered a new rectangle.

2.4.2. Spatial working memory – Morris water maze

A circular tank 100 cm in diameter and 30 cm deep was filled with water (24–26 °C) to a depth of 25 cm. A transparent round platform 10 cm in diameter was placed ~0.5 cm below the surface of the water. In this test of spatial memory the animal must learn to use distinctive distal visual cues surrounding the pool to navigate a direct path to the hidden platform. Animal training took place during a 6 day acquisition phase with three massed trials administered each day. The platform remained in a constant location during the acquisition phase. Animals were placed on the platform for 30 s preceding the start of each training session. The trials were conducted using a pseudorandom protocol in which mice were placed in the water in one of three preset entry locations. Mice were allowed to swim freely for 60 s or until the platform was reached. If the platform was not located during the 60 s, mice were guided to the platform and allowed to remain for 30 s. After completion of three consecutive trials, mice were placed in their