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DIFFERENTIAL RESPONSE TO INTRAHIPPOCAMPAL INTERLEUKIN-4/INTERLEUKIN-13 IN AGED AND EXERCISE MICE

ALYSSA LITTLEFIELD[a,b]* AND RACHEL A. KOHMAN[a]

[a] *University of North Carolina Wilmington, Department of Psychology, Wilmington, NC, USA*

[b] *Rosalind Franklin University of Medicine and Science, Department of Neuroscience, Chicago, IL, USA*

Abstract—Normal aging is associated with low-grade neuroinflammation that results from age-related priming of microglial cells. Further, aging alters the response to several anti-inflammatory factors, including interleukin (IL)-4 and IL-13. One intervention that has been shown to modulate microglia activation in the aged brain, both basally and following an immune challenge, is exercise. However, whether engaging in exercise can improve responsiveness to anti-inflammatory cytokines is presently unknown. The current study evaluated whether prior exercise training increases sensitivity to anti-inflammatory cytokines that promote the M2 (alternative) microglia phenotype in adult (5-month-old) and aged (23-month-old) C57BL/6J mice. After 8 weeks of exercise or control housing, mice received bilateral hippocampal injections of an IL-4/IL-13 cocktail or vehicle. Twenty-four hours later hippocampal samples were collected and analyzed for expression of genes associated with the M1 (inflammatory) and M2 microglia phenotypes. Results show that IL-4/IL-13 administration increased expression of the M2-associated genes found in inflammatory zone 1 (Fizz1), chitinase-like 3 (Ym1), Arginase-1 (Arg1), SOCS1, IL-1ra, and CD206. In response to IL-4/IL-13 administration, aged mice showed increased hippocampal expression of the M2-related genes Arg1, SOCS1, Ym1, and CD206 relative to adult mice. Aged mice also showed increased expression of IL-1β relative to adults, which was unaffected by wheel running or IL-4/IL-13. Wheel running was found to have modest effects on expression of Ym1 and Fizz1 in aged and adult mice. Collectively, our findings indicate that aged mice show a differential response to anti-inflammatory cytokines relative to adult mice and that exercise has limited effects on modulating this response.

*Correspondence to: A. Littlefield, University of North Carolina Wilmington, Department of Psychology, 601 S. College Road, Wilmington, NC 28409-5612, USA.
E-mail addresses: alyssa.littlefield@my.rfums.org (A. Littlefield), kohmanr@uncw.edu (R. A. Kohman).

Abbreviations: Arg1, Arginase-1; BDNF, brain derived neurotrophic factor; Fizz1, found in inflammatory zone 1; IGF, insulin-like growth factor; IL, interleukin; IL-1ra, IL-1 receptor antagonist; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; RT-PCR, real-time polymerase chain reaction; s.c., subcutaneous; SEM, standard error of the mean; SOCS, suppressor of cytokine signaling; TBI, traumatic brain injury; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor; Ym1, chitinase-like 3.

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Key words: M2, anti-inflammatory, wheel running, IL-1ra, microglia, cytokine.

INTRODUCTION

Microglia are the resident immune cells of the central nervous system. In their ramified resting state these cells constantly scan the microenvironment and upon detecting a change, they rapidly activate (Kettenmann et al., 2011). The form of this activation is dependent on the stimulus encountered. Detection of any pathological changes or inflammatory molecules induces microglia to express the classic inflammatory form of activation, referred to as the M1 phenotype (Kreutzberg, 1996). M1 microglia increase levels of the activation markers CD86, major histocompatibility complex II and CD11b, proliferate, and release a host of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α (Kettenmann et al., 2011). Induction of the M1 phenotype provides a rapid and non-specific immune response in order to clear an invading pathogen by triggering inflammation. In contrast, microglia are also capable of expressing an alternative or M2 phenotype. This activation state is neuroprotective, characterized by the release of anti-inflammatory molecules including IL-4, IL-13, and IL-10 as well as neurotrophic factors and is thought to promote healing through the resolution of inflammation (Mosser, 2003; Ponomarev et al., 2007; Pepe et al., 2014). Additionally, the M2 phenotype increases levels of Arginase-1 (Arg1) which contributes to wound healing and matrix deposition, chitinase-like 3 (Ym1), found in inflammatory zone 1 (Fizz1) which promotes deposition of the extracellular matrix, and CD206 a mannose receptor (Cherry et al., 2014). Prior work has shown that microglia can be shifted to this neuroprotective phenotype through exposure to IL-4 and/or IL-13 (Butovsky et al., 2005; Lee et al., 2013). M2 microglia have been further broken down into the functional subphenotypes M2a, which deals with repair/regeneration, M2b, which is immunoregulatory, and M2c, which is associated with acquired-deactivation (Chhor et al., 2013). These M2 categories were originally described in peripheral macrophages, but microglia show similar forms of activation (Mosser, 2003; Mosser and Edwards, 2008). M2a and M2c phenotypes are known to reduce M1 inflammatory cytokines while increasing the anti-inflammatory cytokines IL-10 and IL-4 (Roszer, 2015). Clearly, cells

expressing the M2 phenotype mediate the resolution of inflammation and allow an organism to recover from an insult.

