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Neuroscience Letters 390 (2005) 76–80
Neuroscience Letters
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Macrosialin increases during normal brain aging are attenuated by caloric restriction

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Received 17 May 2005; received in revised form 13 July 2005; accepted 30 July 2005

Abstract

During normal aging, microglia develop an activated phenotype characterized by morphologic changes and induction of CD11b, MHC II, and other inflammatory markers. We show that macrosialin (CD68), a macrophage-specific protein, is increased by aging in selected brain regions of male C57BL/6NNia mice. In corpus callosum and striatum, macrosialin mRNA and protein increased ≥50% (24 months versus 4 months); hippocampus and cerebellum were unchanged. Caloric restriction (CR) attenuated these age-related increases. Since CR attenuates age-related increases in oxidative damage and inflammation, we examined whether oxidized lipoproteins and inflammatory processes regulate macrosialin using murine BV-2 microglial cells as a model. Oxidized low-density lipoproteins (oxLDL) induced macrosialin protein by 50%. Moreover, macrosialin was induced in response to lipopolysaccharide (LPS) plus interferon-γ (IFN-γ) which activates inflammatory pathways in BV-2 cells. Thus, the previously documented increase in oxidized lipoproteins, inflammation, and microglial activation during normal aging may contribute to the age-related increase in macrosialin expression.

Keywords: Macrosialin; Aging; Microglia; Caloric restriction; oxLDL; Inflammation

Macrosialin, the mouse homolog of the human CD68, is a member of the lysosomal/endosomal-associated membrane glycoprotein (LAMP) family and is expressed primarily in monocytic cells [13,14]. Macrosialin is also a member of the scavenger receptor family which recognize a wide range of anionic macromolecules such as oxidatively modified lipoproteins, apoptotic cells, and cell surface antigens of microorganisms [37]. In macrophages, macrosialin is mainly localized in lysosomes and endosomes, and rapidly exchanges with a smaller subfraction of macrosialin on the cell surface [18]. Its localization in late endosomes and predominance in phagocytic macrophages implicates macrosialin in phagocytosis [7,27]. For example, no macrosialin expression is detected in resting vascular endothelial cells, but it can be induced by cholesterol treatment which transdifferentiates vascular endothelia to phagocytic cells with macrophage characteristics [28]. Macrosialin is also induced in mouse peritoneal macrophages by oxidized low-density lipoproteins (oxLDL) [7,38].

Although implicated in phagocytosis, the exact function(s) of macrosialin is not known. Under normal, physiological

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doi:10.1016/j.neulet.2005.07.058

conditions, scavenger receptors function to clear cellular debris; however, in Alzheimer's and other neurodegenerative diseases, scavenger receptors may mediate the recruitment and activation of macrophage cells [15,22,37]. Although less studied than other scavenger receptors, macrosialin may have similar functions [38]. Scavenger receptors may contribute to the disease pathology by inducing the microglial production of reactive oxygen species and inflammation [9,38]. However, little is known about macrosialin expression and function in the brain.

In two transgenic mouse models for Alzheimer disease (AD) (APP23 and Tg2576), macrosialin immunostaining is localized to activated microglial cells around amyloid plaques [5,30]. In human AD brains, macrosialin is localized in cells near amyloid plaques and extraneuronal neurofibrillary tangles [17]. AD-related pathology is also associated with increased oxidative damage and inflammation [2,6,24].

In contrast to AD, glial activation and oxidative damage during normal brain aging are more subtle. Microglia are activated in the absence of amyloid plaques in many brain regions, particularly in myelinated pathways of aging rodents, primates, and humans [10,20,25,33]. These changes in rodents are attenuated by life-long caloric restriction which extends life span and slows many aging processes including the accumulation of oxidative damage [10,23,26,32]. Thus, we hypothesized that normal oxidative and/or inflammatory mechanisms during aging in the brain may regulate macrosialin.

We chose a commonly used aging model, male C57BL/6NNia mice, to study how aging and CR regulate brain macrosialin expression. Animal procedures followed the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23 revised 1996). Ad libitum (AL) fed mice were maintained on the NIH-31 diet consisting of 4% fat. Caloric restricted (CR)-mice, fed 40% less calories than AL mice, were maintained on the NIH-31/NIA Fortified Diet (2.6% fat) from 4 to 24 months. Mice were anesthetized with isofluorane and perfused with saline. Brains were removed and stored at −70 °C. Brain sections (20 μm) were mounted onto polylysine-coated slides. In situ hybridization on fresh frozen sections for macrosialin used anti-sense ^{35}S-labeled cRNA to the 0.7 kb region (264–982 nt.) of mouse macrosialin mRNA. Immunocytochemistry used rat-anti-mouse CD68 (clone FA-11) (Serotec, Oxford, England). In situ hybridization and immunostaining were analyzed by image densitometry [20]. Briefly, regions of interest were outlined and the percent of the area covered by the immunoreactive product within the defined region was estimated using video densitometry (IPLAB Spectrum software, Signal Analytics Corp.). Statistically significant differences between means were determined by ANOVA, followed by Fisher post hoc tests (StatView 5.0, SAS Institute Inc., Cary, NC, USA).

