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Microglia priming by interleukin-6 signaling is enhanced in aged mice

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ABSTRACT

During peripheral infection, excessive production of pro-inflammatory cytokines in the aged brain from primed microglia induces exaggerated behavioral pathologies. While the pro-inflammatory cytokine IL-6 increases in the brain with age, its role in microglia priming is not known. This study examined the functional role of IL-6 signaling on microglia priming. Our hypothesis is that IL-6 signaling mediates primed states of microglia in the aged. An initial study assessed age-related alteration in IL-6 signaling molecules; sIL-6R and sgp130 were measured in cerebrospinal fluid of young and aged wild-type animals. Subsequent studies of isolated microglia from C57BL6/J (IL-$6^{+/+}$) and IL-6 knock-out (IL-$6^{-/-}$) mice showed significantly less MHC-II expression in aged IL-$6^{-/-}$ compared to IL-$6^{+/+}$ counterparts. Additionally, adult and aged IL-$6^{+/+}$ and IL-$6^{-/-}$ animals were administered lipopolysaccharide (LPS) to simulate a peripheral infection; sickness behaviors and hippocampal cytokine gene expression were measured over a 24 h period. Aged IL-$6^{-/-}$ animals were resilient to LPS-induced sickness behaviors and recovered more quickly than IL-$6^{+/+}$ animals. The age-associated baseline increase of IL-1β gene expression was ablated in aged IL-$6^{-/-}$ mice, suggesting IL-6 is a key driver of cytokine activity from primed microglia in the aged brain. We employed in vitro studies to understand molecular mechanisms in priming factors. MHC-II and pro-inflammatory gene expression (IL-1β, IL-10, IL-6) were measured after treating BV.2 microglia with sIL-6R and IL-6 or IL-6 alone. sIL-6R enhanced expression of both pro-inflammatory genes and MHC-II. Taken together, these data suggest IL-6 expression throughout life is involved in microglia priming and increased amounts of IL-6 following peripheral LPS challenge are involved in exaggerated sickness behaviors in the aged.

1. Introduction

Peripheral immune stimulation causes the production of pro-inflammatory cytokines, such as IL-6. The signal for these cytokines is transported to the brain via both neural and humoral pathways, including vagal afferents and direct crossing of the blood-brain barrier (Banks et al., 1994; Maier et al., 2006; Poon et al., 2013; Quan, 2008). In the brain, microglial cells respond to signals from the periphery by producing pro-inflammatory cytokines, which then target neurons to elicit a sickness behavior response that is adaptive in nature (Robert et al., 2006). Microglia appear to be the main cell in the brain that express the IL-6 receptor and potently secrete IL-6 during peripheral immune stimulation (Burton et al., 2013). IL-6 knockout (IL-$6^{-/-}$) mice have shown an overall decrease in the number of activated brain macrophages associated with cortical lesions, suggesting a role for IL-6 in the orchestration of central nervous system inflammation (Penkowa et al., 1999). IL-6 in the central nervous system is also implicated in a myriad of behavioral pathways, including but not limited to neurodegeneration, astrocytosis, and changes in *c-fos* expression (Banks et al., 1994; Campbell et al., 1993; Vallières et al., 1997). In the brain of adult mice, IL-6 plays a pivotal role in mediating lipopolysaccharide (LPS)-induced sickness behaviors (Burton et al., 2011) as well as cognitive deficits (Sparkman et al., 2006; Wei et al., 2015). Although it is known that aged mice experience exaggerated sickness behaviors (Godbout et al., 2005; Kelley et al., 2013), it is yet unclear if IL-6 contributes to these behaviors.

In addition to the classical IL-6 pathway, where IL-6 binds its receptor on the cell membrane, there is an IL-6 trans-signaling pathway.

