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Intracranial IL-17A overexpression decreases cerebral amyloid angiopathy by upregulation of ABCA1 in an animal model of Alzheimer's disease

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ABSTRACT

Neuroinflammation is a pervasive feature of Alzheimer's disease (AD) and characterized by activated microglia, increased proinflammatory cytokines and/or infiltrating immune cells. T helper 17 (Th17) cells are found in AD brain parenchyma and interleukin-17A (IL-17A) is identified around deposits of aggregated amyloid β protein (Aβ). However, the role of IL-17A in AD pathogenesis remains elusive. We overexpressed IL-17A in an AD mouse model via recombinant adeno-associated virus serotype 5 (rAAV5)-mediated intracranial gene delivery. AD model mice subjected to injection of a vehicle (PBS) or rAAV5 carrying the lacZ gene served as controls. IL-17A did not exacerbate neuroinflammation in IL-17A-overexpressing mice. We found that IL-17A overexpression markedly improved glucose metabolism, decreased soluble Aβ levels in the hippocampus and cerebrospinal fluid, drastically reduced cerebral amyloid angiopathy, and modestly but significantly improved anxiety and learning deficits. Moreover, the ATP-binding cassette subfamily A member 1 (ABCA1), which can transport Aβ from the brain into the blood circulation, significantly increased in IL-17A-overexpressing mice. In vitro treatment of brain endothelial bEnd.3 cells with IL-17A induced a dose-dependent increase in protein expression of ABCA1 through ERK activation. Our study suggests that IL-17A may decrease Aβ levels in the brain by upregulating ABCA1 in blood-brain barrier endothelial cells.

1. Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia (Fratiglioni et al., 2000; Kawas et al., 2000; Kukull et al., 2002), characterized by extracellular amyloid β (Aβ) deposition in the brain parenchyma and cerebral blood vessels. Additionally, neuroinflammation is a consistent feature of AD and thought to influence the disease pathogenesis (McManus et al., 2014). Immune responses occurring in brains of AD patients can be driven by resident immune cells and/or brain infiltrating peripheral immunocompetent cells (Ghosh and Geahlen, 2015; Togo et al., 2002). Microglia, the innate immune cells in the central nervous system (CNS), are activated by Aβ deposits and secrete inflammatory cytokines and chemokines, contributing to AD progression (Hickman et al., 2008; Ridolfi et al., 2013; Rodriguez et al., 2016).

Central-memory $CD4^+$ T lymphocytes account for > 80% of the cells in the cerebrospinal fluid (CSF) and routinely penetrate the CNS. Lymphocytes in the CNS are replenished by newly immigrating cells approximately twice per day (Engelhardt and Ransohoff, 2005; Hickey, 2001; Smolders et al., 2013). Numerous lines of evidence indicate the presence of T cells in the brains of AD patients (Ferretti et al., 2016; Lombardi et al., 1999; Monsonego et al., 2003; Shalit et al., 1995; Togo et al., 2002) although their possible roles in AD pathogenesis have been studied less than those of microglia. T cells in the brains of AD patients increased as compared with subjects with non-AD degenerative dementias and age-matched controls (Lueg et al., 2015; Togo et al., 2002). T cells breaching the brain vasculature and parenchyma can be either detrimental or beneficial for brain homeostasis (Kipnis et al., 2004; Monsonego et al., 2006; Ziv et al., 2006). Depletion of regulatory T (Treg) cells in an AD mouse model accelerated the onset of cognitive deficits without altering brain Aβ load (Dansokho et al., 2016). Administration of Aβ-specific type 1 T helper (Th1) cells into the brain lateral ventricle of an AD mouse model enhanced removal of cerebral Aβ deposits and promoted neurogenesis without harmful effects (Fisher et al., 2014). Thus, these T cells play beneficial roles in the AD pathophysiology. In contrast, intravenous

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administration of Aβ-specific Th1 cells caused brain infiltration, increased microglial activation and Aβ deposition, and impaired cognitive function in an AD mouse model but similarly administered Aβ-specific IL-17-producing helper T (Th17) cells infiltrated into the brain without exacerbating microglia activation, Aβ deposition and cognitive deficits in the same mice (Browne et al., 2013).

IL-17A or IL-17 is a signature cytokine of Th17 cells and is involved in anti-microbial host defense and inflammation. A plethora of evidence supports the notion that Th17 cells and IL-17A play a pathogenetic role in certain autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, psoriasis, and inflammatory bowel disease. Respiratory infection with Bordetella pertussis in an AD mouse model (McManus et al., 2014) and intrahippocampal administration of Aβ42 into rats (Zhang et al., 2013) induced infiltration of Th17 cells and expression of IL-17A in the brain, which were associated with exacerbation of an AD-like phenotype in these experimental animals. It is interesting to note that plasma IL-17A levels have been identified as one of the plasma biomarkers for AD diagnosis and neocortical Aβ load (Burnham et al., 2014; Doecke et al., 2012). However, the role of IL-17A in AD pathophysiology is unclear. In this study, we investigated the effects of IL-17A overexpression via intracranial delivery of recombinant adeno-associated virus serotype 5 (rAAV5)-mediated IL-17A on Aβ load, microglia activation, glucose metabolism and behavioral function using a transgenic animal model of AD.