Macrosialin mRNA and protein increased with regional specificity in 24-month-old versus 4-month-old mice. Macrosialin mRNA increased 400% in the corpus callosum and 40% in the striatum, but did not change in the hilus (Fig. 1A). Protein levels generally paralleled the mRNA, but the changes were smaller. The corpus callosum displayed widespread macrosialin immunoreactivity as parallel arrays in microglia adjacent to nerve bundles. In striatum, immunostaining in 24-month-old AL mice was largely at the periphery of the corticostriatal bundles (arrow in Fig. 1B inset). Macrosialin immunoreactivity increased in the corpus callosum of aged mice by 75%, whereas the trend in the striatum was not significant (Fig. 1B). Macrosialin staining in most other regions [hippocampus (dentate gyrus, CA1, CA3, and hilus), cerebral cortex and cerebellum] was too low for reliable quantification. Caloric restriction during the adult life span attenuated age-related increases in macrosialin mRNA and protein in the corpus callosum (Fig. 1A and B).

In peritoneal macrophages, macrosialin expression is regulated by atherogenic diets and by inflammation and can be induced by oxidized low-density lipoproteins (oxLDL) [7]. To examine effects of oxLDL on macrosialin, BV-2 microglial cells [4] were treated with 30 μg/ml oxLDL [7] or native LDL. By 48 h, oxLDL, but not native LDL, induced macrosialin protein by 50% (Fig. 2A). Neither oxLDL nor native LDL altered macrosialin mRNA (not shown).

As another approach, responses to lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria and a potent inflammatory agent which also induces protein oxidation in BV-2 cells [19], was also examined. IFN-γ (10 ng/ml; R&D Systems, Minneapolis, MN, USA) was added with 100 ng/ml LPS (Sigma, St. Louis, MO, USA) to maximize responses. Although LPS alone had no effect on macrosialin mRNA, LPS plus IFN-γ induced mRNA 50% by 24 h (Fig. 2B) and protein by 40 h (Fig. 2C). LPS plus IFN-γ also induces nitric oxide by 24 h (data not shown [36]). However, nitric oxide did not alter macrosialin expression, since treatment of BV-2 cells with sodium nitroprusside, a nitric oxide generator, did not alter macrosialin mRNA or protein (data not shown). Moreover, macrosialin may not mediate nitric oxide production in response to LPS [3]. Therefore, the LPS induction of macrosialin and nitric oxide production may be independent events.

These findings show that macrosialin expression increases during normal brain aging. We hypothesize that oxidized lipoproteins and/or inflammation are possible regulators of macrosialin expression during aging. Macrosialin changes show brain regional selectivity, with the greatest induction of mRNA and protein in the white matter of the corpus callosum and striatum. These age changes were attenuated by CR, which increases lifespan in experimental animal models with corresponding attenuation of the load of oxidatively modified proteins and lipids [11]. These results extend the age-related activation of microglia in the corpus callosum and in corticostriatal tracts by the surface membrane receptors, CD11b and MHC II [10,20], to the scavenger receptor macrosialin.

