Brain, Behavior, and Immunity 53 (2016) 72–83

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

A(H1N1) vaccination recruits T lymphocytes to the choroid plexus for the promotion of hippocampal neurogenesis and working memory in pregnant mice

Fangfang Qi [a,1], Junhua Yang [a,1], Yucen Xia [a], Qunfang Yuan [a], Kaihua Guo [a], Juntao Zou [a], Zhibin Yao [a,b,*]

[a] Department of Anatomy and Neurobiology, Zhongshan School of Medicine, Sun Yat-sen University, PR China
[b] Guangdong Province Key Laboratory of Brain Function and Disease, Zhongshan School of Medicine, Sun Yat-sen University, PR China

ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form 30 October 2015
Accepted 9 November 2015
Available online 11 November 2015

Keywords:
T cell
Chemokine
Trafficking
Microglia
TCR
M2 polarization

ABSTRACT

We previously demonstrated that A(H1N1) influenza vaccine (AIV) promoted hippocampal neurogenesis and working memory in pregnant mice. However, the underlying mechanism of flu vaccination in neurogenesis and memory has remained unclear. In this study, we found that T lymphocytes were recruited from the periphery to the choroid plexus (CP) of the lateral and third (3rd) ventricles in pregnant mice vaccinated with AIV (Pre+AIV). Intracerebroventricular delivery of anti-TCR antibodies markedly decreased neurogenesis and the working memory of the Pre+AIV mice. Similarly, intravenous delivery of anti-CD4 antibodies to the periphery also down-regulated neurogenesis. Furthermore, AIV vaccination caused microglia to skew toward an M2-like phenotype (increased Arginase-1 and Ym1 mRNA levels), and elevated levels of brain-derived growth factor (BDNF) and insulin-like growth factor-1 (IGF-1) were found in the hippocampus, whereas these effects were offset by anti-TCR antibody treatment. Additionally, in the CP, the expression level of adhesion molecules and chemokines, which assist leukocytes in permeating into the brain, were also elevated after AIV vaccination of pregnant mice. Collectively, the results suggested that the infiltrative T lymphocytes in the CP contribute to the increase in hippocampal neurogenesis and working memory caused by flu vaccination, involving activation of the brain's CP, M2 microglial polarization and neurotrophic factor expression.

1. Introduction

A growing body of literature has indicated that the adaptive peripheral immune response plays a vital role in neuronal plasticity and behavioral function (Yirmiya and Goshen, 2011; Ziv and Schwartz, 2008). Rolls et al. (Rolls et al., 2008) reported that pregnancy induced a decrease in hippocampal neurogenesis in wild-type mice. However, pregnancy failed to induce such decreases in nude mice, although they had lower neurogenesis levels than wild-type mice before pregnancy. Therefore, Rolls et al. proposed that the decreased hippocampal neurogenesis might be mediated by pregnancy-induced natural suppression of the immune response, particularly the adaptive immune response.

Interestingly, we recently reported that A(H1N1) influenza vaccination during pregnancy promoted hippocampal neurogenesis and working memory, both in the offspring (Xia et al., 2014a) and the pregnant mice (Xia et al., 2014b). The exact mechanism, based on immune cells, is not fully understood, although the systemic T helper (Th)1/Th2 balance was involved.

Nude mice have been shown to have impaired spatial learning abilities and hippocampal neurogenesis. However, these impairments can be restored by reconstituting these immune-deficient mice with T lymphocytes from their wild-type counterparts. Reconstitution of immune-deficient mice with B lymphocytes did not affect their impaired spatial learning abilities, indicating that cognitive improvement was T cell-dependent (Wolf et al., 2009a; Ziv et al., 2006). At the same time, several other researchers have addressed the potential relationship between T cells and learning behavior (Brynskikh et al., 2008; Kipnis et al., 2004). A recent study showed that $CD4^+$ T cell subsets, but not $CD8^+$ T cell subsets, were sufficient for maintaining normal learning and memory (Radjavi et al., 2014). Access of immune cells to the choroid plexus (CP) through the blood–cerebrospinal fluid barrier (BCSFB) is more feasible than to the CNS parenchyma under physiological conditions (Derecki et al., 2010; Shechter et al., 2013). Importantly, adhesion

