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Matrix metalloproteinase-12 contributes to neuroinflammation in the aged brain

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ABSTRACT

During aging the brain displays an increased proinflammatory status, which is associated with the pathogenesis of aging-related diseases such as Alzheimer's and Parkinson diseases. Matrix metalloproteinases (MMPs) facilitate the migration of inflammatory cells in tissues and modulate their inflammatory activity. In this study, we screened expression of MMPs in 3-, 10-, and 18-month-old mice and observed that cerebral MMP-12 expression was strongly upregulated during aging. We compared the neuroinflammation of 3-, 10-, and 18-month-old MMP−12-deficient versus wild type mice by counting microglia and measuring inflammatory gene transcripts in the brain and observed that MMP-12 deficiency reduced neuroinflammation during aging. In order to identify potential mechanisms, we analyzed the inflammatory activity of microglia directly isolated from adult mouse brains or cultured from newborn mice. We observed that MMP-12 deficiency increased the inflammatory activity of adult brain-derived microglia, but did not affect cultured microglia. We found greater numbers of CD11b/CD45^{high} cells in the parenchyma of MMP-12 wild type than in the parenchyma of MMP−12-deficient mouse brains. Thus, our study suggested that the upregulated cerebral MMP-12 during aging enhances aging-associated neuroinflammation by facilitating recruitment of bone marrow-derived microglia into the brain.

1. Introduction

During aging the central nervous system (CNS) displays a progressively increased inflammatory status, a process referred to as "Inflamm-aging" (Franceschi et al., 2000). Microglia, the key inflammatory cells in the CNS, produce higher amounts of both pro- and anti-inflammatory cytokines in aged human and mouse brains than in young brains (Lee et al., 2000; Letiembre et al., 2007; Lu et al., 2004; Sierra et al., 2007; Ye and Johnson, 1999). Apart from the inflammatory overactivation, microglia also expand the population by local proliferation and by replenishment from the bone marrow-derived precursor cells (Ajami et al., 2007; Conde and Streit, 2006; Prinz et al., 2011). Notably, bone marrow-derived microglia are more efficient in amyloid-β clearance than their resident counterparts in Alzheimer's disease (AD) mouse models (Simard et al., 2006; Prinz et al., 2011).

The effects of enhanced neuroinflammation on the aged brain are unclear. In aging-related diseases, such as AD and Parkinson disease, long-lasting inflammation is associated with neurodegeneration (Akiyama et al., 2000; Mrak and Griffin, 2005; Hirsch and Hunot, 2009). We recently observed that reducing proinflammatory activation in the brain by the ablation of microglial Toll-like receptor 2 or its downstream signaling molecule, MyD88, ameliorates neuronal deficits in Alzheimer's amyloid precursor protein transgenic mice, an AD animal model (Hao et al., 2011; Liu et al., 2012). Upregulation of proinflammatory activation with increased amounts of tumor necrosis factor (TNF)-α or interleukin (IL)-1β in substantia nigra triggers dopaminergic neuron death in mice (Deleidi et al., 2010; De Lella Ezcurra et al., 2010). However, anti-inflammatory activation characterized by expression of genes such as *il-10, mannose receptor C type 1, arginase 1,* and *chitinase-3-like protein 3* in the brain was also observed in AD patients and in AD animal models (Colton et al., 2006; Liu et al., 2012). Anti-inflammatory activation could protect neurons from degeneration and facilitate their regeneration (Liu et al., 2012; Ma et al., 2010).

Matrix metalloproteinases (MMPs) are a growing family of zinc-bound proteases. Although most MMPs are expressed at low levels in the adult CNS, the levels of certain MMPs increase in inflammation-related diseases, such as AD, Parkinson disease, and multiple sclerosis (MS), or after stroke or spinal cord trauma (Lorenzl et al., 2002; Yong et al., 2001, 2005). These MMPs facilitate migration of inflammatory cells by disrupting extracellular matrix and modulate inflammatory reactions by cleaving inflammatory

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cytokines and chemokines (Dean et al., 2008; Parks et al., 2004; Yong et al., 2001, 2005). Macrophage metalloelastase, or MMP-12, is a 54-kDa elastinolytic protease that is expressed by microglia and infiltrated macrophages in the CNS in association with disease-caused neuroinflammation (Crocker et al., 2008; Dasilva and Yong, 2008; Nuttall et al., 2007; Power et al., 2003; Toft-Hansen et al., 2004; Vos et al., 2003). MMP-12 could play a dual role in the inflammatory disease pathogenesis. After spinal cord trauma or intracerebral hemorrhage, or in Theiler's murine encephalomyelitis, MMP-12 exaggerates disease progression by increasing permeability of the blood-brain/spinal barrier and recruitment of macrophages into the CNS (Hansmann et al., 2012; Wells et al., 2003, 2005); however, in experimental autoimmune encephalomyelitis (EAE), an animal model of MS, MMP-12 inhibits pathogenesis by enhancing anti-inflammatory effects (Goncalves DaSilva and Yong, 2009; Weaver et al., 2005).

We hypothesized that (1) the inflammatory activation associated with aging alters the expression of cerebral MMPs; and (2) the changed expression of MMPs modulates neuroinflammation. We screened the cerebral expression of most known MMPs in mice and observed that MMP-12 was the most upregulated MMP during aging. We then compared the extent of neuroinflammation in aged MMP-12-deficient compared with wild type mice to identify the role of MMP-12 in "Inflamm-aging."

