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Acute isolation and transcriptome characterization of cortical astrocytes and microglia from young and aged mice

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ABSTRACT

Astrocytes and microglia become reactive in many neurological disorders resulting in phenotypic and functional alterations. Both cell types might also display functional changes during normal aging. To identify gene signatures and changes in basal cellular functions of astrocytes and microglia in relation to aging, we isolated viable astrocytes and microglia from young adult and aged mouse cortices and determined their gene expression profile. Aged astrocytes, compared with young astrocytes, showed an increased inflammatory phenotype and increased 'zinc ion binding.' Young astrocytes showed higher expression of genes involved in 'neuronal differentiation' and hemoglobin synthesis. Astrocyte expression of genes involved in neuronal signaling remains high throughout age. Aged microglia had higher expression of genes involved in 'vesicle release,' 'zinc ion binding,' and genes within the tumor necrosis factor-ligand family and young microglia had increased transcript levels of C-C motif chemokines. These data provide a transcriptome database of cell-type enriched genes of astrocytes and microglia from adult mice and give insight into the differential gene signature of astrocytes and microglia in relation to normal aging.

1. Introduction

Astrocytes and microglia are the 2 major populations of glial cells in the brain. Astrocytes are indispensible players in neural communication. They recycle glutamate, regulate central nervous system blood flow, release gliotransmitters, and express ionotropic and metabotropic neurotransmitter receptors. In addition, they are active players in central nervous system immune responses (Oberheim et al., 2012; Ransohoff and Brown, 2012; Volterra and Meldolesi, 2005; Wang and Bordey, 2008). As such, understanding astrocyte physiology is crucial for our insight into normal and pathological brain functioning. In response to a variety of brain diseases and brain traumas, astrocytes can undergo morphological and functional changes, a process known as astrogliosis (Eng and Ghirnikar, 1994; Sofroniew and Vinters, 2009). Aging has been linked to a mild increase in inflammation, reactive gliosis, and oxidative stress. Furthermore, aging has been related to blood–brain barrier dysfunction and a dysregulated Ca^{2+} homeostasis. Changes in astrocytes are implicated in all of these age-related alterations (Lee et al., 2000; Møller et al., 2010; Popescu et al., 2009; Salminen et al., 2011; Simard et al., 2003). To gain more insight into the physiological and pathological processes in astrocytes during normal aging, we first optimized isolation methods to obtain pure and viable astrocytes from brains at all ages, which then allowed characterization of the transcriptome and the age-dependent changes therein.

Microglia are the professional immune cells of the brain and function as surveyors, constantly scanning their environment for insults or other signals indicative of a disruption in brain homeostasis (Kettenmann et al., 2011; Nimmerjahn et al., 2005; Perry et al., 2010). These cells are capable of acquiring either a pro- or anti-inflammatory phenotype in response to damage (Lucin and Wyss-Coray, 2009; Melief et al., 2012). It is proposed that microglia are involved in maintenance of synapses, with their processes continuously scanning synapses and the microenvironment (Nimmerjahn et al., 2005; Wake et al., 2009). Several studies have shown that microglia, during aging, acquire a

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"primed" phenotype characterized by an increase in expression of major histocompatibility complex II, proinflammatory cytokines, and pattern recognition receptors (reviewed in Norden and Godbout, 2013 and Wong, 2013). Primary microglia have been isolated based on their expression of CD11b and CD45 from young rats (Sedgwick et al., 1991), young (2.5–3-month-old) mice (Olah et al., 2012), and human tissue (Becher and Antel, 1996; Melief et al., 2012), but a genome-wide analysis of their age-related changes has not been presented.

For isolation of astrocytes from the mouse brain, previous studies have used fluorescent reporters under astrocyte-specific promoters such as glial fibrillary acidic protein (GFAP), S100b, ALDH1L1, solute carrier family 1, member 1 (GLAST), solute carrier family 1, member 2 (GLT-1) (Cahoy et al., 2008; Lovatt et al., 2007; Yang et al., 2011; Zamanian et al., 2012). Additionally, an antibody against the astrocytic glutamate transporter, GLT-1, has been used to isolate astrocytes from 10–12-week-old mice followed by gene expression profiling (Lovatt et al., 2007). Cahoy and colleagues provided a gene list from astrocytes isolated from the whole brain, containing white and gray matter astrocytes from mouse pups of postnatal day 1-30 (P1-P30) (Cahoy et al., 2008). Aged cortical astrocytes might be substantially different from postnatal or young mature astrocytes and, to our knowledge, an inventory of genes highly enriched and expressed in cortical astrocytes from aged mice has thus far not been provided or been compared with young mature astrocytes. In addition, we also provide transcriptome data on young and aged microglia.

In this study, we used an optimized isolation protocol suitable for cell isolation from the cortex of aged mice to obtain astrocytes, using myelin removal and an antibody against GLT-1, and to obtain microglia, using antibodies against CD11b and CD45, for fluorescence-activated cell sorting (FACS). Application of this protocol followed by gene expression profiling, enabled us to identify age-dependent changes. We define young as glia isolated from 2.5-month-old young adult mice brains and aged as glia isolated from 15–18-month-old (middle-aged/old mice; based on http://research.jax.org/faculty/harrison/ger1vLifespan1.html). Our findings give a unique insight into the basal gene signature of these 2 glial cell populations and highlight important cellular functions and their transcriptional alterations in relation to aging.

