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Bi-phasic gliosis drives neuropathology in a Sandhoff disease mouse model

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ABSTRACT

Microgliosis and astrogliosis are known to be exacerbating factors in the progression of the lysosomal storage disorder Sandhoff disease. We have also found evidence for excitotoxicity via glutamate receptors in Sandhoff disease. To view the interaction of these cascades, we measured cerebellar expression of markers for gliosis, apoptosis, and excitatory synapses over the disease course in a Sandhoff disease mouse model. We observe a 2-stage model, with initial activation of microgliosis as early as 60 days of age, followed by a later onset of astrogliosis, caspase-mediated apoptosis, and reduction in GluR1 at approximately 100 days of age. These results implicate immune cells as first responders in Sandhoff disease.

1. Introduction

Sandhoff disease (SD) is caused by a mutation in the *Hexb* gene, the product of which is a subunit of two lysosomal enzymes - β-hexosaminidase A and β-hexosaminidase B - which leads to accumulations of the gangliosides GM2 and GA2 in neuronal tissues (Itoh et al., 1984; Sandhoff et al., 1971). $Hexb^{-/-}$ mice are well studied and are representative of Sandhoff disease and the closely related Tay-Sachs disease (TSD), with mice displaying motorneuronal and behavioural deficits, reaching endpoint at approximately 17–19 weeks (Phaneuf et al., 1996).

The disease is largely viewed as perpetuated through the activation of microglia/central nervous system (CNS) infiltrating peripheral blood mononuclear cells (PBMC) and astrocytes, with many genes related to gliosis upregulated in the disease in both humans and mouse models (Jeyakumar et al., 2003; Myerowitz et al., 2002; Sargeant et al., 2012). It has been observed that microgliosis occurs before neuronal apoptosis in the brainstem and spinal cord of SD mice, and thus it is suggested that the chronic accumulation of microglia and macrophages - themselves unable to process gangliosides - leads to increased neurotoxicity (Wada et al., 2000). Microglial/macrophage contributions to the disease have been shown to be lessened in double knockouts (DKO) involving inflammatory genes including $Hexb^{-/-}Ccr2^{-/-}$, $Hexb^{-/-}Mip1\alpha^{-/-}$ and $Hexb^{-/-}Tnf\alpha^{-/-}$, resulting in increased lifespans of SD mouse models (Abo-Ouf et al., 2013; Kyrkanides et al., 2008; Wu and Proia, 2004). Related proteins, macrophage inflammatory protein-1 alpha and beta (MIP-1α, MIP-1β), and tumour necrosis factor receptor 2 (TNFR2) have been identified as strong biomarkers for gangliosidoses in human patients (Utz et al., 2015). Interestingly, ablating *Ccr2*-provoked infiltration of PBMCs (subsequently lowering the number of TNFα expressing cells in the brain) appears to have no effect on apoptotic pathways or astrocyte activation (Kyrkanides et al., 2008). This is surprising since microglial activation can lead to the release of various cytokines, including interleukin-6 (IL6) and tumour-necrosis factor-alpha (TNFα), which may induce an apoptotic pathway in neurons, and since we ourselves have observed a reduction in astrocytes and apoptosis in the cerebellum of $Hexb^{-/-}Tnf\alpha^{-/-}$ mice relative to $Hexb^{-/-}$ mice (Abo-Ouf et al., 2013; Shishodia and Aggarwal, 2002). It is possible that the remaining TNFα in $Hexb^{-/-}Ccr2^{-/-}$ mice is sufficient for the astroglial and apoptotic pathways, or the effect is tissue specific. Astrogliosis appears to be driven by one or more pathways involving Sphk1/S1P receptors, and ERK (but not AKT), and ganglioside buildup in SD astrocytes is sufficient to activate proliferation (Kawashima et al., 2009; Kyrkanides et al., 2008; Wu et al., 2008). While the importance of microgliosis and astrogliosis in SD are clear, their relationship to each other during their progression in the development of the disease is not, warranting further study.

Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CNS, central nervous system; DKO, double knockout; GFAP, glial fibrillary acidic protein; IL-6, interleukin-6; NF-L, neurofilament-L; PBMC, peripheral blood mononuclear cells; PSD-95, post-synaptic density protein-95; SD, Sandhoff disease; TSD, Tay-Sachs disease; TNFα, tumour necrosis factor-alpha; WT, wild-type.

