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Full-length Article

Scavenger Receptor-A deficiency impairs immune response of microglia and astrocytes potentiating Alzheimer's disease pathophysiology

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ABSTRACT

Late onset Alzheimer disease's (LOAD) main risk factor is aging. Although it is not well known which age-related factors are involved in its development, evidence points out to the involvement of an impaired amyloid-β (Aβ) clearance in the aged brain among possible causes. Glial cells are the main scavengers of the brain, where Scavenger Receptor class A (SR-A) emerges as a relevant player in AD because of its participation in Aβ uptake and in the modulation of glial cell inflammatory response. Here, we show that SR-A expression is reduced in the hippocampus of aged animals and APP/PS1 mice. Given that Aβ deposition increases in the aging brain, we generated a triple transgenic mouse, which accumulates Aβ and is knockout for SR-A (APP/PS1/SR-A$^{-/-}$) to evaluate Aβ accumulation and the inflammatory outcome of SR-A depletion in the aged brain. The lifespan of APP/PS1/SR-A$^{-/-}$ mice was greatly reduced, accompanied by a 3-fold increase in plasmatic pro-inflammatory cytokines, and reduced performance in a working memory behavioral assessment. Microglia and astrocytes lacking SR-A displayed impaired oxidative response and nitric oxide production, produced up to 7-fold more pro-inflammatory cytokines and showed a 12-fold reduction in anti-inflammatory cytokines release, with conspicuous changes in lipopolysaccharide-induced glial activation. Isolated microglia from young and adult mice lacking SR-A showed a 50% reduction in phagocytic activity. Our results indicate that reduced expression of SR-A can deregulate glial inflammatory response and potentiate Aβ accumulation, two mechanisms that could contribute to AD progression.

1. Introduction

The aging of the population is a major challenge in public health due to the increasing prevalence of age-related chronic diseases, including several neurodegenerative diseases. Aging is characterized by a chronic inflammatory state (Franceschi et al., 2007) and is the most prevalent risk factor for developing Alzheimer's disease (AD). In addition, increasing evidence shows that impaired amyloid-β (Aβ) clearance and neuroinflammation are contributing factors for developing late onset Alzheimer's disease (LOAD) (Gallina et al., 2015; Mawuenyega et al., 2010; McIntee et al., 2016; Patterson et al., 2015).

Glial cells are responsible for the innate immune response in the brain, being also involved in Aβ clearance, and have attracted attention in the last decade as mediators of the neuroinflammation observed in AD (Krabbe et al., 2013; Medeiros and LaFerla, 2013; Olabarria et al., 2010; Streit et al., 2009). There is robust evidence showing that microglia and astrocytes develop an inflammatory phenotype as the brain ages (Lucin and Wyss-Coray, 2009; Mosher and Wyss-Coray, 2014; Rozovsky et al., 1998), showing an impaired capability to respond correctly to different stimuli such as pathogen associated molecules, like lipopolysaccharide (LPS), and also Aβ (Frank et al., 2010; Lue et al., 2001; Tichauer et al., 2014). These observations led the proposal of new hypotheses placing glial dysregulation as a possible cause for AD (Rodríguez et al., 2016; von Bernhardi, 2007; von Bernhardi et al., 2015).

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Scavenger receptor have emerged as important actors in AD pathophysiology (Cornejo and von Bernhardi, 2013; El Khoury et al., 1998); in especial Scavenger Receptor class A (SR-A), given their participation in Aβ phagocytosis (Frenkel et al., 2013) and in the outcome of inflammatory activation of glial cells (Godoy et al., 2012; Murgas et al., 2014). Several studies have shown that SR-A deficiency induces accumulation of Aβ (Frenkel et al., 2013; Lifshitz et al., 2013; Yang et al., 2011). Furthermore, there is evidence that neuroinflammation induced by increased Aβ reduces SR-A expression in microglia (Hickman et al., 2008), which could result in a vicious cycle in which Aβ uptake and regulation of glial response by SR-A become impaired, further inducing Aβ accumulation and a pro-inflammatory environment in the brain. Even though the role of SR-A in Aβ uptake is well established, little is known about the way SR-A orchestrates the glial response in the presence of Aβ.

In agreement with previous reports, we observed a reduction in SR-A expression in the hippocampus of aged WT mice, reduction that in APP/PS1 mice was already present at young ages, suggesting that SR-A participation could be reduced by aging and Aβ accumulation. Thus, we generated a triple transgenic mouse that accumulates Aβ and is knockout for SR-A (APP/PS1/SR-A$^{-/-}$), to elucidate the relevance of SR-A for Aβ accumulation and glial inflammatory outcome in the aged brain. Here we show that SR-A deficiency resulted in impairments in the inflammatory response of microglia and astrocytes in a model of Aβ accumulation both *in vivo* and *in vitro*. The absence of SR-A changed the production of reactive oxygen species (ROS) and nitric oxide (NO), the levels of pro- and anti-inflammatory cytokines, and the phagocytic activity of microglial cells. SR-A-deficient APP/PS1 mice showed a high mortality rate, high levels of plasmatic inflammatory cytokines at young ages, increased Aβ plaques and CD68 expression in the hippocampus. Working memory was mildly impaired in APP/PS1/SR-A$^{-/-}$ mice assessed with the 6 arms radial water maze (6ARWM) behavioral test.