* Corresponding author at: #74, Zhongshan No. 2 Road, Guangzhou 510080, PR China.
E-mail address: yao.zb@163.com (Z. Yao).
[1] These authors contributed equally to the work and should be regarded as co-first authors.

http://dx.doi.org/10.1016/j.bbi.2015.11.006
0889-1591/

molecules and chemokines are required for the recruitment of T cells to the CP in an immune activation model (Kunis et al., 2015).

Microglia, as the innate resident immune cells in the CNS, actively participate in many aspects of adult brain function, including neurogenesis, synaptic plasticity and long-term potentiation (Di Filippo et al., 2008; Sato, 2015; Tishkina et al., 2014). Recent studies have reported that microglia can be divided into a pro-inflammatory M1-like phenotype and an anti-inflammatory, neuroprotective M2-like phenotype, according to their expressions of special cytokines and receptors (Cherry et al., 2014; Pepe et al., 2014; Tang and Le, 2015). Importantly, the M2 microglia exert beneficial effects on hippocampal neuronal function via the regulation of T cells secreting IL-4 and IFN-γ (Butovsky et al., 2005, 2006). In this study, we showed that infiltrative T cells in the CP contributed to increased hippocampal neurogenesis and working memory in pregnant mice vaccinated with the A(H1N1) influenza vaccine (AIV).

2. Materials and methods

2.1. Animals and vaccination

Female C57BL/6 mice (8 week old) were purchased from the Laboratory Animal Center of Sun Yat-sen University. Half of them were randomly mated with healthy males in same-sex pairs. The presence of a vaginal plug was designated as gestational day 0 (GD0), and the delivery day was designated postnatal day 0 (PD0). Each pregnant mouse was immunized with inactivated AIV in the quadriceps via a single intramuscular injection containing 3 μg of hemagglutinin in the first trimester (GD2.5) according to our previous research (Xia et al., 2014a,b), or treated with an equal amount of sterilized PBS (n = 10 per group). The split inactivated AIV was obtained from the Center for Disease Control and Prevention (CDC, Guangdong, China). The efficacy and safety of the vaccine have been widely confirmed. All of the experiments were undertaken in accordance with the Guide of the Institutional Animal Ethics Committee of Sun Yat-sen University. Each experimental animal was individually maintained in a specific pathogen-free room on 12 h light/dark cycles, with food and water provided ad libitum.

2.2. Hemagglutination inhibition (HI) assay

Mouse blood was collected using the tail vein bleeding method, followed by centrifugation (5000 rpm, 10 min) to remove blood clots after overnight incubation at 4 °C. The antibody titers were determined via a hemagglutination inhibition (HI) assay, according to an established procedure using chicken erythrocytes (Zhu et al., 2009). Briefly, sera were treated with a receptor-destroying enzyme at 37 °C for 18 h and were heated at 56 °C for 30 min. They were then diluted with PBS in serial two-fold dilutions in 96-well plates. We recorded the highest dilution that caused complete hemagglutination inhibition against four hemagglutination units (HAUs) of virus as the HI titer.

2.3. Administration of 5-bromo-2deoxyuridine (BrdU) and tissue preparation

For cell proliferation analysis, all of the mice received three BrdU (Sigma–Aldrich, St. Louis, MO, USA) injections (50 mg/kg, i.p., once every 2 h) at GD14, according to previous research (Ziv et al., 2006). The mice were deeply anesthetized with chloral hydrate (120 mg/kg, i.p.) and were perfused intracardially with 0.9% NaCl, followed by pre-cooled phosphate-buffered 4% paraformaldehyde 2 h after the third BrdU injection. The brains were dissected, postfixed overnight at 4 °C for 10 h and equilibrated in 30% sucrose solution. Then, 40-μm-thick frozen sections of the hippocampus were collected using a Leica SM2000R sliding microtome (Leica Microsystems, Richmond Hill, Ontario, Canada) for histological analysis.

