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Human postmortem brain-derived cerebrovascular smooth muscle cells express all genes of the classical complement pathway: A potential mechanism for vascular damage in cerebral amyloid angiopathy and Alzheimer's disease

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Abstract

Deposition of amyloid around blood vessels, known as cerebral amyloid angiopathy (CAA), is a major pathological feature found in the majority of Alzheimer's disease (AD) cases, and activated complement fragments have been detected on CAA deposits in AD brains. In this study, we demonstrate for the first time that human cerebrovascular smooth muscle cells (HCSMC) isolated from cortical vessels derived from postmortem brains can express mRNAs for complement genes C1qB, C1r, C1s, C2, C3, C4, C5, C6, C7, C8 and C9, the components of the classical complement pathway. Secretion of the corresponding complement proteins for these genes was also demonstrated, except for C1q and C5. Of particular significance was the observation that treatment of HCSMC with aggregated amyloid beta (Aβ) 1–42 increased expression of complement C3 mRNA and increased release of C3 protein. Aβ treatment of HCSMC also increased expression of C6 mRNA. Interferon-γ induced expression and release of complement C1r, C1s, C2 and C4. As HCSMC are closely associated with Aβ deposits in vessels in the brain, their production of complement proteins could amplify the proinflammatory effects of amyloid in the perivascular environment, further compromising brain vascular integrity.

Keywords: Vasculature; Inflammation; Amyloid; Toxicity; Neurodegeneration; Cell culture; Gene expression

Introduction

There is now strong evidence that damage of the cerebrovasculature is involved in the etiology of Alzheimer's disease (AD) (Luchsinger et al., 2005; Shi et al., 2000; Yip et al., 2005; Zlokovic et al., 2005). Cerebrovascular pathology is a common feature in AD brains and encompasses a variety of lesions including changes in endothelial and vascular smooth muscle cells, macroscopic and micro-infarction, hemorrhage, and white matter changes related to small vessel disease. Cerebral amyloid angiopathy (CAA) is found in approximately 90% of AD cases and consists of deposits of amyloid beta (Aβ) peptide in and around the perivascular spaces of vessels (Premkumar et al., 1996). This eventually leads to the death of smooth muscle cells and endothelial cells in the vicinity of amyloid deposits (Vonsattel et al., 1991). The presence of Aβ deposits in the vasculature can also lead to proinflammatory responses including microglia/macrophage activation (Giulian et al., 1995) and complement activation (McGeer et al., 1989). Although Aβ peptide can be directly toxic to HCSMC (Davis et al., 1999; Wilhelmus et al., 2005), the involvement of other mechanisms in mediating cerebrovascular damage, including inflammatory processes, has been indicated (Miao et al., 2005).

The complement system is one of the components of the innate immune system, and consists of a series of functionally-linked proteins, which, when activated, interact to amplify the immune response to targeted molecules (van Beek et al., 2003).

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The components of the complement system can mediate cytolysis if the complete pathway is activated. During the activation process, complement fragments are produced that can bind to invading microorganisms or cellular debris and target them for phagocytosis, or promote chemotaxis of inflammatory cells to sites of tissue damage through interaction with specific complement receptors (Aderem and Underhill, 1999; Gasque et al., 2002). Activation of the complement system in AD and in brains affected by other neurodegenerative diseases has been extensively characterized (Gasque et al., 2002; McGeer and McGeer, 2002; van Beek et al., 2003). Aβ plaques and other hallmark features of AD, including vascular amyloid deposits, are tagged with different complement fragments, indicating that the complement system is activated in the AD brain (McGeer et al., 1989; Rogers et al., 1992; Shen et al., 2001; Veerhuis et al., 1996). The presence of activated complement protein fragments C1q, C3c, C4d and C5b-9 on cerebrovascular-associated amyloid in AD brains has been observed (Verbeek et al., 1998), though the source of these proteins was not determined.

Aβ peptide can activate the classical complement pathway by binding C1q in the absence of specific antibodies (Rogers et al., 1992; Webster et al., 1997), as well as activating the alternative complement pathway in the absence of C1q (Bergamaschini et al., 1999; Bradt et al., 1998). Studies have shown localized expression of different complement mRNAs within the brain (Johnson et al., 1992; Walker and McGeer, 1992), with significant increases of complement mRNA expression in brain regions selectively affected by AD (Yasojima et al., 1999). It is now known that neurons, microglia, astrocytes, oligodendrocytes and microvascular endothelial cells have the capacity to express many of the genes and gene products of complement pathway proteins (Gasque et al., 1995; Hosokawa et al., 2003; Klegeris et al., 2000; Shen et al., 1997; Terai et al., 1997; Thomas et al., 2000; Walker et al., 1995, 1998).