Abbreviations: IL-6, interleukin-6; LPS, lipopolysaccharide; sIL-6R, soluble IL-6 receptor; gp130, glycoprotein 130; sgp130, soluble glycoprotein 130; MHC-II, major histocompatibility complex class II; IL-1B, interleukin-1β; TNF-α, tumor necrosis factor alpha; CD45, cluster of differentiation 45; CD68, cluster of differentiation 68; STAT3, signal transducer and activator of transcription 3

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In this pathway, IL-6 binds the soluble IL-6 receptor (sIL-6R) in extra cellular fluid; this complex then binds gp130 present on the cell membrane of any cell (Rose-John and Neurath, 2004).Therefore, an IL6R−/gp130+ cell is able to utilize IL-6 signaling (Rose-John and Neurath, 2004). Our previous work has identified this mechanism in sickness and cognitive behaviors in adult mice (Burton et al., 2011; Burton and Johnson, 2012, p. 20). However, little has been done to investigate the effects of this mechanism on sickness and other behaviors in aged mice.

Microglia are derived from early myeloid lineage cells and represent approximately 10–12% of the total central nervous system cell population (Ginhoux et al., 2010). Normally, in the absence of any stimuli, microglia are quiescent and in an immune surveillance state (Nimmerjahn et al., 2005). Once activated, microglia possess a macrophage-like phenotype, including inflammatory cytokine production, phagocytosis, and antigen presentation (Ransohoff and Perry, 2009). This neuroinflammatory process is normally transient, with microglia returning to a resting state as the immune stimulus is resolved. However, in various brain environments, from neurodegenerative disease to aging, it is proposed that elements render microglia in a “primed” or “reactive” state, wherein a subsequent local or peripheral immune challenge causes an exaggerated and protracted cytokine production (D'Avila et al., 2018; Dilger and Johnson, 2008; Godbout and Johnson, 2009; Norden and Godbout, 2013). Furthermore, markers of primed microglia, such as major histocompatibility complex class II (MHC-II) and CD68, are increased in the brain during pathology and normal aging (Burton et al., 2016; Frank et al., 2006a; Ogura et al., 1994; Safaiyan et al., 2016; Wong et al., 2005). Although typically confined to professional antigen presenting cells, MHC-II can be induced in other cells types if exaggerated amounts of pro-inflammatory substances, such as IL-6, are present in the extracellular environment (Holling et al., 2004). Ex-vivo or peripheral immune stimulation results in an exaggerated cytokine response in microglia that express higher levels of MHC-II in aged mice (Henry et al., 2008; Njie et al., 2012). Furthermore, studies have also shown IL-6 induced MHC-II expression in peripheral monocyte-derived cells (Shafer et al., 2002; Vassiliadis and Papadopoulos, 1995). Taken together, these findings suggest that enhanced microglial priming in aged populations may be due to the influence of IL-6.

Evidence from previous studies also shows that microglia from aged mice are potent producers of IL-6 (Godbout and Johnson, 2004; Ye and Johnson, 1999). Decreasing the amount of IL-6 after or during peripheral stimulation also decreases the amounts of other pro-inflammatory cytokines (Godbout et al., 2004). Further findings show that exaggerated pro-inflammatory cytokines in aged mice interact with the brain microenvironment, leading to more severe sickness behaviors (Godbout et al., 2005), depressive-like behaviors (Godbout et al., 2008), and deficits in hippocampal-dependent learning and memory when compared with younger animals (Barrientos et al., 2006; Chen et al., 2008). Interestingly, when the pro-inflammatory arm of IL-6 is inhibited during peripheral immune stimulation, aged mice are refractory to cognitive deficits (Burton et al., 2011). Although these data lay the groundwork to support the notion that microglial priming via MHC-II plays a central role in exaggerated neuroinflammation and behavioral deficits, IL-6-specific involvement during aging has yet to be determined. This study therefore seeks to bridge multiple gaps, including establishing a causative role of IL-6 signaling in the protracted sickness phenotype associated with aging.