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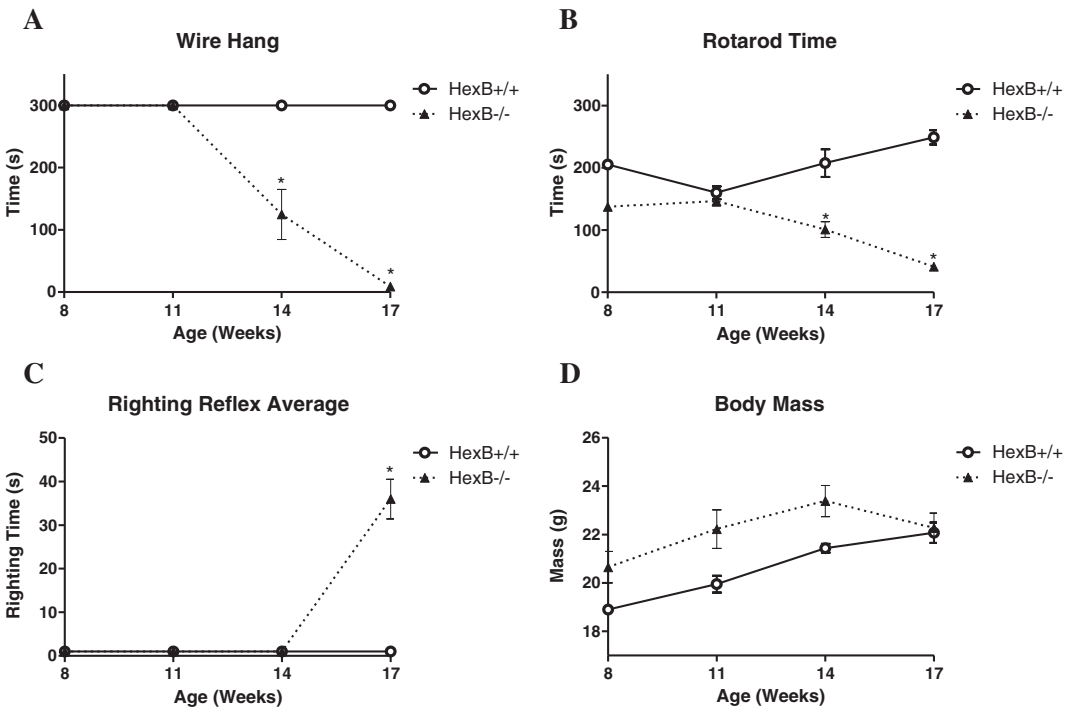

Fig. 1. Decline in Sandhoff disease mouse behavioural performance occurs during specific time point. *Hexb*$^{-/-}$ mouse deterioration in wire hang and rotarod performances occur during the same 11–14 week period, and closely precede the loss of the righting reflex (indicating endpoint). Body mass is not significantly affected during this time period. Bars = mean ± SE. *t*-Tests, n = 3 for each group, *P < 0.05.

Because of the importance of gliosis in SD, neuronal aspects of disease progression have been largely ignored in favor of inflammatory mechanisms. However, in Alzheimer's disease, AMPAR dysregulation may lead to neuronal death through excitotoxicity, and Sandhoff patients have been shown to share common traits with Alzheimer's disease, including intraneuronal accumulations of cellular products such as amyloid-beta (Abad et al., 2006; Hynd et al., 2004; Keilani et al., 2012). TNFα has been shown to increase insertion of both calcium-permeable GluR1 in hippocampal pyramidal neurons, and calcium-impermeable GluR2 in motor neurons (Ogoshi et al., 2005; Rainey-Smith et al., 2010). Increased neuronal activity through calcium-permeable AMPARs can cause excitotoxicity, while decreased neuronal activity can lead to caspase-mediated apoptosis in cerebellar granule neurons (Clayton et al., 2012; Van Damme et al., 2007). Due to the involvement of TNFα in SD, and its various roles in AMPAR distribution, AMPARs such as GluR1 make interesting targets for study.

Immunohistochemical time course experiments have been performed in the brainstem and spinal cord of SD mice for glial and apoptotic markers, but did not include measurement of molecular expression levels, and this study seeks to expand on that work (Wada et al., 2000). To identify key developmental time points for various aspects of SD - including gliosis, neurological markers, and neuronal death - we have analysed expression of disease markers over the progression of the illness in mouse models. We demonstrate a two-stage model of Sandhoff disease, with an early onset of microgliosis followed by a later increase in astrogliosis and apoptosis, and a reduction in GluR1.

2. Materials and methods

2.1. Mice

Hexb$^{-/-}$ mice were generated as previously described, on a C57BL/6 background. Control wild-type (WT) mice are C57BL/6 (Phaneuf et al., 1996).

2.2. Genotyping

Genotyping of mice was performed via PCR on tail samples, using the following primers at previously described: For *Hexb*- Hexb-R: CAATCGGTGCTTACAGGTTTCATC, HexbWT-F: GGTTTCTACAAGAGACA TCATGGC, HexbKO-F: GATATTGCTGAAGAGCTTGGCGGC.

2.3. Behaviour testing

Hexb$^{+/+}$ and *Hexb*$^{-/-}$ female mice were tested at 8, 11, 14 and 17 weeks of age, to observe changes in behaviour during the expected major period of decline in the health of *Hexb*$^{-/-}$. Measurements were taken of wire hang, rotarod, righting reflex, and body mass. Wire Hang: Mice were placed on top of a wire mesh, which was then inverted, 30 cm above a padded surface. Hang time was measured from the point of inversion, until time to fall - to a maximum of 5 min. The best time of 3 consecutive trials was used. Rotarod: Mice were placed on a rotating rod (Accuscan EzRod with EzRod v.120 software), and time to fall was measured via photo-sensors. Rotarod rotations were increased from 0 rpm to a maximum of 40 rpm over 360 s. Mice were placed on the rod at 4 rpm. The best time of 3 consecutive trials was used. Righting Reflex: Mice were positioned on their backs (with a slight tilt to the left and then repeated to the right) on a solid surface, and time for mouse to right itself onto its feet was measured. The average of measurements for the left side and right side righting reflex was used a measurement of cerebellar, pons, and mesencephalon function.

2.4. Western blot

The cerebella of female WT and *Hexb*$^{-/-}$ mice at 60, 80, 100, and 120 days were lysed via sonication in proteinase inhibitor-containing RIPA. Laemmli sample buffer (6×) was added to the lysates and the samples were heated at 95 °C for 5 min. Samples were resolved on a