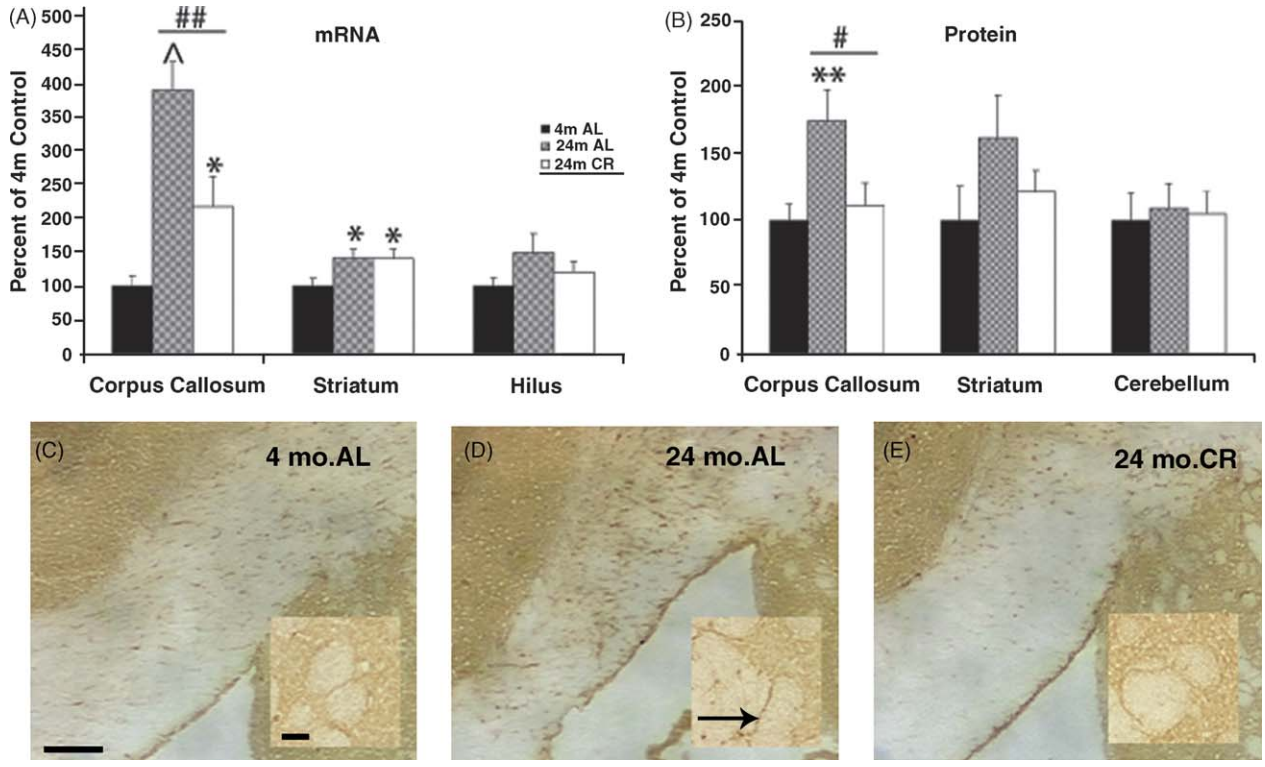

Fig. 1. Effects of age and caloric restriction on macrosialin mRNA (A) and protein (B) expression in several brain regions of C57BL/6 mice. Macrosialin mRNA expression was analyzed by in situ hybridization. Protein expression was analyzed by immunocytochemistry. Signal intensity was expressed as a percentage of the 4-month AL group. Bars represent mean ± S.E.M. for 10 animals per group. $^{*}p < 0.05$ relative to 4-month AL, $^{**}p < 0.01$ relative to 4-month AL, $^{\wedge}p < 0.0001$ relative to 4-month AL, $^{\#}p < 0.05$ relative to 24-month AL, $^{\#\#}p < 0.01$ relative to 24-month AL. (C–E) Macrosialin immunoreactivity in the corpus callosum and corticostriatal bundles (insets) of (C) 4-month AL, (D) 24-month AL, and (E) 24-month CR C57BL/6 mice. Arrow identifies macrosialin immunostaining at periphery of corticostriatal bundle in 24-month AL mice. Bars = 100 μm for micrographs, 30 μm for insets.

The age-related increases of macrosialin we observed in the corpus callosum may be important to changes in myelinated pathways during normal brain aging. Magnetic resonance imaging diffusion tensor imaging studies on adult humans consistently show that white matter is structurally altered within the corpus callosum [1,29]. Over 4 years of follow-up, elderly men showed 0.9% annual rate of decrease in the corpus callosum size which was correlated with a decline in word recognition by the Stroop test. In addition, the size of the corpus callosal splenium correlated with performance in the Mini-Mental State Examination, a test of cognitive function [35].

We propose that the altered gross structure of white matter is due, in part, to the increased gliosis during aging. The number of glia (astrocytes and microglia) increases in the corpus callosum of aging mice [34]. This can also be extended to monkeys where there was an increased density of activated microglia in the corpus callosum of cognitively impaired old monkeys [31]. In addition, we have shown that the regions with greatest activation of astrocytes and microglia were the corpus callosum and striatum [20]. The present study adds macrosialin to the expanding list of age-related markers of glial activation in white matter.

Several studies have associated brain oxidative damage to cognitive impairments. The cognitively impaired old monkeys with increased in activated microglial cells also had increased inducible nitric oxide synthase and nitrated proteins in white matter. Most of the nitrated proteins were associated with myelinated axons [31]. Another study in mice showed an increased relationship between markers for brain oxidative stress and performance in tests to assess neuromuscular or synaptic functions [21]. We have also detected increased nitrotyrosine immunostaining in aging corpus callosum of mice (unpublished data).

