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Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impairment in an Alzheimer's disease mouse model through modulation of neuroinflammation

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Abstract

Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSC) have a potential therapeutic role in the treatment of neurological disorders, but their current clinical usage and mechanism of action has yet to be ascertained in Alzheimer's disease (AD). Here we report that hUCB-MSC transplantation into amyloid precursor protein (APP) and presenilin1 (PS1) double-transgenic mice significantly improved spatial learning and memory decline. Furthermore, amyloid-β peptide (Aβ) deposition, β-secretase 1 (BACE-1) levels, and tau hyperphosphorylation were dramatically reduced in hUCB-MSC transplanted APP/PS1 mice. Interestingly, these effects were associated with reversal of disease-associated microglial neuroinflammation, as evidenced by decreased microglia-induced proinflammatory cytokines, elevated alternatively activated microglia, and increased anti-inflammatory cytokines. These findings lead us to suggest that hUCB-MSC produced their sustained neuroprotective effect by inducing a feed-forward loop involving alternative activation of microglial neuroinflammation, thereby ameliorating disease pathophysiology and reversing the cognitive decline associated with Aβ deposition in AD mice.

Keywords: Alzheimer's disease; Human umbilical cord blood-derived mesenchymal stem cell; Amyloid-β; microglia; Spatial learning and memory; Microglial neuroinflammation

Genetic studies in familial Alzheimer's disease (AD) suggest that amyloid β-peptide (Aβ) plays a key pathogenic role in AD, and have connected the Aβ plaque with formation of intracellular tau tangles, another neurotoxic feature of AD (Huang and Jiang, 2009; Mattson, 2004). Aβ plaques are potent activators of microglia and astrocytes, 2-cell types that respond to cerebral amyloidosis by chronic proinflammatory activation (Praticò and Trojanowski, 2000). Numerous reports indicate that the neuroinflammatory process contributes to the pathogenesis of AD. Thus, therapeutic strategies aimed at manipulating this inflammatory cascade, including Aβ immunization (Games et al., 2000; Schenk et al., 1999) and modulation of microglial activation

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(Tan et al., 1999), have been evaluated, and are able to reduce AD-like pathology and improve behavioral impairment in AD transgenic mouse models. Currently, however, no treatment is available for AD patients to modulate neuroinflammation and prevent the cell death that results in inevitable decline.

Administration of cells isolated from human umbilical cord has produced beneficial effects in animal models for neurodegenerative diseases, including AD, by using cell replacement or immunomodulatory strategies (Chen et al., 2006; Nikolic et al., 2008). Recent developments in stem cell technology raise the prospect of cell replacement therapy for neurodegenerative disorders. For example, human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSC) are under intense investigation as a potential therapeutic source of neurons to replace damaged or lost cells in neurological diseases (Harris, 2008; Hirko et al., 2008). Cord blood cells also have been shown to antagonize proinflammatory T_h1, and stimulate anti-inflammatory T_h2 responses by immunomodulation in neurological disorders (Vendrame et al., 2005). Although hUCB-MSC have been suggested as a potential therapeutic approach for several neurological disorders (Harris, 2008; Hirko et al., 2008), the actual therapeutic impact of hUCB-MSC on AD neuropathology, especially cognitive impairments, and their mechanism of action has not yet been ascertained.

Many studies including those on the human post-mortem brain, as well as neuroimaging analysis in AD patients and in rodent transgenic models, have provided evidence that microglia are attracted to and surround senile plaques in AD (Van Groen et al., 2009; Wiley et al., 2009). However, their exact role in the pathogenesis of AD remains to be elucidated. Some studies have also indicated that Aβ can activate microglia to produce cytokines and neurotoxins, hence promoting neurodegeneration (El Khoury et al., 2003; Meda et al., 1995). In contrast, others have suggested that microglia have a neuroprotective role, secreting neurotrophic agents and eliminating toxic Aβ by phagocytosis (Jimenez et al., 2008; Simard et al., 2006). One study observed the existence of an age-dependant phenotypic change of microglial activation in the hippocampus of an AD mouse model, from an alternative (expressing IL-4) activation state to a classic cytotoxic (expressing IL-1β and TNF-α) phenotype (Jimenez et al., 2008). Interestingly, more recent work in an ischemic mouse model confirmed that microglia can switch phenotypes to become "alternatively activated" such that anti-inflammatory effects predominate, and that this is promoted by adult stem cell transplantation (Ohtaki et al., 2008). Our previous report also showed that intracerebral transplantation of bone marrow stem cells can increase microglial activation and reduce Aβ deposits in an acutely induced AD model. The activated microglia were located near the Aβ deposits, and their morphology was changed from ramified to ameboid as an action of the microglial phagocytosis (Lee et al., 2009).

Here, we examined whether hUCB-MSC transplantation into the hippocampus of an AD mouse model could have beneficial effects through microglia activation, and whether these microglia are "alternatively activated" by the immunomodulatory properties of the transplanted hUCB-MSC. We found that hUCB-MSC transplantation promoted alternative microglial activation by opposing proinflammatory and stimulating anti-inflammatory pathways, rescued cognitive impairment, and reduced Aβ deposits, β-secretase 1 (BACE-1), and tau pathology in the brain.

1. Methods

1.1. Animals

A double transgenic mouse model of AD was used for the evaluation of hUCB-MSC intracerebral transplantation. APP/PS1 double transgenic and nontransgenic mice were generated from matings between single transgenic mice expressing human mutant APP (Hsiao et al., 1996) and mutant PS1 (Duff et al., 1996). The single APP and PS1 transgenic mice were originally obtained from Taconic and Jackson Laboratory, respectively. Given the existence of gender differences in Aβ deposition in this model, we used only males in the present study. All procedures were in accordance with an animal protocol approved by the Kyungpook National University Institutional Animal Care and Use Committee (IACUC).

1.2. Isolation and culture of hUCB-MSC

Human UCB samples were collected from the umbilical vein of deliveries with informed maternal consent. In all cases, UCB harvests were processed within 24 hours of collection, with viability of more than 90%. Isolation and expansion of hUCB-MSC were performed according to our previous report (Kim et al., 2009). Differentiation characteristics of hUCB-MSC, including their ability to form osteogenic, chondrogenic, and adipogenic lineages were tested before the onset of this study.

1.3. Implantation of guide cannula

One week before the first hUCB-MSC injection, each mouse underwent surgery to implant a guide cannula into its brain (Lee et al., 2009). Detailed methodology is described in the Supplemental Methods section.

1.4. Transplantation of hUCB-MSC into the hippocampal brain region

hUCB-MSC or PBS were administered once every 2 weeks (n = 15 per group). APP/PS1 mice were treated starting at 7 months, 1 week of age, and finishing at 8 months, 1 week of age (total three times once every 2 weeks