Experimental Gerontology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Aging leads to altered microglial function that reduces brain resiliency increasing vulnerability to neurodegenerative diseases

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ARTICLE INFO

Article history:
Received 14 November 2016
Received in revised form 27 January 2017
Accepted 31 January 2017
Available online xxxx

Keywords:
Microglia
Polyphenol

ABSTRACT

Aging is the primary risk factor for many neurodegenerative diseases. Thus, understanding the basic biological changes that take place with aging that lead to the brain being less resilient to disease progression of neurodegenerative diseases such as Parkinson's disease or Alzheimer's disease or insults to the brain such as stroke or traumatic brain injuries. Clearly this will not cure the disease *per se*, yet increasing the ability of the brain to respond to injury could improve long term outcomes. The focus of this review is examining changes in microglia with age and possible therapeutic interventions involving the use of polyphenol rich dietary supplements.

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Aging is the primary risk factor for neurodegenerative diseases and is associated with increased morbidity and mortality from acute and chronic injuries that lead to cognitive decline. One factor thought to contribute to this loss of resilience is a biological background of elevated inflammation that is characteristic of aged organisms. However the underlying molecular alterations that lead to inflammation and the therapeutic approaches to improve resiliency are not fully understood (Bennet et al., 1996; Michaud et al., 2013; Moll et al., 2014; Niccoli and Partridge, 2012). Aging is a complex process that involves cellular senescence, a gradual loss of tissue homeostasis, both of which contribute to reduced organ function. Aging involves multiple mechanisms that lead to diminished organism homeostasis. It is becoming clear that the "environment" of the aged brain as well as the peripheral organs has a profound effect on the function of the brain. These age related changes can compromise the brain's regenerative capacity in response to the CNS challenges that arise from acute injuries such as stroke or head injuries, or chronic diseases like Parkinson's Disease and Alzheimer's Disease. Two major biological processes that characterize this aged "environment" are oxidative stress and inflammation; microglia are one of the primary cell types in the brain that contribute to both oxidative stress and inflammation. Microglia are constantly sensing the environment and responding to numerous signals that indicate the health status of the surrounding neurons and other glial cells. In young brain these responses are appropriately balanced and microglia can effectively protect the CNS from immunologic insults, like invading pathogens, while avoiding the damage associated with sustained activation. In the aged brain microglia have been reported to be in a "primed" state where they have an increased response to pro-inflammatory cytokines such as interleukin (IL)1-β and tumor necrosis factor (TNF) α. In this primed state they also show a blunted response to anti-inflammatory signals such as IL-10 and IL-4 (Fenn et al., 2012; Lee et al., 2013; Norden et al., 2015).

1. Microglial changes with age

Microglia are continually assessing the microenvironment and can respond to a variety of stimuli by rapidly moving between activation states. These activation states were initially termed M1 or classical pro-inflammatory and M2 or alternative activation. There is an ongoing balance of expression of cytokines from microglia depending on the surrounding signaling molecules. However, it is important to mention that it is becoming clear that microglial phenotype is quite complex. Some researchers have suggested that microglia can be categorized into a further subdivision of phenotypes M2a, M2b and M2c in an attempt to clarify some of these differences, as these have been used to classify macrophage responses to varying stimuli (Wilcock, 2012). It has also been shown that even this classification is likely too simple and that at any given time microglia can express markers of many of the subtypes of activation and perhaps we should abandon the dogma of trying to put microglia into a box (Heneka et al., 2015; Morganti et al., 2016). It has been demonstrated that in the aged brain, microglia do not respond to the environment in the same manner as young and there are high levels of IL1β and TNFα and low levels of IL-10 even under basal conditions (Gemma et al., 2005; Gemma et al., 2002; Michaud et al., 2013; Monje et al., 2003). To demonstrate this, Lee et al. stimulated microglial activation in the brains of young and old mice (Lee et al., 2013) by treating with cocktails containing either pro-inflammatory compounds (IL1β + IL12) or the anti-inflammatory compounds IL-4 + IL-13. This

