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Existence of different types of senile plaques between brain and spinal cord of TgCRND8 mice

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ABSTRACT

Conflicting findings exist regarding the formation of diffuse and dense-core β-amyloid (Aβ) plaques in Alzheimer's disease (AD). In the present study, we characterized Aβ plaque types in the brain and spinal cord of TgCRND8 mice, which express a transgene incorporating both the Indiana mutation (V717F) and the Swedish mutations (K670N/M671L) in the human amyloid-β protein precursor (APP) gene. By combining immunohistochemistry and thioflavin S staining, we were able to define dense-core and diffuse plaques in neocortex of the brain and spinal cord of 9 week-, 5 month-, 10 month- and 20-month-old TgCRND8 mice. The senile plaques in the neocortex were predominantly dense-core plaques, even in the youngest mice. However, diffuse plaques were instead detected in spinal cord of the mice, regardless of age. Our results that relative predominance of dense-core plaques in the neocortex and diffuse plaques in the spinal cord of TgCNRD8 mice of all disease durations argue against the notion that diffuse plaques may represent an early stage in the evolution of dense-core plaques. Furthermore, we also found that the ratio of Aβ42/Aβ40 of the brain was much higher than that of the spinal cord by Aβ ELISA assay. Our findings strongly indicate that diffuse and dense-core plaques may form via independent processes in AD and Aβ42 is more prone to form dense-core plaques than is Aβ40.

Introduction

β-amyloid (Aβ) deposition in the brain is well accepted as a pathological hallmark of Alzheimer's disease (AD), although there is considerable diversity of opinions regarding the significance of plaque generation in relation to the etiopathogenesis of the disease (O'Brien and Wong, 2010; Kocherhans et al., 2010; Chakrabarty et al., 2010a, 2010b). The heterogeneity of plaque morphology has also been noted by a number of early investigators in the field of AD research (Wisniewski and Terry, 1973; Ulrich, 1985), although the significance of these plaque isoforms attached to the pathomechanisms of the disease is yet to be determined (Armstrong, 1998; Dickson and Vickers, 2001; D'Andrea and Nagele, 2010; Chlan-Fourney et al., 2011). Of the many descriptive names for plaques presented in the literature, two of the most common subtypes viz. dense-core and diffuse types, have generally been classified based on their distinctive appearance in the immunohistochemical preparations of postmortem AD brains (D'Andrea and Nagele, 2010). Dense-core plaques, but not diffuse plaques, are of the β-sheet/fibrillar conformation of the Aβ peptide and thus can be identified by β-sheet-sensitive dyes such as thioflavin-S (Chlan-Fourney et al., 2011). This type of plaques is frequently surrounded by dystrophic neurites and reactive microglia and astrocytes (reviewed by Reitz et al., 2011).

It has been suggested that the remnants of nuclear elements in the dense-core plaques such as nucleotides and/or ATP/ADP (D'Andrea et al., 2001; Honda et al., 2001) may activate astrocytes and microglia (Nagele et al., 2003, 2004). Diffuse plaques, on the other hand, are largely devoid of dystrophic neurites or activated microglia and astrocytes. Moreover, it is generally agreed, despite with limited experimental support, that extracellular amyloid deposits first form diffuse plaques that evolve over time into dense-core plaques (Mackenzie et al., 1995; Griffin et al., 1995). In conflict

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with the hypothesis that diffuse plaques may represent an early stage in the evolution of dense-core plaques, plaques in the striatum and cerebellum retain their predominantly diffuse nature in AD regardless of disease duration (Joachim et al., 1989; Wolf et al., 1999; Wang et al., 2002). Diffuse plaques in the cerebellum and striatum do not appear to evolve into dense-core plaques over the course of AD (Wolf et al., 1999). Therefore, it seems that the morphological phenotype of β-amyloid plaques is host tissue-dependent. Another line of evidence lending support to this suggestion is that different ratio of dense-core/diffuse plaques was formed in different source of tissues (Meyer-Luehmann et al., 2003), although the mechanisms underlying such differences remain obscure.

TgCRND8 mouse is a model of aggressive AD-related amyloidosis as they express a transgene incorporating both the Indiana mutation (V717F) and the Swedish mutation (K670N/M671L) in the human amyloid-β protein precursor (APP) gene (Chishti et al., 2001). Although considerable knowledge has been generated about the beta-amyloid plaques in the brain, much less is known about their characteristics in the spinal cord. Only a few studies have investigated these plaques in the spinal cord of another AD mouse models (Wirths et al., 2006, 2007; Wirths and Bayer, 2008; Jawhar et al., 2012). However, the comparative analysis between spinal cord and brain beta-amyloid plaques has not been performed so far. In this study, we therefore investigated whether there is a homogeneity of the senile plaques in the brain and spinal cord of TgCRND8 mice in terms of their origin; or indeed there exists different senile plaques between brain and spinal cord in this AD model. The answer to this question through well designed and executed experimental work is of importance as it would provide much sought after evidence to judge whether it is the case that extracellular amyloid deposits first form diffuse plaques that evolve over time into dense-core plaques, or that morphological phenotype of β-amyloid plaques is host tissue-dependent.

