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Insulin-degrading enzyme deficiency accelerates cerebrovascular amyloidosis in an animal model

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ABSTRACT

Cerebrovascular amyloidosis (CA) may result in intraparenchymal bleeding and cognitive impairment. It was previously shown that transforming growth factor-β1 (TGF-β1) expression under an astrocyte promoter resulted in congophilic vascular deposits and vascular pathology. A reduction in insulin-degrading enzyme (IDE) activity was previously suggested to play a role in the accumulation of congophilic vascular deposits in the microvasculature of Alzheimer's disease (AD) cases. Here, we aim to investigate the link between TGF-β1 and IDE activity in the development of CA. We found that TGF-β1 can reduce IDE expression in a mouse brain endothelial cell line (ECs). Furthermore, we discovered that IDE activity in the brains of TGF-β1 transgenic (Tg) mice was significantly reduced compared with that of the control mice in an age-dependent manner. In addition, TGF-β1/IDE$^{-/-}$ mice showed significantly greater levels of cerebrovascular pathology compared with TGF-β1 mice. We have previously shown that 16-month-old TGF-β1 mice have a significant reduction in synaptophysin protein levels, which may lead to cognitive impairment. Here we discovered a significant reduction in synaptophysin protein already at the age of seven in the hippocampus of TGF-β1/IDE$^{-/-}$ mice compared with TGF-β1 mice. Further investigation of TGF-β1–mediated IDE activity in ECs may provide useful therapeutic intervention targets for cerebrovascular diseases such as CA.

1. Introduction

Cerebrovascular amyloidosis (CA) is a disease of the small blood vessels in the brain, in which deposits of amyloid proteins on blood vessel walls may lead to stroke, brain hemorrhage, or dementia (Korczyn et al., 2012; Smith and Greenberg, 2009). Although the most common form of cerebrovascular amyloid is the deposition of β-amyloid (Aβ) (1–40), other proteins have been linked to familial forms of CA, such as amyloid precursor protein (APP), cystatin C, prion protein, gelsolin, and transthyretin (Burgermeister et al., 2000; Lacombe et al., 2004). Most cases of CA are sporadic and can be detected to various degrees in approximately half of all individuals beyond 70 years of age (Itoh et al., 1993; Yamada et al., 1987). In addition, CA can be detected in up to 90% of Alzheimer's disease (AD) patients (Yamada et al., 1987).

Transforming growth factor-β1 (TGF-β1), which promotes the synthesis of extracellular matrix proteins and contributes to vascular remodeling following injury, cell growth, cell proliferation, cell differentiation, and apoptosis, has been implicated in the cerebrovascular pathology of AD (Wyss-Coray et al., 2000a; Wyss-Coray et al., 2000b). Levels of TGF-β1 are increased and positively correlated with the degree of cerebrovascular amyloidosis in human AD brain tissue. Furthermore, TGF-β1 immunoreactivity in AD brains with CA is elevated along the cerebral blood vessels. Transgenic overexpression of TGF-β1 under the control of an astrocyte glial fibrillary acidic protein (GFAP) promoter in mice causes an age-related (starting approximately 6 months of age) deposition of congophilic amyloid around cerebral blood vessels and prominent perivascular astrocytosis (Wyss-Coray et al., 1995; Wyss-Coray et al., 2000a; Wyss-Coray et al., 1997), leading to vascular alterations and dysfunction at 15 months of age (Wyss-Coray et al., 2000b). It was previously shown that in TGF-β1 mice, the region displaying the greatest number of intraparenchymal vessels with CA pathology was clearly the hippocampus, and the density of CA positive vessels in the cortex was approximately only 20% of those in the hippocampus (Gaertner et al., 2005). These data suggest an important role for cerebrovascular amyloid pathology in the progression of neurodegenerative diseases, such as AD (Lifshitz and Frenkel, 2009).

The severity of cerebrovascular amyloid deposition has been linked to the activity of several enzymes known to degrade amyloid deposition. Insulin-degrading enzyme (IDE), also known as insulysin, is a large zinc-binding protease that is known to cleave multiple short polypeptides and has an important role in regulating insulin and Aβ metabolism (Im et al., 2007). IDE has been

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localized to several places, including the cytosol, peroxisomes, endosomes, proteasome complexes, and the surface of cerebrovascular endothelial cells (ECs) (Dorfman et al., 2010; Gao et al., 2004). Studies of genetically inherited forms of AD show a reduction in the catalytic activity of IDE (Kim et al., 2007) among affected individuals. The defect in Aβ proteolysis by IDE also contributes to Aβ accumulation in the cortical microvasculature of AD cases with CA (Morelli et al., 2004). In animal models, deficits in IDE function lead to impaired Aβ degradation in the brain (Farris et al., 2003; Miller et al., 2003), whereas overexpression of IDE reduces Aβ levels and retards or completely prevents amyloid plaque formation in the brain (Wang et al., 2006). Nevertheless. there was no evidence that linked between the elevation of TGF-β1 and IDE activity in ECs. We hypothesis that TGF-β1 may impair IDE activity resulting in CA like pathology. We report that an IDE deficiency in TGF-β1 Tg mice leads to the early onset of disease and rapid disease progression.