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http://dx.doi.org/10.1016/j.exger.2017.01.027
0531-5565/Published by Elsevier Inc.

study not only demonstrated that the aged brain responds more dramatically to the pro-inflammatory cocktail, but it also has an impaired or diminished response to the anti-inflammatory stimuli. This observation has been replicated with isolated microglia and has been termed "priming" (Fenn et al., 2012; Norden et al., 2015). This may be important in terms of response to neurodegenerative diseases such as AD and PD, as arginase 1, one marker of the alternative activation state, is necessary for Aβ plaque reduction. In a recent paper it was demonstrated that arginase-1 positive microglia phagocytose Aβ and if arginase-1 expression was prevented with an IL-4Rα blocking antibody then this impaired the ability of microglia to remove reduce amyloid plaques (Fenn et al., 2014).

Many investigations have begun to assess the age related shifts in gene and protein expression in isolated microglia in order to understand the molecular underpinnings of the changes in microglial function with age. A recent study using RNAseq comparing isolated microglia to whole brain identified the microglial sensome (Hickman et al., 2013). These authors then examined the microglial sensome in aged cells and a large number of the down regulated genes were related to detecting endogenous ligands whereas those related to the recognition of host defense genes were up-regulated. These findings further suggest that aged microglia do not sense and respond to the microenvironment in the same manner as microglia from young animals. Another recent study using gene microarrays of isolated microglia demonstrated up-regulation of a number of pathways including NFκB related genes and identified Sirt1 epigenetic regulation of IL1β as important in aged microglia (Cho et al., 2015). This study looked at myeloid-specific knockdown of Sirt1 and demonstrated alterations in microglial function. One caveat however is that peripheral macrophages were also altered using this approach, thus the specific role of microglia *versus* macrophages/monocytes that are known to cross into the brain was not delineated.

A pathway known to regulate microglial priming is the transcription factor nuclear factor erythroid related factor 2 (Nrf2) signaling cascade. Although Nrf2 is normally involved in the cellular response to oxidative injury, when this molecule is knocked out in mice, microglial phenotype shifts towards exaggerated pro-inflammatory responses (Lastres-Becker et al., 2012), precisely reflecting the primed microglial phenotype that is observed with normal aging. Recent evidence linking Nrf2-antioxidant response element (ARE) to microglial function include the critical role of Nrf2-ARE in promoting phagocytosis in microglia/macrophages as shown by a reduction in phagocytosis using a Nrf2 decoy to block Nrf2 actions (Zhao et al., 2014). It is also well established that there is a decline in microglial phagocytosis with age (Norden et al., 2015); again, indicating that alterations in Nrf2 expression can recapitulate the normal age-related changes in glial function.

2. Impact of inflammation on neural plasticity

The impacts of the changes in microglial function with age are numerous. There is strong evidence for cell non-autonomous effects on stem cell niches, with much of the evidence for this coming from studies using heterochronic parabiosis wherein the circulation of two animals of different ages is combined and early studies in our lab using the technique of *in oculo* transplantation. These latter experiments excised embryonic CNS tissues and transplanted the grafts into in the anterior chamber of the eye in rats of various ages (Granholm et al., 1987; Willis et al., 2005; Willis et al., 2010). The technique of *in oculo* transplantation uses fetal brain tissue is grown in the chamber of the eye where it becomes innervated by the blood and nerves from the host retina. In this manner you can study the development of brain tissues, such as the hippocampus in hosts of various ages, thus the environment of the host influences the growth of the brain tissue. When hippocampus is grown in the anterior chamber of aged rats it develops more slowly and does not attain morphology similar to that observed. When aged hosts are used one difference is that there are higher levels of pro-inflammatory cytokines that have a negative impact on the growth of the brain tissues. The "environment" of the aged host can be manipulated by feeding the older rats diets enriched in polyphenols, such as blueberries. This lowers the levels of cytokines and increases the growth rate of the hippocampal tissue. A more organotypic morphology is also observed under these conditions, similar to what is observed when the hippocampal tissue grows in a young host (Granholm et al., 1987; Willis et al., 2005; Willis et al., 2010). These observations reiterate that the aged environment either lacks some vital factors necessary to perpetuate optimal cellular function or contains negative factors that inhibit proper cellular function.