Given the fact that Aβ is upregulated in aged primate brains (Zhao et al., 2016), and that Aβ accumulation could reduce SR-A expression (Hickman et al., 2008), our results suggest that SR-A reduced expression together with Aβ accumulation impair the immune response mediated by microglia and astrocytes, promoting a pro-inflammatory environment and a reduced Aβ clearance, favoring a vicious cycle for AD development.

2. Materials and methods

2.1. Reagents

LPS (from Escherichia coli 0111:B4), Thioflavin S and antibody against α-tubulin were purchased from Sigma (ThermoFisher, USA). HiLyte Fluor 555-labeled-Aβ$_{(1-42)}$ was purchased from Anaspec (USA). Percoll was obtained from GE Healthcare Life Sciences (USA). The antibody against ionized calcium-binding adapter molecule 1 (Iba1) was from Wako (Japan). Antibody against glial fibrillary acidic protein (GFAP) and the fluorescent mounting medium were purchased from Dako (Agilent Technologies, Denmark). Antibody against SR-A was from R&D Systems (USA). Antibody against CD68 was from AbD Serotec (USA). Alexa Fluor 488-conjugated secondary antibody anti-rabbit, 5-(6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H$_2$DCF DA) and Hoechst 33258 were from Molecular Probes (ThermoFisher, USA). ELISA kits for cytokine detection were purchased from eBioscience (Affymetrix, USA). Cell culture media, antibiotics and serum were purchase from Gibco (ThermoFisher, USA). Flow cytometry buffers Cytofix/Cytoperm and Perm/Wash were obtained from BD biosciences (USA).

2.2. Mice

APP/PS1 bi-transgenic mice (B6.Cg-Tg(APPswe,PSEN1dE9)85Dbo/J, C57Bl6/J background) were purchased from Jackson Laboratories (USA). SR-A$^{-/-}$ and SR-A$^{+/+}$ mice (129/ICR background) were kindly facilitated by Dr. Guillermo Merino, and their developer, Dr. Tatsuhiko Kodama (Research Center for Advanced Science and Technology, University of Tokyo, Japan) and kept at the institutional animal facility. Mice were housed at a maximum density of 5 adult mice per cage, in a temperature-regulated room with a 12 h light/dark cycle, and with free access to food and water. APP/PS1/SR-A$^{-/-}$ triple transgenic mice were generated by backcrossing heterozygous APP/PS1 and homozygous SR-A$^{-/-}$ animals for more than 12 generations. Animals were genotypified after weaning (primers and genotyping examples in Supplemental Fig. S1a). All procedures were performed following the animal handling and bioethical requirements defined by the Pontificia Universidad Católica de Chile School of Medicine Ethics Committee and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.3. Glial cultures

Mixed glial cell cultures, containing astrocytes and microglia, were obtained from the cerebral cortex of 1-to-2 day old WT, APP/PS1, SR-A$^{-/-}$ or APP/PS1/SR-A$^{-/-}$ mice, as previously described (Giulian and Baker, 1986). Briefly, cortices were rinsed with Ca^{2+}/Mg^{2+}-free Hank's balanced salt solution (HBSS); meninges were removed, and tissue was minced and incubated with 0.25% trypsin-EDTA in HBSS at 37 °C for 10 min. The tissue was mechanically dissociated, and cells were seeded in 75 cm^2 cell culture flasks (one brain per flask) in Dulbecco's Modified Eagle's Medium DMEM/F12 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. Cultures were incubated in a water saturated, 5% CO_2 atmosphere at 37 °C.

After 14 days in culture, microglia were purified by differential adhesion with 60 mM Lidocaine at 37 °C for 10 min (Bronstein et al., 2013). Microglia were resuspended and seeded in a 1:1 mixture of supplemented DMEM/F12 and conditioned media (Conditioned culture medium was obtained from mixed glial cell cultures, centrifuged at 200 g for 10 min, and kept sterile for later use). After 24 h of seeding, the medium was replaced by fresh supplemented DMEM/F12. Astrocytes were purified by differential adhesion after trypsinization of the attached cells after the isolation of microglial cells. This procedure yield cultures that were highly enriched in astrocytes (95% or more) or microglia (over 99%). Cell identity was evaluated by immunocytochemistry against Iba1 (1:500) for microglia, and GFAP (1:500) to identify astrocytes (data not shown).

Glia were plated in 6-well plates at a density of 2.5×10^5 cells per well for Western blot, in 96-well black plates with optical bottom at a density of 4×10^4 cells per well for ROS detection, and in 24-well plates at a density of 1×10^5 cells for NO and ELISA determination of cytokines. For immunofluorescence, 4×10^4 cells were seeded on 12 mm glass coverslips.

2.4. Western blot

Hippocampal tissue was homogenized in ice-cold RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and protease inhibitors, pH 7.5). Samples were separated by electrophoresis in 12% poly-acrylamide gels and transferred to a nitrocellulose membrane. After transference, the membrane was treated with blocking buffer (0.05% Tween 20, 5% milk in PBS) and then incubated with goat anti SR-A (1:500), and mouse anti α-tubulin (1:1000) primary antibodies. Supplemental Fig. S1b

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