2. Methods

2.1. Animals

MMP−12-deficient (B6.129X-*Mmp12*^{tm1Sds}/J, MMP-12$^{-/-}$) and wild-type C57BL/6J (MMP-12$^{+/+}$) mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and kept in specific pathogen-free conditions at the University of the Saarland. MMP-12$^{-/-}$ mice were first crossed to MMP-12$^{+/+}$ mice to produce MMP-12$^{+/-}$ mice. The heterozygous mice were then intercrossed to produce MMP-12$^{-/-}$ and MMP-12$^{+/+}$ mice. Afterward the breedings consisted of crosses between homozygous mice. Animal studies were performed with the approval of the local research ethics committee in accordance with national and international guidelines. All efforts were made to minimize the number of animals used and to ensure that any suffering was kept to a minimum.

2.2. Tissue preparation

MMP−12-deficient or wild type C57BL/6 mice at different ages (3, 10, and 18 months old) were sacrificed via inhalation of isoflurane (Abbott, Wiesbaden, Germany). Mice were then rapidly perfused transcardially with ice-cold phosphate buffered saline. The brain was removed and divided. The left hemibrain was immediately fixed in 4% paraformaldehyde (Sigma, Schnelldorf, Germany) for immunohistochemistry. A 0.5 mm thick piece of tissue was sagittally cut from the middle side of right hemisphere and homogenized in Trizol (Invitrogen, Karlsruhe, Germany) for RNA isolation. The remainder of the right hemisphere was used to prepare a single cell suspension for flow cytometry or to isolate proteins for biochemical analysis and MMP-12 activity assay. All samples for protein and RNA investigation were snap frozen and stored at −80 °C immediately until use.

2.3. Isolation of adult microglia for transcriptional analysis and flow cytometry

The brain tissues derived from 18-month-old MMP-12-deficient and wild type C57BL/6 mice were homogenized in precooled 5 ml Hanks' balanced salt solution (HBSS; Invitrogen) using a 24 gauge needle, 2 mL syringe, and passed through 70 μm mesh (BD, Heidelberg, Germany) according to the published protocol (Hao et al., 2011). To isolate microglial RNA, the cells were pelleted by centrifuging the brain homogenate and resuspended in 200 μL HBSS containing 10% fetal calf serum and 20 μg/mL CD11b antibody (clone M1/70; BD). After incubation for 1 hour, CD11b-positive cells were selected by Dynabeads-conjugated sheep anti-rat IgG (Invitrogen) and lysed in RTL Plus buffer (Qiagen, Hilden, Germany). To evaluate CD45-positive cells in the brain, the pelleted cells were resuspended in 200 μL of HBSS containing 25 μg/mL rat anti-mouse CD16/CD32 antibody (clone 2.4G2; BD), 10% fetal calf serum, and 0.1% sodium azide. Cells were then stained with Alexa647-conjugated CD11b and fluorescein isothiocyanate-conjugated CD45 (clone 30-F11; BD) antibodies or relevant isotype controls. The CD45/CD11b-positive cells were detected and analyzed on the BD FACS Canto II flow cytometry (Franklin Lakes, NJ, USA). The CD11b-positive cell population was identified using side scatter and CD11b gating. Positive staining with fluorescein isothiocyanate-conjugated CD45 antibody was determined by comparison of fluorescence levels with the relevant isotype control.

2.4. Quantitative RT-PCR to measure transcripts of MMPs and inflammatory genes

Total RNA was isolated from the brain hemisphere by Trizol and from adult microglia by RNEasy Mini Kit (Qiagen). First-strand cDNA was synthesized by priming total RNA with random primers (Invitrogen) and using Moloney murine leukemia virus reverse transcriptase according to the instructions of the manufacturer (Sigma). For quantification, quantitative real-time polymerase chain reaction (PCR) was performed using the Applied Biosystems 7500 Fast real-time PCR system (Foster City, CA, USA). For measurements of inflammatory gene transcripts, the Taqman gene expression assays of mouse TNF-α, IL-1β, inducible nitric oxide synthases (iNOS), chemokine (C-C motif) ligand 2 (CCL-2), IL-10, and 18s RNA (all assays were from Applied Biosystems) were used. To detect gene transcription of MMPs (MMP-1a, -2, -3, -7, -8, -9, -10, -11, -12, -13, -14, -15, -16, -17, -19, -20, -21, -23, -24, -25 and -28) and their endogenous tissue inhibitors (TIMP-1, -2, -3 and -4), SYBR green I dye-based quantitative PCR was performed using the previously published primers (Wells et al., 2003; see Supplementary Table 1). To evaluate the proliferative capacity of microglia, the transcripts of cyclin genes were measured by reverse transcription and real-time PCR with the following primers: cyclin A2: 5′-GCCTTCCACTTGGCTCTCTA-3′ and 5′-AAGGTCCACAAGACA AGGCT-3′; cyclin D1: 5′-TGAGAACAAGCAGACCATCC-3′ and 5′-TGA ACTTCACATCTGTGGCA-3′; and cyclin E1: 5′-CCAAGGGAGAGAGA CTCGAC-3′ and 5′-GGCAATTTCTTCATCTGGGT-3′.

The amount of double-stranded PCR product synthesized in each cycle was measured by detecting the FAM dye freed from the Taqman probes or SYBR green that binds to double-stranded DNA. Threshold cycle (Ct) values for each test gene from the replicate PCRs were normalized to the Ct values for the 18s RNA or the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) control from the same cDNA preparations. Transcription rations were calculated as $2^{(\Delta Ct)}$, where ΔCt is the difference between Ct (18s or GAPDH) and Ct (test gene).

2.5. MMP-12 Western blot and activity assay

The brain was homogenized in working buffer, consisting of 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 5 mM $CaCl_2$, 1% Triton X-100, and 0.05% Brij 35 (all regents were from Sigma). Protein concentrations were measured using the Bio-Rad Protein Assay (Bio-Rad