Prominent support of the cell non-autonomous influences on stem cell vitality has also been generated using the technique of heterochronic parabiosis (Conboy et al., 2005; Villeda et al., 2011). These researchers used this method to surgically conjoin a young and aged animal, allowing the fusion of the two the vascular systems, and exposing each parabiont to the circulating factors of the other. This work demonstrated that the systemic milieu of aged mice reduces function of neural stem cells, hematopoietic stem cells, muscle satellite stem cells, and liver stem cells, (Conboy et al., 2005; Mayack et al., 2010; Villeda et al., 2011). Furthermore, when serum from old rats or mice is used to treat cultures of stem cells from various niches in a model of parabiosis in a dish, the serum from old animals has a negative impact on stem cell proliferation and there are changes in fate determination that recapitulate aging (Mayack et al., 2010; Villeda et al., 2011; Villeda et al., 2014). Several possible factors that are altered in old blood have been identified as possible negative and positive influences on the stem cell niches. For example, Villeda et al. initially suggested that CCL11 (eotaxin) is increased similarly in human and parabiont plasma and may be one of the negative regulators in the aged blood on neurogenesis (Villeda et al., 2011). Another recent paper suggests beta2-microglobulin is also a pro-aging factor (Smith et al., 2015), while several other reports have suggested that growth differentiation protein 11 (GDF-11) is decreased with age. Treatment with GDF-11 has a positive effect on several stem cell niches such as the liver and muscle (Katsimpardi et al., 2014; Sinha et al., 2014).

In addition to circulating factors, one of the main non-autonomous factors impacting the neurogenic niche is influences from surrounding cellular components such as microglia. Several studies have shown that aged microglia negatively impact the stem cell niche in a Nrf2-ARE dependent manner (L'Episcopo et al., 2013; Piccin et al., 2014). Specifically the role of microglia on the aged stem cell niche has become an area of active investigation. A recent study suggests that the cells from the neurogenic niche of aged animals negatively impacts neural stem cells from the subventricular zone *ex vivo* (Piccin et al., 2014). In contrast, adding neurogenic niche cells from the young animal had a rejuvenating effect on stem cells isolated from the aged niche. Other studies have shown that aged microglia directly impact the niche. Aged microglia grown in culture with young neural progenitors induce senescence in these NPC's as reflected in decreased proliferation and maturation (L'Episcopo et al., 2013). This influence of the niche was linked to Nrf2 function and treatments that reduced microglial M1 phenotype were associated with increased Nrf2 expression, however a causal link was not established (L'Episcopo et al., 2013). In addition, restoration of the anti-inflammatory response may be just as important as reducing pro-inflammatory responses and this aspect of changes in microglia is less well studied.

3. Approaches to modulating the systemic milieu and local inflammatory cell influences on the stem cell niche

With the summary of the literature included above, it is established that several cell non autonomous sources have a negative impact on the stem cell niche are present with age. We have examined a number of strategies for modulating the non-autonomous mechanisms. One of the approaches we have used to mitigate these detrimental age-related changes in the CNS is a dietary intervention using polyphenol rich diets.

Please cite this article as: Bickford, P.C., et al., Aging leads to altered microglial function that reduces brain resiliency increasing vulnerability to neurodegenerative diseases, Exp. Gerontol. (2017), http://dx.doi.org/10.1016/j.exger.2017.01.027