2. Materials and Methods

2.1. Mice

Heterozygous TGF-β1 mice were generously received from Tony Wyss–Coray's laboratory (Wyss-Coray et al., 1995) and maintained on an inbred C57BL/6 mice genetic background (The Harlan laboratories). Heterozygous IDE mice were kindly received from Dennis Selkoe's laboratory (Farris et al., 2003) and bred to C57BL/6 mice for seven generations and then to each other to create homozygous deletion mice on a C57BL/6 background. $IDE^{-/-}$ mice were maintained in our lab and inbred with heterozygous TGF-β1 mice. The male mice (WT, $IDE^{-/-}$, TGF-β1 and TGF-β1/$IDE^{-/-}$) were analyzed at 3, 7 and 9 months of age. All animal care and experimental use was in accordance with the Tel Aviv University guidelines and approved by the university's animal care committee.

2.2. Cell culture

The bEnd5 EC line (a kind gift from Dr. Britta Engelhardt, Bern, Switzerland) is derived from a mouse brain and was generated by the immortalization of cells with the polyoma middle T oncogene (Laschinger and Engelhardt, 2000). Previous studies showed that bEnd5 cells are an appropriate endothelial cell line for use as a BBB in vitro model (Laschinger and Engelhardt, 2000) and suggested that these cells may offer a useful means for studying the alterations in mouse brain endothelial cell biology and signaling pathways that occur during brain pathology (Yang et al., 2007). The bEnd5 EC line and the murine macrophage-like RAW264.7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum (FCS), 1 mM sodium pyruvate, 4 mM L-glutamine, 1% (v/v) nonessential amino acids, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 12.5 U/mL nystatin. The cell lines were maintained at 37 °C, with 5% CO_2 and a 95% relative humidity. We use 10 ng/ml TGF-β1, as suggested by previous publications on TGF-β1 signaling in EC (Albinana et al., 2011; Vinals and Pouyssegur, 2001) to evaluate the effect of TGF-β1 on IDE expression.All of the medium components were obtained from Biological Industries (Beit–Haemek, Israel).

2.3. Immunohistology and quantitation of amyloid load

Seven-month-old mice were sacrificed (transcardially punctured and saline-perfused), and their brains were rapidly excised and frozen. The brains (left hemisphere) were cut into 14-μm coronal sections at −20 °C, and the sections were used for histological examination. For the quantification of the frequency and severity of CA, the brain sections (1.44 mm lateral to bregma) were stained with Congo red (Sigma–Aldrich) and visualized by fluorescence microscopy (Nikon Eclipse 80i). A quantitative analysis was restricted to the area of the hippocampus, and 4–5 consecutive sections (14-μm) per animal were used. The stained hippocampal blood vessels were counted and analyzed (20 × microscope objective) throughout the section. The collected data are presented as the total number of congo-positive blood vessels in the section. For quantification, we analyzed congophilic amyloid depositions that were rated above severity grade one, as previously described (Winkler et al., 2001). For immunostaining, the following antibodies were used: rabbit pan-laminin (pan-Ln) (1:1000; Novus Biological) and rat CD31 (1:75; BD Biosciences). The relative fluorescence intensity of laminin was quantified by using ImageJ software. The percentage of pixels that had a staining intensity greater than the corresponding threshold was then used to integrate the percentage area occupied by laminin staining in the cortex.

2.4. Microhemorrhage

Staining for deposits was performed on 3–5 adjacent 14-μm-thick coronal sections of mouse brains that contained similar regions located approximately 1.5 mm lateral to the bregma and that were collected from seven-month-old Tg mice and age-matched WT littermates. The sections were stained with Perls' Prussian blue with a working solution (equal parts of freshly made 5% potassium ferrocyanide and 5% hydrochloric acid) for 60 min at room temperature, washed in deionized water, and counterstained with nuclear fast red. Perls' Prussian blue-stained clusters of hemosiderin staining were qualitatively evaluated (presence/absence) in sections throughout the neocortex, hippocampus, and thalamus.

2.5. Real-time PCR analysis

bEnd5 cells were cultured in six-well culture plates for 72 h in DMEM supplemented with FCS. Then, the cultured medium was replaced with serum-free DMEM, and the cells were further incubated in the presence of either TGF-β1 (10 ng/mL) or vehicle for 1.5 h or 24 h. Total RNA was isolated using the MasterPure™ RNA Purification Kit (Epicentre). A probe for IDE TaqMan real-time PCR (# Mm00473077_m1) was purchased from Applied Biosystems (Foster City, CA, USA). Real-time PCR was performed using an Applied Biosystems PRISM 7300 thermal cycler according to the manufacturer's instructions. The mouse actin gene, a housekeeping gene, was used to normalize each sample. A quantitative analysis was performed by the $2^{-\Delta\Delta ct}$ method and statistically analyzed using Graphpad Prism Software. RAW 264.7 macrophage cells were plated in 12-well plates in three different conditioned media: (1) Endothelial cell (EC)-conditioned medium: the supernatant of ECs grown for 24 h in DMEM; (2) TGF-β1 stimulated EC-conditioned medium: the supernatant, collected after 24 h, of ECs that were stimulated with 10 ng/mL TGF-β1 for 2 h, after which the medium was replaced with serum-free medium containing 0.5% bovine serum albumin (BSA); and (3) control medium: serum-free DMEM containing 0.5% BSA. Twenty-four hours later, the total RNA was isolated and analyzed for the presence of IDE using real-time PCR, as previously described.

2.6. Isolation of brain endothelial cells

Brain endothelial cells were isolated from either $IDE^{-/-}$ or WT mice using percoll separation and specific magnetic bead columns, as previously described (Cardona et al., 2006). Briefly, the brains of animals perfused with PBS without Ca_2^+ and Mg_2^+ were minced with a scalpel blade and digested for 30 min at 37 °C in RPMI 1640 containing 2 mM L-glutamine, dispase and collagenase type three (Roche); this step was followed by an additional 30 min