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Neuronal driven pre-plaque inflammation in a transgenic rat model of Alzheimer's disease

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ABSTRACT

Chronic brain inflammation is associated with Alzheimer's disease (AD) and is classically attributed to amyloid plaque deposition. However, whether the amyloid pathology can trigger early inflammatory processes before plaque deposition remains a matter of debate. To address the possibility that a pre-plaque inflammatory process occurs, we investigated the status of neuronal, astrocytic, and microglial markers in pre- and post-amyloid plaque stages in a novel transgenic rat model of an AD-like amyloid pathology (McGill-R-Thy1-APP). In this model, we found a marked upregulation of several classical inflammatory markers such as COX-2, IL-1β, TNF-α, and fractalkine (CX3CL1) in the cerebral cortex and hippocampus. Interestingly, many of these markers were highly expressed in amyloid beta-burdened neurons. Activated astrocytes and microglia were associated with these Aβ-burdened neurons. These findings confirm the occurrence of a proinflammatory process preceding amyloid plaque deposition and suggest that Aβ-burdened neurons play a crucial role in initiating inflammation in AD.

1. Introduction

Alzheimer's disease (AD) is the most frequent cause of cognitive decline and dementia, affecting more than 36 million people worldwide (World Alzheimer Report, 2012).

The principal pathologic features found in postmortem brains of AD sufferers are extracellular amyloid plaques, formed by aggregated amyloid-beta (Aβ) peptides, and neurofibrillary tangles composed of paired filaments of abnormally phosphorylated tau protein (Cuello et al., 2007; Grundke-Iqbal et al., 1986; Masters et al., 1985; Selkoe, 2001).

At advanced stages of the pathology, in the presence of extracellular amyloid plaques, inflammation is an invariable component of the disease and might contribute to CNS dysfunction, injury, and ultimately loss of neurons (Haass and Selkoe, 2007). This overt inflammatory process is characterized by abundant reactive astrocytes and activated microglia surrounding amyloid plaques, by increased inflammatory molecules (cytokines and chemokines), and by the participation of the complement system (Akiyama et al., 2000).

The notion that inflammatory processes could play a role in the early AD pathology followed the discovery that patients with arthritis receiving lifelong treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) had a lower incidence of AD (Andersen et al., 1995; McGeer et al., 1990; Stewart et al., 1997); for a review: (Klegeris and McGeer, 2005). The data from these retrospective studies suggest that an inflammatory process should be present in the preclinical phases of the pathology, and that its inhibition could retard or prevent the onset of the clinical stages of the disease. In addition, in a prospective study, the NSAID naproxen diminished the incidence of AD in high-risk cohorts when given before the phase of subjective cognitive impairment (Breitner et al., 2011). However, prospective trials applying NSAIDs to clinically diagnosed AD cases have failed to slow down disease progression, suggesting that preventing inflammation at the clinical, more advanced stages is less likely to result in a beneficial outcome (Aisen et al., 2003; Martin et al., 2008; Thal et al., 2005). The previously mentioned observations and the fact that minocycline, an anti-inflammatory tetracycline, corrects pre-plaque inflammation and reduces the amyloid component in a tg mouse model of AD (Ferretti et al., 2012a) would indicate that inflammation has a

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prominent disease-aggravating role in the initial phases of the disease. As most research efforts have been placed on characterizing the late, plaque-associated inflammatory response, little is known on the early inflammation occurring in preclinical AD stages.

The data on biomarkers emerging from familial and sporadic AD cases make a compelling case for the occurrence of a silent AD pathology starting decades before clinical diagnosis (Bateman et al., 2012; Jack et al., 2013). As an acute injection of Aβ oligomers is sufficient to provoke the upregulation of inflammatory markers in a normal rat brain (Bruno et al., 2009), it is conceivable that, in the evolution of the AD pathology, a proinflammatory process is triggered by the early increase of soluble Aβ, before plaque deposition. We took advantage of a new transgenic (tg) rat model that reliably develops a progressive AD-like amyloid pathology to thoroughly characterize the inflammatory state of their brains at stages before and after amyloid plaque deposition.

The McGill-R-Thy1-APP tg rat model expresses the human APP_{751} transgene bearing the Indiana and Swedish mutations under the control of the murine Thy-1.2 promoter (Leon et al., 2010). As opposed to most tg mouse models containing multiple transgene copies, this rat model carries 1 copy per allele. This minimal transgenic charge is sufficient to reproduce an AD-like amyloid pathology and consequent cognitive impairments. Homozygous animals already display behavioral alterations at 3 months, a stage where Aβ immunoreactivity is limited to the intraneuronal compartment. The homozygous tg rats might display isolated amyloid plaques in the subiculum starting at the age of 6- to 8-month-old and invariably develop the full amyloid pathology as from 8- to 9-month-old.

We have used this animal model, closer to the human species than mice, to investigate whether a “pre-plaque” inflammatory process occurs before the overt extracellular aggregation of Aβ-amyloid peptides. Our investigations support the notion that Aβ-burdened neurons are key players in initiating this early inflammation. Such process would be akin to the earliest possible stages of the “silent AD” pathology in the human.