As the brain ages, microglia become primed toward the inflammatory M1 state (Sierra et al., 2007). These age-related changes translate to an increase in basal levels of inflammatory cytokines as well as a prolonged neuroinflammatory and behavioral response following an immune challenge (Godbout et al., 2005; Sierra et al., 2007; Dilger and Johnson, 2008). An attenuated response to regulatory factors that limit microglial cell activation likely contributes to the development of low-grade chronic inflammation within the aged brain. (Fenn et al., 2012; Lee et al., 2013; Norden and Godbout, 2013). For instance, aged animals show reduced expression of CD200, which is released by neurons and reduces microglial cell activation (Frank et al., 2006). Additionally, following exposure to the bacterial endotoxin lipopolysaccharide (LPS), microglia from aged mice exhibit prolonged down-regulation of the fractalakine receptor. Activation of the fractalakine receptor helps maintain microglia in a resting state as well as attenuate inflammation during recovery from an immune challenge (Wynne et al., 2010; Norden and Godbout, 2013). Further, Fenn et al. (2012) report that exposing M1 activated microglia from adult mice to IL-4 induced the M2 anti-inflammatory phenotype as evidenced by increased levels of Arg1, IL-10, suppressor of cytokine signaling (SOCS)-1, and SOCS3. However, M1 microglia from aged mice were unresponsive to IL-4 exposure and maintained a classically activated phenotype. In addition, aged mice failed to show an increase in the surface expression of IL-4 receptor-alpha following an immune challenge (Fenn et al., 2012), indicating that age-related deficits in the IL-4 and IL-13 signaling pathways likely contribute to aberrant microglia activation. Lee et al. (2013) administered an IL-4/IL-13 cocktail without prior cell activation and found that three days post treatment aged mice had lower expression of Fizz1 and failed to induce Arg1, Ym1, and insulin-like growth factor (IGF)-1 compared to adult and middle-aged mice, providing further evidence that induction of the M2 response following stimulation with IL-4/IL-13 is diminished in the aged.

One possible intervention for attenuating the age-related dysfunction of microglia is exercise. In aged animals exercise has been shown to down-regulate microglia activation, attenuate LPS-induced IL-1β production, decrease microglia proliferation, and increase the proportion of microglia that co-label with IGF-1 and brain derived neurotrophic factor (BDNF) (Nichol et al., 2008; Barrientos et al., 2011; Kohman et al., 2012; Littlefield et al., 2015). However, reductions in LPS-induced cytokine expression are not consistently seen. For example, prior work found that voluntary wheel running did not attenuate LPS-induced reduction in BDNF or increases in TNF-α, IL-1β, IL-6, and IL-10 in aged mice (Martin et al., 2013, 2014). In the absence of an immune challenge, exercise has been shown to increase levels of anti-inflammatory cytokines such as IL-10 as well as neurotrophic factors such as BDNF in the brain of young mice (de Almeida et al., 2013). Collectively, the evidence indicates that exercise may modify microglia activation in the aged brain, potentially attenuating the age-related priming toward the classic inflammatory phenotype. Whether exercise is capable of modulating how microglia in the aged brain respond to M2-inducing signals is currently unknown.

Age-related changes in immune function appear to alter the response to M2-inducing stimuli. Exercise has been shown to attenuate certain aspects of the age-related priming of microglia toward the M1 phenotype. Whether exercise alters the ability of aged subjects to express the M2 phenotype is presently unknown. The objective of the current study was to determine whether prior exercise increases microglia responsiveness to anti-inflammatory cytokines in aged animals. Specifically, we determined whether exercise in the form of voluntary wheel running alters hippocampal expression of M2 (i.e., Arg1, Ym1, Fizz1, IL-1 receptor antagonist [IL-1ra], transforming growth factor-β [TGF-β], CD206, and SOCS1) and M1 (i.e., IL-1β) associated genes in adult and aged mice following infusion of the anti-inflammatory cytokines IL-4 and IL-13.

EXPERIMENTAL PROCEDURES

Experimental subjects

Subjects were 31 adult (5-month-old) and 28 aged (23-month-old) C57BL/6J male mice. Aged mice were purchased from the National Institute on Aging rodent colony maintained by Charles River and adult mice were bred in-house from breeding stock purchased from The Jackson Laboratory (Bar Harbor, Maine). Mice were individually housed under a reverse light/dark cycle. Throughout the experiment mice were given free access to food and water. Experimental procedures and animal care were in accordance with the *Guide for the Care and Use of Laboratory Animals* and an approved protocol reviewed by the Institutional Animal Care and Use Committee at the University of North Carolina Wilmington.

Experimental design

Half of the adult and aged mice were semi-randomly assigned to the exercise condition and were individually housed in polypropylene cages (36 cm L × 20 cm W × 14 cm H) containing a running wheel (23 cm diameter; Respironics, Bend, OR, USA). Mice had 24-hour access to the running wheel. The individual wheel cages were connected to a computer running the Vital View software (Respironics, Bend, OR, USA) that collected the number of wheel rotations per minute. The remaining adult and aged mice were assigned to the control condition and were housed individually (29 cm L × 19 cm W × 13 cm H) without a running wheel. Following eight weeks of exercise or control housing, all mice received bilateral hippocampal injections of either an M2 promoting cytokine cocktail (containing IL-4 and IL-13) or vehicle (0.2 M phosphate-buffered saline (PBS)), procedure described below. Within an age group mice were assigned to receive the cytokine

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