2.4. Immunofluorescence

BrdU-positive proliferation was measured as previously described (Xia et al., 2014a). In brief, brain slices were pretreated with 50% formamide/2× saline-sodium citrate (SSC) at 65 °C for 2 h, incubated in 2 N HCl at 30 °C for 40 min and then blocked in 1% bovine serum albumin (BSA) and 0.25% Triton X-100 (Sigma–Aldrich, St. Louis, MO, USA) at 37 °C for 1 h. The slices were then incubated in rat anti-BrdU monoclonal antibodies (1:400, Oxford Biotechnology, UK) at 37 °C for 2 h, followed by overnight incubation at 4 °C. For DCX, Tbr2, BDNF, IGF-1, Arginase-1 (Arg), T cells (GD14), Iba-1, GFAP, Nestin (GD 20-21) and NeuN (PD 7) staining, sections were blocked for 30 min in a solution of 1% BSA as described above. The primary antibodies and dilutions were goat anti-DCX (1:400, Santa Cruz Biotechnology, CA, USA), rabbit anti-Tbr2 (Abcam, Cambridge, MA, USA), rabbit anti-BDNF (1:200, Abcam, Cambridge, MA, USA), goat anti-mouse IGF-1 (1:200, BD Bioscience, San Jose, CA, USA), rabbit anti-Arginase-1 (1:200, Proteintech, Chicago, IL, USA), mouse anti-TCR (1:1000, Acris antibodies, Germany), mouse anti-CD4 (Sigma–Aldrich, St. Louis, MO, USA), rabbit anti-Iba-1 (1:1000; Wako Chemical, Japan), mouse anti-GFAP (1:10,000; Sigma–Aldrich, St. Louis, MO, USA), mouse anti-Nestin (1:500, Abcam, Cambridge, MA, USA) and mouse anti-NeuN (1:1000; Sigma–Aldrich, St. Louis, MO, USA). The next day, the sections were stained with the secondary antibodies, including Alexa Fluor 594 donkey anti-rat, Alexa Fluor 488 donkey anti-goat, Alexa Fluor 555 goat anti-rabbit, Alexa Fluor 488 goat anti-rabbit and Alexa Fluor 488 goat anti-mouse antibody (1:400; Molecular Probes, Eugene, OR, USA) at 37 °C for 2 h. In addition, bisbenzimide H33258 fluorochrome trihydrochloride (Hoechst) (Enzo Life Sciences, New York, USA) was used to label the cell nuclei.

2.5. Confocal and MBF stereo investigations

Quantitative analyses of $BrdU^+$ proliferating cells and DCX^+ neuronal progenitor cells were performed using a Stereo Investigator stereological system (MicroBrightField Inc., Williston, VT, USA) following standard stereotaxic coordinates. All of the stereological analyses were performed under the 40× objective of a Nikon microscope. The coefficient of error (CE) was kept at less than 10% to obtain reliable results.

2.6. Isolation of microglia from the hippocampus

Pregnant mice were administered AIV or PBS as described above (n = 6 per group). Seven days after AIV vaccination, mice were transcardially perfused with cold 0.9% NaCl to remove the circulatory lymphocytes and macrophages. The CP tissues from the lateral and 3rd ventricle and the hippocampus were dissected and minced into small pieces. Single-cell suspensions were generated by incubating the tissue with 0.25% trypsin at 37 °C for 1 h. The single-cell suspensions were washed and centrifuged at 800*g over a 30%/70% Percoll gradient. Purified cells were determined on an FACSCalibur flow cytometer (BD influx™, Franklin Lakes, NJ, USA) using anti-CD11b primary antibody, followed by incubation with streptavidin-PE secondary antibody (BIO-RAD, Hercules, CA, USA).