2. Methods

2.1. Animals

Female and male homozygous rats expressing the APP Swedish and Indiana mutation (McGill-R-Thy1-APP) ranging from the age of 4- to 18-month-old were used. Their genotype was determined using quantitative polymerase chain reaction technique as in Leon et al. (2010). We used at least 12 animals per group and time point. Age-matched wild-type (wt) littermates served as control animals. Based on the progression of the pathology, 4- to 6-month-old rats were used to investigate the pre-plaque period as animals of 13- to 18-month-old were used to study the post-plaque pathology.

All animals were housed in a controlled environment (temperature 22 °C, humidity 50%–60%, 12 hours light and/or12 hours dark schedules), and all procedures were approved by the McGill Animal Care Committee, following the guidelines of the Canadian Council on Animal Care.

2.2. Processing of the brains

Tissue was processed as previously described (Ferretti et al., 2012b). Briefly, tg McGill-R-Thy1-APP (Leon et al., 2010) and wt littermates were deeply anesthetized with equithesin (2.5 mL/kg intraperitoneally) and perfused for 1 minute with ice-cold saline solution (pH 7.4). Brains were quickly removed and separated into right and left hemispheres on ice. The left hemisphere was dissected into cortex, hippocampus, and cerebellum. These brain areas were snap-frozen in liquid nitrogen and stored at −80 °C for biochemical analysis. The right hemisphere was immersion-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 hours at 4 °C before being cut into 40-μm thick coronal sections with a freezing sledge microtome (SM 2000 R, Leica, Wetzlar, Germany). Free-floating sections were stored in cryoprotectant (37.5% ethylene glycol and 37.5% sucrose in phosphate buffered saline [PBS]) at −20 °C until processed for immunohistochemistry.

2.3. Western blotting

Cortical samples from tg and wt rats of 4-, 6- and 13-month-old were homogenized in lysis buffer (Cell Signaling, Danvers, MA, USA) containing a complete protease inhibitor cocktail (Roche Diagnostic, Manheim, Germany). The amount of protein was measured using a conventional Bradford assay (Bradford, 1976). Proteins (60–100 μg) were loaded in 10%–15% sodium dodecyl sulfate-polyacrylamide gels according to the molecular weight of the protein of interest and wet-transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) for 1 hour before incubation with the primary antibodies at 4 °C overnight. In 6-month-old animals, the following primary antibodies were used: anti- IL-1β, 1:500 (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA), anti-COX-2, 1:500 (Cayman Chemicals, Ann Arbor, MI, USA and Cell Signaling Technology Inc, Danvers, MA, USA), anti-glial fibrillary acidic protein (GFAP), 1:20,000 (Sigma-Aldrich, Oakville, Ontario, Canada), and βIII-tubulin, 1:50,000 (Promega, Madison, WI, USA). In 13-month-old animals, the following antibodies were used at the same dilution as previously mentioned, anti-GFAP, anti-COX-2, and βIII-tubulin. Subsequently, membranes were washed in TBS-T and incubated with secondary antibodies—either a peroxidase-conjugated goat anti-rabbit IgG or a goat anti-mouse IgG 1:10,000 (Jackson ImmunoResearch, West Grove, PA, USA) in 5% nonfat milk in TBS-T for 90 minutes. Immunoreactive bands were visualized with ECL prime (GE Healthcare Life Sciences, Baie d'Urfe, Quebec, Canada) using either Kodak Biomax XAR imaging film kit with exposure times varying from 5 to 20 minutes or a Storm PhosphoImager 840 (Molecular Dynamics, Sunnyvale, CA, USA). Groups (wt and tg) were obtained simultaneously and normalized with respect to βIII-tubulin immunoreactivity. All experiments were performed at least in triplicate. The software GelProPlus (Media Cybernetics Inc, Rockville, MD, USA) was used to quantify the optical density of each band.

2.4. Cytokine arrays

Cytokine arrays (EA-4002, EA-1201, Signosis Inc, Sunnyvale, CA, USA) were used, according to manufacturer's instructions, to compare levels of cytokines and chemokines between wt and tg animals at the age of 4- and 13-month-old. These arrays allow monitoring the expression of cytokines, including proinflammatory and anti-inflammatory molecules and chemokines, semi-quantitatively by optical densities. A total of 12 wt and 12 tg cortical homogenates of 4- and 13-months old rats were prepared as in Section 2.2. Based on the average protein concentration in each sample, these homogenates were pooled to obtain a final solution of 0.3 μg/μL of total protein concentration per group (wt and tg). This concentration was chosen according to preliminary trials and dilutions. To dilute the pooled homogenates, PBS solution was used. Briefly, 100 μL of sample was incubated in the anti-cytokine antibody-coated wells for 2 hours before adding a biotin-labeled antibody mixture followed by a horseradish peroxidase (HRP)-streptavidin conjugate. Four washes were done in between