In the present study, we characterized and compared the regional features of β-amyloid plaques in the neocortex and spinal cord of TgCRND8 mice of various ages with an explicit aim to identify the phenotypes of plaques in the neocortex of the brain and in the spinal cord. We found that unlike in the neocortex of the brain, the predominant phenotype of plaques in the spinal cord is diffuse plaques. We have also found a relatively lower ratio of Aβ42/Aβ40 in the spinal cord when compared to that of the brain of TgCRND8 mice. This discrepancy might contribute to the difference in predominant types of Aβ plaques in neocortex and spinal cord. Our experimental findings strongly support that diffuse and dense-core plaques may form via independent processes in AD. This is the first report on the types of senile plaques in the spinal cord of TgCRND8 mice which, we believe, is a useful tool to study possible mechanisms associated with Aβ plaque formation.

Materials and methods

Mice

All animal husbandry procedures performed were approved by the Committee on the Use of Live Animals for Teaching and Research of the University of Hong Kong. To generate CRND8 mice, male TgCRND8 mice were mated with female C57BL/6J mice. All mice were housed with three to five animals in a cage and maintained with food and water ad libitum and a 12 h light/dark cycle.

Perfusion and tissue processing

The 9 week-, 5-, 10 and 20-month-old TgCRND8 mice ($n = 5$) were perfused transcardially under deep anesthesia with a saline solution, followed by fixative solution composed of 4% paraformaldehyde. The brains and cervical cords were removed and kept in the same fixative overnight, followed by immersion in 30% sucrose solution. Brains and spinal cords were then sectioned coronally and horizontally (30 μm) using a frozen-stage equipped microtome. Plaques were classified as dense core (immunoreactive for the anti-Aβ antibody, Bam-10, as well as positive to thioflavin-S positive staining) or diffuse (immunoreactive for the anti-Aβ antibody, but negative to thioflavin-S staining) as described previously (Chlan-Fourney and others, 2011).

For immunostaining of Bam-10 (1:2000, Sigma–Aldrich, Poole, UK), which recognizes the epitope residing within amino acid residues 1–12 of Aβ protein, standard immunofluorescence was performed on floating sections according to procedures described previously (Yuan et al., 2007). Briefly, the sections were incubated with the primary antibody against Bam-10 in 0.1 M phosphate buffered saline (PBS) (pH 7.4) containing 10% normal goat serum and 0.2% Triton X-100 for overnight at room temperature. Following incubation with the primary antibody, antigens were visualized using Alexa 568-conjugated secondary antibody (1:800 Molecular probes). For double staining of Bam-10/thioflavin-S or Bam-10/GFAP, the sections were then incubated with either 1% thioflavin-S (Sigma–Aldrich, UK) for 5 min for the detection of β-sheet-rich Aβ-containing plaques (Orly Lazarov 2002) or rabbit polyclonal antibody against GFAP (1:500, Sigma–Aldrich, Poole, UK) and then the Alexa 488-conjugated secondary antibody (1:800 Molecular probes) for the detection of astrocytes. Each step was followed by three washes (20 min, RT) in PBS. Finally, the sections on gelatin-coated glass slides were coverslipped in mounting medium (Dako, Denmark). Fluorescent images were captured with a Zeiss microscope (Zeiss, Gottingen, Germany) equipped with a Spot digital camera (Diagnostic Instruments, Sterling Heights, MI, USA).

Determination of Aβ plaque burden

Brains and cervical cords were sectioned in 30 μm thickness using a microtome. Plaque deposition levels were examined in neocortex and cervical spinal cord. Five animals were used in each group for counting. Images of 100× magnification were captured using a Zeiss microscope equipped with a SPOT camera and SPOT software (RT Color diagnostic Instrument INC, Michigan, USA) on four sections per animal. By using ImageJ software, pictures were binarized to 8-bit black and white pictures and a fixed intensity threshold was applied to define the DAB staining. Measurements were performed for a percentage area covered by Bam-10 DAB staining.

In order to quantify the relative proportion of each plaque type, Bam-10 immunohistochemistry- or thioflavin S labeling was examined in the entire cerebral cortex and cervical cord of TgCRND8 mice as described previously (Doggui et al., 2010). The ratio of dense-core plaques/senile plaques was calculated by dividing total area of thioflavin S staining by total area of Bam-10 immunostaining. Four coronal sections of either brain or spinal cord from each mouse ($n = 5$) were examined.

Assessment of Aβ levels

The levels of Aβ40 and Aβ42 peptides were analyzed using human β-Amyloid Aβ40 and Aβ42 Aβ colorimetric sandwich ELISA kits (Wako Pure Chemical Industries Ltd., Japan).

Sandwich Aβ ELISA assay was performed as described previously (Lord et al., 2011; Durairajan et al., 2012). Neocortex or spinal cord at cervical level of TgCRND8 mice at the age of 20 months was homogenized in Tris-buffered saline (20 mM Tris and 137 mM NaCl, pH 7.6) supplemented with 1% protease inhibitor cocktail (Sigma). After sonication, samples were centrifuged