In this study, we demonstrate for the first time that HCSMC derived from cerebral vessels from human postmortem brains have the potential to express the complete range of mRNAs of the classical complement pathway, and to secrete most of the corresponding proteins. This may be of significance as HCSMC are closely associated with deposited Aβ in the basement membrane around vessels; the production and subsequent activation of complement in this perivascular environment could contribute to vascular dysfunction.

Table 1
Polymerase chain reaction primer sequences

Gene	Fragment size (bp)	Cycle no.
C1QB-F CCCAGGGATAAAAGGAGAGAAA	358	38
C1QB-R GGCGTGGTAGGTGAAGTAGTAGAG		
C1R-F CCTCCCTGACAACGATACCTTCTA	215	26
C1R-R CGTCCTGCTTTAGAGATGGGTGT		
C1S-F AGAGAAGATTTTGATGTGGAAGCA	444	26
C1S-R ACAGGTTGACATTTCAGTTTGGA		
C2-F CTCTACCTGCTCCTGGACTGTT	304	30
C2-R GTCGCATTTGGTTGTTCATCAT		
C3-F CCTGGCTCCACAGTTCTCTATCG	383	27
C3-R GTTCCCTCCACTTTCTTCCCGTA		
C4-F TGGTTCCTATGCGGCTTGGTTGT	324	26
C4-R GAGGCTTCCACTCTCTGCTTCAAT		
C5-F TGGCATTAGCAGCAGTGGACAGT	313	34
C5-R CAGGCTCCATCGTAACAACATTTC		
C6-F GGCGTGTATGACCTTCTCTATC	382	34
C6-R CACAGGGGATGTTTCTTACC A		
C7-F ACTGTTGAGGGGACCCATT	457	28
C7-R ACCGTAAATCTTCTCCACATCTG		
C8-F ACTGCGACCCTCTTGACTCTG	311	37
C8-R AGGACCCCTGTGTCTCCATAG		
C9-F AATGAGCCCCTGGAGTGAATGGT	180	38
C9-R ATTTCCGCAGTCATCCTCAGCAT		
β-actin-F CCACGAAACTACCTTCAACTCC	262	22
β-actin-R ACTCGTCATACTCCTGCTTGCT		

Table 2
Details of antibodies used

Antibodies	Supplier	Dilution	Application
C1Q	Dako (R)	1:5000	W
C1R	MP (G)	1:5000	W
C1S	MP (G)	1:5000	W
C2	ART (G)	1:5000	W
C3	ART (G)	1:10,000	W
C4	ART (G)	1:10,000	W
C5	ART (G)	1:5000	W
C6	ART (G)	1:5000	W
C7	ART (G)	1:5000	W
C8	ART (G)	1:10,000	W
C9	ART (G)	1:5000	W
SMA	Sigma (M)	1:5000	ICC
CD31	Dako (M)	1:1000	ICC
LN3	MP (M)	1:500	ICC
GFAP	Dako (R)	1:2000	ICC

Abbreviations: Dako; Dako North America, Carpinteria, CA: MP; MP Biomedicals, Solon, OH: ART; Advanced Research Technologies, San Diego, CA; Sigma; Sigma Chemical Company, St. Louis, MO: (R), rabbit polyclonal: (G), goat polyclonal: (M), mouse monoclonal: W, western blots: ICC; immunocytochemistry: SMA, smooth muscle α-actin.

Materials and methods

Human brain vessel isolation

HCSMC were cultured from human postmortem brains using the following protocol to firstly isolate viable human brain vessels. In this study, HCSMC isolated from 9 separate human postmortem cases were used in the described experiments. Except where noted, all isolates produced similar results. Human brain tissues were received by the Sun Health Research Institute Brain Donation program within 3h of death of the donor. Collection of human brain tissues in this program had received approval from the Institutional Review Board of the Sun Health Corporation. Brains used in this study were from 4 AD, 2 Parkinson's disease with dementia and 3 non-demented (ND) donors (mean age 85yr ± 5.3 (std. dev.); postmortem delay 2.3h ± 0.4 (std. dev.)). For vessel isolation, gray matter (approximately 25g) was dissected from coronal slices of frontal cortex. Tissue was homogenized in Hanks balanced salt solution (HBSS) using a wide-bore Dounce homogenizer. Tissue homogenate was then centrifuged (250g, 10min), and resuspended in Dextran 70 (Sigma-Aldrich, St. Louis, MO) to a final concentration of 15%. Homogenates were centrifuged (5800g/15min) and the resulting vessel-enriched pellets were recovered. This fraction was digested for 60–90min in 0.1% collagenase type II (Worthington Biochemicals, Lakewood, NJ) at 37°C and then filtered sequentially through 100, 70 and 40μm mesh filters (BD, Bedford, MA). HCSMC were cultured from vessels retained on the 100 and 70μm mesh filters. These vessels were plated onto collagen type-I coated culture