2. Methods

2.1. Mice

C57Bl/6 mice were sacrificed at an age of 15–18 months for aged cells and at 2.5 months for young cells (for optimization some 3–5-month-old mice were used). All animals were housed in standard conditions with access to water and food ad libitum. Animal handling and experimental procedures were reviewed and approved by the ethical committee for animal care and use of the Royal Netherlands Academy for Arts and Sciences, acting in accordance with the European Community Council directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize suffering and number of animals used.

2.2. Isolation of different glial cell populations

Preparation of single-cell suspension was done based on earlier protocols (Lovatt et al., 2007). The general procedure was: the mice were anesthetized using pentobarbital and transcardially perfused with Hank's Balanced Salt Solution without calcium or magnesium (HBSS) (Invitrogen). The cortices were dissected out and kept in ice-cold HBSS. After mechanical dissociation using a Tissue Chopper (McIlwain; Ted Pella, Inc), the tissue was subjected to enzymatic dissociation using papain (Worthington) at a final concentration of 8 U/mL in combination with DNase I at 80 Kunitz units/mL final concentration (Sigma-Aldrich; D4263) in a piperazine-N,N′-bis(ethanesulfonic acid); 1,4-piperazinediethanesulfonic acid (PIPES)-based buffer with the addition of L-cystein-HCL and ethylenediaminetetraacetic acid (EDTA) in an incubator at 37 °C for 50 minutes on a rocker, and another 15 minutes of incubation after addition of an extra 25 Kunitz units/mL DNase I. The mix was spun at 200*g* for 15 minutes and the pellet was triturated to obtain a single-cell suspension.

2.2.1. Percoll density gradient and myelin removal

Singe cells from the enzymatic degradation were washed and resuspended in Minimum Essential Media (MEM) with 1% bovine serum albumin (BSA) and filtered through a 70-μm single-cell strainer (BD Bioscience), followed by a separation step, in which the cell suspension was overlaid on an isotonic (90%) Percoll layer and spun at 200*g*, at 4 °C for 15 minutes, using low brake. The top phase was discarded, and the Percoll layer containing the cells and the myelin layer were collected and diluted in 5 times the volume using MEM/1% BSA, followed by centrifugation at 200*g* at 4 °C for 10 minutes. The supernatant containing the myelin was discarded and the pellet was resuspended in cold MACS buffer containing 1 volume dilution of phosphate buffered saline (PBS), 2 mM EDTA, and 0.5% BSA. To get rid of the myelin, Myelin Removal Beads II (Miltenyi Biotec; 130-096-733) were used according to the manufacturer's protocol before staining of the cells with the FACS antibodies. In brief: cells were resuspended in 1 mL MACS buffer and incubated with myelin removal beads at 4 °C for 15 minutes; cells were washed and loaded on a magnetic column, the column was washed 3 times with MACS buffer, and the cells in the flow through were used for further staining and sorting.

2.2.2. FACS sorting procedure

Pelleted cells were resuspended in PBS (0.5% BSA) and stained with unconjugated purified rabbit polyclonal GLT-1 (1:100; custom made; Biomatik; 0.6 mg/mL), mouse anti-O4 (1:400; Chemicon; MAB345), CD11b-APC (1:200; eBioscience; 17-0112-82), CD45-PE (1:200; eBioscience; 12-0451-82), Fc-receptor block CD16/32 (1:200; BD Pharmingen; 553142) and polyclonal rabbit immunoglobulin G-isotype control (1:150; Thermo Scientific; NC-100-P1) (at 4 °C for 30 minutes). After washing, the secondary anti-rabbit biotin antibody (1:100; Jackson ImmunoResearch; 111-065-144) and PE-conjugated goat anti-mouse (1:125; eBioscience; 12-4010-82) (for visualization of O4 staining) was added an incubated at 4 °C for 30 minutes, after an additional washing step, streptavidin APC-Cy7 (1:125; Biolegend; 405208) was added and incubated at 4 °C in the dark for 15 minutes. The cells were washed and resuspended in (GKN-BSA; 8 g/L NaCl, 0.4 g/L KCl, 1.77 g/L $Na_2HPO_4.2H_2O$, 0.69 g/L $NaH_2PO_4.H_2O$, 2 g/L D-(1)-glucose, pH 7.4) with 0.3% bovine serum albumin (Roche Diagnostics) and the cell viability dye 7AAD (BD Bioscience) was added. Using the BD FACS Aria I, (BD Bioscience), GLT1-positive cells were sorted on $GLT1^+/CD11b^-$ expression and aged microglia cells were sorted on $CD11b^+/CD45^+$ expression after gating away the dead cells based on the 7AAD signal. The sorted cells were either directly subjected to RNA isolation or resuspended in Dulbecco's Modified Eagle Medium (DMEM)/F-10 (Invitrogen), 10% fetal calf serum (FCS), 1% penicillin-streptomycin (Penstrep; Invitrogen) for culturing.

2.3. Immunochemistry

Cytospins were generated from the different cell populations from the FACS-sort; $GLT\text{-}1^+$ cells, $CD11b^+/CD45^+$ cells, and from the negative populations; 10–20,000 cells per cytospin