2. Materials and methods

2.1. Experimental animals and intracranial injection of rAAV-IL-17A

A rAAV vector, rAAV5-IL-17A, was prepared as described previously (Yang et al., 2016). A control rAAV vector encoding LacZ, rAAV5-LacZ, was similarly prepared. B6.Cg-Tg (APPswe, PSEN1dE9) 85Dbo/J mice (TgAPPswe/PS1dE9 mice) purchased from Jackson Laboratory, were used as an AD mouse model to study a potential role of IL-17A in the AD pathogenesis. For intracranial injection, 2-month-old TgAPPswe/PS1dE9 mice were anesthetized by Nembutal® (pentobarbital) and placed on a stereotaxic instrument with a motorized stereotaxic injector (Stoelting, Wood Dale, IL). A midline incision was made to expose the bregma. A hole in the skull was made by a drill 0.5 mm posterior to the bregma and 1.0 mm right to the midline. rAAV5-IL-17A (1.5×10^{10} vector genomes (vg) in 10 μl PBS/mouse) was injected unilaterally into the right lateral brain ventricle at the depth of 2 mm at a rate of 1 μl/min. Mice subjected to rAAV5-IL-17A injection are referred to as IL-17A-overexpressing mice (n = 8). Mice similarly treated with 10 μl PBS or rAAV5-LacZ (1.5×10^{10} vg/mouse) served as controls and are referred to as control mice (n = 9): Four PBS-injected and five rAAV5-LacZ-injected mice were combined together because no phenotypic differences between two controls were found (more information in Supplementary Fig. 1). Due to the difference in brain amyloid content between male and female TgAPP/PS1 mice (Akhter et al., 2015; Gallagher et al., 2013; Sierksma et al., 2013; Taniuchi et al., 2007; Wang et al., 2003), we used only male mice for this study. All animal protocols were prospectively approved by the Institutional Animal Care and Use Committee of the University of Illinois College of Medicine at Peoria.

2.2. Behavioral tests

2.2.1. Behavioral schedule and statistics

A battery of behavioral tests was performed on experimental mice at 12 months of age as previously described (Lim et al., 2011). After measuring body weight and adapting mice to handling, the tests were conducted over a 16-day period as follows: spontaneous alternation (days 1–10), open-field (days 6–8), elevated plus-maze (days 9 and 10), and Morris water maze (days 11–16). In each test, whenever possible, the apparatus was wiped clean with a wet cloth and dried before the next mouse was introduced to minimize odor cues. For T-maze, Mann-Whitney *U* test was used to assess the chance of alternation compared with theory (50%) and unpaired *t*-test was used for the intergroup differences. Intergroup differences were assessed by 2×3 analysis of variance (ANOVA) for 2 independent groups and 3 days of testing with repeated measures on the second factor for the open-field and 2×2 ANOVA for two groups and 2 days of testing for the elevated plus-maze. The unpaired *t*-test was used for the probe test of the Morris water maze and a 2×5 ANOVA for 2 independent groups and 5 days of testing with repeated measures on the second factor was used for 5-day acquisition of the Morris water maze. Results are expressed as mean ± standard error of the mean (SEM). In all cases, $P < 0.05$ was considered to be significant.

2.2.2. Exploration and anxiety

Spontaneous alternation was measured in a T-maze, made of white acrylic and consisting of a central stem flanked on each side by 2 arms. The maze width was 9 cm, the wall height 20 cm, and each arm 30 cm in length. On the initial trial, the mice were placed in the stem with the right arm blocked by a plastic barrier (forced choice). After entering the available arm, the mice were kept in it for 1 min by closing the barrier behind them. The mice were then retrieved and after removing the barrier, placed back in the stem for a free-choice trial, either into the same arm or the opposite arm (4-paw criterion). On the following 10 days, the same 2-trial procedure was repeated, except that the blocked arm was switched from right on odd days to left on even days.

The number of alternations and the latencies before responding during the choice trial were measured. In the absence of any decision within 1 min, the mice were briefly prodded from behind, usually not more than once, far from the choice point so that a response could be recorded on every trial.

Motor activity was measured in the open-field chamber that was made of white acrylic with a 50 cm × 50 cm surface area. Each mouse was placed in a corner of the open-field. The activity in central (25 cm × 25 cm surface area) and peripheral zones was recorded in a 5-min session for 3 consecutive days and analyzed by video tracking software (SD Instruments, San Diego, CA). The distance traveled and the time spent resting (<2 cm/s), moving slow (2–5 cm/s), or moving fast (>5 cm/s) in each zone were measured, as well as the time spent in the periphery and center of the apparatus.

The elevated plus-maze apparatus consisted of 4 arms in a cross-shaped form 70 cm in length with a 10 cm × 10 cm central region. Two of the arms were enclosed on 3 sides by walls (10 cm in height) facing each other, while the other two were open, except for a minimal border (0.5 cm in height) used to minimize falls. A mouse was placed in the central region and then the number of entries and the time spent inside enclosed and open arms were measured in a 5-min session on 2 consecutive days with the same video tracking system. The open/total arm entries and duration ratios were also calculated.

2.2.3. Spatial learning and memory

The Morris water maze consisted of a pool of blue opaque plastic, 116 cm in diameter with 75-cm high walls, filled with water (20 °C) at a height of 31 cm. Powdered milk was evenly spread over the water surface to camouflage the escape platform (8 × 8 cm) made of white plastic and covered with a wire mesh grid to ensure a firm grip. The watered milk was removed every day after a few hours of training and the pool rinsed with clean water. The pool was con-