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Down syndrome individuals with Alzheimer's disease have a distinct neuroinflammatory phenotype compared to sporadic Alzheimer's disease

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ABSTRACT

Down syndrome (DS) is the most common genetic cause of intellectual disability and is primarily caused by the triplication of chromosome 21. The overexpression of amyloid precursor protein gene may be sufficient to drive Alzheimer's disease (AD) neuropathology that is observed in virtually all individuals with DS by the age of 40 years. There is relatively little information about inflammation in the DS brain and how the genetics of DS may alter inflammatory responses and modify the course of AD pathogenesis in this disorder. Using the macrophage classification system of M1, M2a, M2b, and M2c inflammatory phenotypes, we have shown that the early stages of AD are associated with a bias toward an M1 or M2a phenotype. In later stages of AD, markers of M1, M2a and M2c are elevated. We now report the inflammatory phenotype in a DS autopsy series to compare this with the progression in sporadic AD. Tissue from young DS cases (under 40 years of age, pre-AD) show a bias toward M1 and M2b states with little M2a or M2c observed. Older DS cases (over 40 with AD pathology) show a distinct bias toward an M2b phenotype. Importantly, this is distinct from sporadic AD where the M2b phenotype has been rarely, if ever observed in postmortem studies. Stimulated by immune complex activation of microglial cells and toll-like receptor activation, the M2b phenotype represents a unique neuroinflammatory state in diseased brain and may have significant implications for therapeutic intervention for persons with DS.

1. Introduction

Down syndrome (DS) is a genetic disorder resulting from the triplication of chromosome 21 (Lejeune et al., 1959). It is the most common chromosomal abnormality in humans and is characterized by both physical and neurological features (Lott, 1992). Improved education and health care have significantly increased both the life span and the quality of life for DS individuals. The amyloid precursor protein gene lies on chromosome 21, and thus is triplicated in DS (Korenberg et al., 1990). The vast majority of DS individuals older than 40 years will develop significant amyloid pathology that is indistinguishable from Alzheimer's disease (AD) including beta-amyloid (Aβ) plaques and neurofibrillary tangles (Leverenz and Raskind, 1998; Wisniewski et al., 1985). By 50–60 years many, but not all, of these individuals will develop dementia (Johannsen et al., 1996; Schupf and Sergievsky, 2002). With the emergence of anti-amyloid agents as potential preventative therapies for AD, there is excitement in the field regarding the translation of these approaches to people with DS. Our goal is to better understand the aging DS brain to inform the potential for translation of AD therapies to DS.

Neuroinflammation is thought to contribute to the progression and severity of AD. Originally described by Alois Alzheimer in 1907 (Alzheimer et al., 1995), microglia have long been known to surround amyloid plaques in the AD brain. Later, mechanistic studies

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described the contribution of microglia to AD pathogenesis including clearance of Aβ via phagocytosis, neurotoxicity via proinflammatory cytokine production and increased amyloid deposition via neuronal cytokine signaling (Colton and Wilcock, 2009; McGeer and McGeer, 1998; Streit et al., 1988). More recently, clear phenotypes have been characterized for neuroinflammatory processes that are multifaceted (Mandrekar-Colucci and Landreth, 2010). Work in our laboratory shows that the AD brain exhibits broad heterogeneity in its neuroinflammatory state (Sudduth et al., 2013b). We use the markers of macrophage phenotypes M1, M2a, M2b, and M2c to identify the bias of a given sample toward one state or another along a spectrum of possible responses (Edwards et al., 2006; Mosser and Edwards, 2008). When we examine early stage AD brain, we find not only heterogeneity, but there was an apparent bias that distinguished 2 populations within the early AD group. One half of the early AD cases show a bias toward a proinflammatory, M1 phenotype, whereas the other half of the early AD cases show a bias toward a wound-repair, M2a phenotype (Sudduth et al., 2013b). In contrast, late-stage AD showed a more homogeneous neuroinflammatory state, with markers of M1, M2, and M2c being elevated. Interestingly, the M2b phenotype was never seen in the AD samples.

Examination of the inflammatory genes that are expressed on chromosome 21 led us to hypothesize that the brain in DS may show a bias toward an M1 phenotype (Wilcock, 2012). We examined neuroinflammatory markers in postmortem DS frontal cortex, both in young individuals, before AD development, and older, with established AD neuropathology. We compared these to age-matched controls as well as sporadic AD samples.

2. Materials and methods

2.1. Tissue samples

Autopsy brain tissue was obtained from several sources including the University of California at Irvine Alzheimer's Disease Research Center, the Maryland Developmental Disorders Brain Bank, and the University of Kentucky Alzheimer's Disease Center. Tissue characteristics are summarized in Table 1. Human tissue collection and handling conformed to University of Kentucky and/or University of California Irvine Institutional Review Board guidelines. All cases selected ranged from 1 to 88 years based, in part, on the availability of frozen frontal cortex. Control cases were subsequently selected to match for age and post mortem interval (PMI) (as closely as possible) to the DS cases. AD cases were selected based on PMI only to match DS with AD cases [DS cases that had sufficient neuropathology for a postmortem diagnosis of AD; (Hyman et al., 2012)]. Since individuals with DS with AD typically come to autopsy at younger ages than sporadic AD cases, it was not possible to match for age at death. As a result, tissues from 6 autopsy groups were available: young controls (age-matched to young DS; N = 16), middle-aged controls (MidC, age-matched to DS with AD; N = 27), old controls (age-matched to sporadic AD; N = 9), DS (N = 7), DS with AD (N = 29), and sporadic AD (N = 9). Both males and females were included in the study, but given the challenges of matching cases, we did not match for gender. The level of premorbid intellectual disability was not available for all of the DS cases, thus it was not possible to control for this variable in the analysis.