2. Experimental procedures

2.1. BV.2 microglial cell culture

The murine microglia cell line, BV.2 (a gift from Linda Van Eldik, Northwestern University, Evanston, IL; used at UIUC) has been used as a model to investigate the neuroimmune system (Jang et al., 2008; Zorina et al., 2010). Cells were maintained in 150-cm^2 tissue culture flasks (BD Falcon, Franklin Lakes, NJ) in Dulbecco's Modified Eagle's Media (DMEM) (Bio-Whittaker, Cambrex, MD) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT), 200 mM glutamine, and 100 units/mL penicillin/streptomycin (Invitrogen, Carlsbad, CA) at 37 °C in a humidified incubator under 5% CO_2. Confluent cultures were passed by trypsinization. Cells were centrifuged (5 min (min) at 27 °C, 200 × *g*) and culture medium was removed. In all experiments, cells were re-suspended in DMEM supplemented with 10% FBS and seeded in six-well plates (BD Falcon, Franklin Lakes,NJ) at a population of 5×10^5 cells per well overnight at 37 °C in a humidified incubator under 5% CO_2 before treatments. Cells were treated with sterile saline containing 0.1% bovine serum albumin (BSA) (vehicle) or 25 ng/mL sIL-6R (R&D systems, Minneapolis, MN) for 1 h (h) followed by treatment with recombinant 100-1000 pg/mL IL-6 (R&D systems, Minneapolis, MN) or 100 ng/mL LPS (serotype 0127:B8, obtained from Sigma, St. Louis, MO) for 12–24 h. Cells were then washed with ice cold phosphate buffered saline (PBS) and prepared for flow cytometric analysis or gene expression. BV.2 microglial cells were assayed for the surface markers Cd11b and MHC-II as described previously, with a few modifications (Burton et al., 2011). In brief, Fc receptors were blocked with anti-CD16/CD32 antibody (eBioscience, San Diego, CA) in a PBS/1% BSA/sodium azide solution and incubated with anti-CD11b APC and anti- MHC-II PE (eBioscience, San Diego, CA), fluorescently labeled isotype antibodies for APC and PE (eBioscience, San Diego, CA), and unstained samples were used for controls. Expression of surface receptors was determined using a Becton-Dickinson LSR II Flow Cytometer (Red Oaks, CA). Thirty thousand events were collected; flow data were analyzed using FCS Express software (De Novo Software, Los Angeles, CA).

2.2. Animal studies

Adult (3–5 months) and aged (22–24 months) male C57BL/6 (IL-$6^{+/+}$) and IL-6 knockout B6.129S2-Il6tm1 Kopf/J (IL-$6^{-/-}$) (Kopf et al., 1994) mice were used. All mice were purchased from Jackson Laboratory (Bar Harbor, ME) and were 2-months old upon receipt. Mice were housed in polypropylene cages and maintained at 21 °C under a reverse-phase 12-h light-dark cycle with ad libitum access to water and rodent chow. At the end of each study, mice were examined post mortem for gross signs of disease (e.g., tumors or splenomegaly). Data from mice determined to be unhealthy were excluded from the analysis (< 5%).

All procedures were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the University of Texas at Dallas Institutional Animal Care and Use Committee.

2.3. Experimental protocols

Mice were handled 1–2 min each day for 7 days before experimentation to acclimate them to handling. To assess the effects of systemic LPS on sickness behavior and pro-inflammatory gene expression in the hippocampus, mice were injected intraperitoneally (IP) with sterile saline or 3.30 mg/kg body weight (100 μg) LPS (serotype 0127:B8, obtained from Sigma, St. Louis, MO). An array of sickness behaviors that assess motivation and survivability measures (locomotor activity, food intake, and weight loss) (Kent et al., 1992) were recorded starting 4 h after LPS administration and continued until 24 h. Mice were killed by CO_2 asphyxiation 24 h later. The brain was rapidly removed and dissected to obtain hippocampal tissue. Hippocampal tissue was snap frozen in liquid nitrogen and stored at -80° C for later analysis. In some cases, microglia from whole brain, to be used in flow cytometry experiments, were also isolated from these animals.