CD68 is also increased in degenerating white-matter brain diseases. For example, multiple sclerosis patients typically show increased CD68 expression in lesions [8,16]. In two less common human acute demyelinating diseases, acute hemorrhagic leucoencephalitis and acute disseminated encephalomyelitis, CD68-positive cells correlate with damaged axons adjacent to veins and venules [12]. The increase of macrosialin expression in white matter during ‘normal aging’ reported here indicates the sensitivity of this microglial response to mild aging changes in white matter.

The present findings add to the understanding of macrosialin (CD68) expression in brain diseases by showing

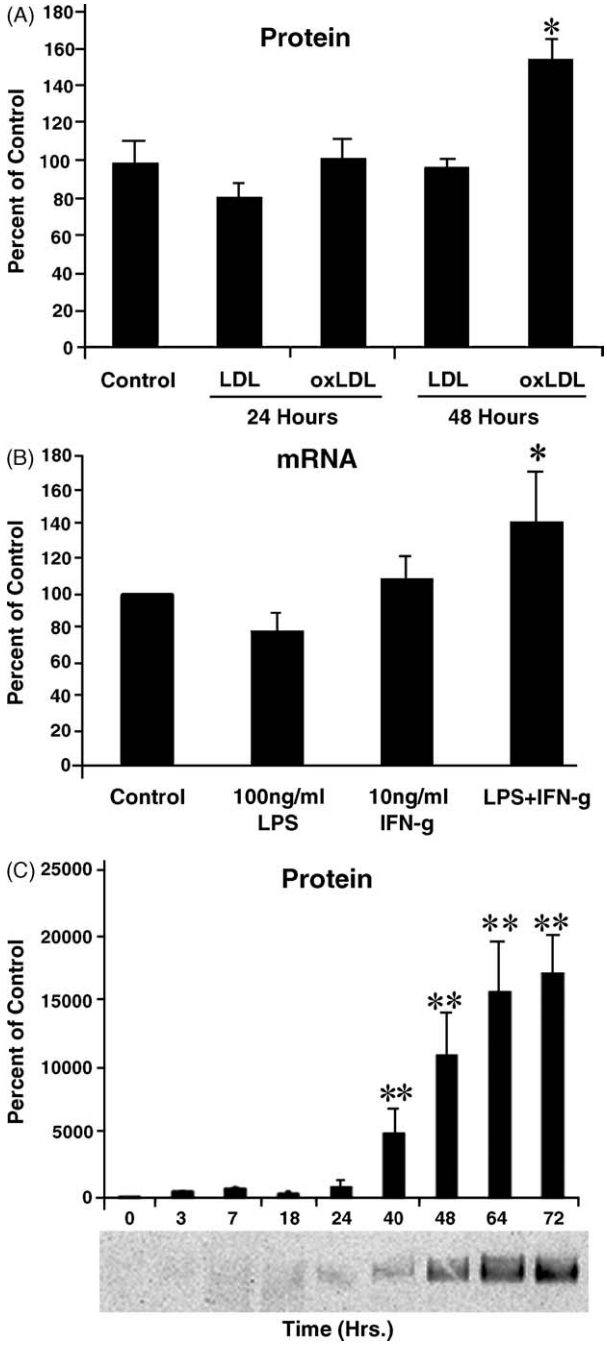

Fig. 2. (A) Macrosialin protein levels in BV-2 cells treated with 30 μg/ml LDL or oxLDL as analyzed by Western blots. Results are representative of data from duplicates of three experiments. (B and C) Lipopolysaccharide plus interferon-γ induces changes in macrosialin mRNA and protein in BV-2 cells. (B) Macrosialin mRNA expression were determined by Northern blot analysis. (C) Macrosialin protein levels were determined by Western blot analysis. Normalized values are expressed as a percentage of the control. $*p < 0.05$ relative to control. $**p < 0.001$ relative to control. Below the graph is a representation of one of the Western blots used to quantify the data.

that macrosialin is induced during normal brain aging independent of disease. These white matter-rich regions are also areas prone to oxidative damage and changes in the structural integrity of myelin. Future studies will determine the role of increased microglial activation, as indicated by markers such as macrosialin, in age-related cognitive decline.

Acknowledgements

This research was funded by the grants to C.E.F. (AG-13499, Alzheimer's Association, and John Douglas French Alzheimer's Foundation), A.M.W. (AG-00093), and N.V.P. (AG-05901).

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