Table 1
Group characteristics of samples used for the study

Group	N	Age[a]	PMI[a]
Young controls (frozen)	16 (12M, 4F)	20.5 (8.8)	17.8 (5.1)
Young controls (fixed)	9 (5M, 4F)	20.7 (3.8)	12.8 (2.1)
Middle-aged controls (frozen)	26 (20M, 6F)	49.7 (10.1)	13.6 (4.9)
Middle-aged controls (fixed)	13 (7M, 6F)	51.06 (2.4)	14.1 (1.9)
Old controls (frozen)	8 (2M,6F)	81.6 (4.7)	3.3 (1.1)
Old controls (fixed)	9 (3M, 6F)	81.6 (4.7)	3.3 (1.1)
Young DS (frozen)	9 (7M, 2F)	26.4 (14.3)	17.7 (7.2)
Young DS (fixed)	7 (4M, 3F)	26.4 (14.3)	17.7 (7.2)
DS with AD (frozen)	29 (14M,15F)	52.0 (6.9)	6.8 (6.2)
DS with AD (fixed)	11 (6M, 5F)	55.8	8.0 (2.0)
Sporadic AD (frozen)	10 (6M, 4F)	80.0 (7.1)	6.8 (6.2)
Sporadic AD (fixed)	8 (6M, 2F)	81.3 (2.8)	5.5 (1.0)

Key: AD, Alzheimer's disease; DS, Down syndrome; PMI, Post mortem interval; SD, standard deviation.
[a] Note: results presented are mean (SD).

2.2. Quantitative real-time reverse transcription polymerase chain reaction

Frozen brain tissue was pulverized using a mortar and pestle on dry ice with liquid nitrogen and the brain powder was stored at −80 °C. RNA was extracted from approximately 80 mg frozen pulverized tissue using the TRIzol Plus RNA purification system (Life Technologies, Grand Island, NY, USA) according to the manufacturer's instructions. RNA was quantified using the BioSpec-nano spectrophotometer (Shimadzu, Columbia, MD, USA) and complementary DNA was reverse transcribed using the complementary DNA High Capacity kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Real-time polymerase chain reaction was performed using TaqMan Gene Expression probes shown in Table 2 (Applied Biosystems). All gene expression data were normalized to 18S rRNA expression. Fold-change was determined using the $2^{(-\text{delta delta Ct})}$ method (Livak and Schmittgen, 2001).

2.3. Aβ enzyme-linked immunosorbent assays

Aβ was extracted from tissue measured as previously described (Beckett et al., 2010). Briefly, frozen cortical samples were extracted sequentially in ice cold phosphate-buffered saline (PBS, pH 7.4) with a complete protease inhibitor cocktail (Amresco, Solon, OH, USA) and centrifuged at 20,800*g* for 30 minutes at 4 °C. After centrifugation, the supernatant was collected and the pellets were sonicated (10 × 0.5 seconds pulses at 100W, Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA, USA) in 2% sodium dodecyl sulfate (SDS) with complete protease inhibitor and centrifuged at 20,800*g* for 30 minutes at 14 °C. The supernatant was again collected and the remaining pellets were sonicated in 70% formic acid (FA), followed by centrifugation at 20,800*g* for 1 hour at 4 °C. Samples were stored at −80 °C until time of assay.

FA-extracted material was initially neutralized by a 1:20 dilution in tris-phosphate buffer (1 M Tris base, 0.5 M Na_2HPO_4), followed by a further dilution as needed (1:5–1:20, for a final dilution of 1:100–1:400) in antigen capture (AC) buffer (20-mM Na_3PO_4, 0.4% Block Ace (AbD Serotec, Raleigh, NC, USA), 0.05% NaN_3, 2-mM EDTA,

Table 2
TaqMan probes used to measure gene expression changes

Gene of interest	PMID	TaqMan ID
IL-1β	NM_000576.2	Hs00174097_m1
IL-6	NM_000600.3	Hs00174131_m1
TNFα	NM_000594.2	Hs00174128_m1
IL-12	NM_000882.3	Hs99999036_m1
CHI3L1	NM_001276.2	Hs00609691_m1
IL-1Ra	NM_173841.2	Hs00893626_m1
MRC1	NM_002438.2	Hs00267207_m1
IL-10	NM_000572.2	Hs99999035_m1
CD86	NM_175862.4	Hs01567025_m1
FCγR1B	NM_000566.3	Hs00174081_m1
TGFβ	NM_000660.4	Hs99999918_m1

Key: ID, identification; PMID, pubmed identification.