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Suppression of NK and CD8$^+$ T cells reduces astrogliosis but accelerates cerebellar dysfunction and shortens life span in a mouse model of Sandhoff disease

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ABSTRACT

Sandhoff disease is an inherited lysosomal storage disease, resulting from the deficiency of lysosomal β-hexosaminidase A and B enzyme activity. The *Hexb* −/− mouse model recapitulates human disease and leads to fatal neurodegeneration and neuroinflammation. IL-15 is important for the proliferation of NK, NK T, and CD8$^+$ cytotoxic/memory T cells. In order to determine how changes to IL-15-dependent immune cell populations would alter the course of Sandhoff disease in mice, we generated a *Hexb* −/− *Il-15* −/− double knockout mouse and used motor behaviour tests, analyzed peripheral blood and brain leukocyte immunophenotypes, cytokine secretion, as well as examined markers of microgliosis, astrogliosis and apoptosis. *Hexb* −/− *Il-15* −/− mice had an accelerated neurodegenerative phenotype, and reached the humane endpoint at 118 ± 3.5 d, compared to *Hexb* −/− mice (127 ± 2.2 d). The performance of *Hexb* −/− *Il-15* −/− mice declined earlier than *Hexb* −/− mice on the rotarod and righting reflex motor behaviour tests. *Hexb* −/− mice had a significantly higher prevalence of pro-inflammatory monocytes in the blood relative to C57BL/6 mice, but this was unaltered by IL-15 deficiency. The prevalence of NK cells and CD8$^+$ T cells in *Il-15* −/− and *Hexb* −/− *Il-15* −/− mice was decreased compared to wild type and *Hexb* −/− mice. While *Hexb* −/− mice displayed an increase in the prevalence of CD4$^+$ and CD8$^+$ T cells in brain leukocytes compared to C57BL/6 mice, there was a decrease in CD8$^+$ T cells in *Hexb* −/− *Il-15* −/− compared to *Hexb* −/− mice. In addition, circulating IL-17 and IL-10 levels were significantly higher in *Hexb* −/− *Il-15* −/− mice, suggesting heightened inflammation compared to *Hexb* −/− mice. Interestingly, astrogliosis levels were significantly reduced in the cerebellum of *Hexb* −/− *Il-15* −/− mice compared to *Hexb* −/− mice while microgliosis was not affected in brains of *Hexb* −/− *Il-15* −/− mice. Our study demonstrated that IL-15 depletion dramatically reduced numbers of NK and CD8$^+$ T cells as well as astrocytes but accelerated disease progression in Sandhoff mice. These results pointed to interactions between NK/CD8$^+$ T cells and astrogliosis and potentially a protective role for NK/CD8$^+$ T cells and/or astrocytes during disease progression. This observation supports the notion that expanding the IL-15-dependent NK and CD8$^+$ T cells populations with IL-15 therapy may have therapeutic benefits for Sandhoff disease.

1. Introduction

Lysosomal storage within the central nervous system (CNS) triggers a series of pathogenic processes including neuronal apoptosis and inflammatory activation of microglia and astroglia (reviewed in (Bosch and Kielian, 2015)). Lysosomal β-hexosaminidase A and B enzyme activity is critical in neurons to allow the catabolism of gangliosides GM2, GA2, and globosides. In humans, the absence of β-hexosaminidase A leads to Tay Sachs disease (OMIM 272800) and the absence of β-hexosaminidase A and B leads to Sandhoff disease (OMIM 268800). Both disorders display similar clinical symptoms of neurodegeneration including motor function dysfunction, ataxia, spasticity, seizures, visual loss, and deafness. The *Hexb* −/− mouse model recapitulates the two human diseases showing the accumulation of gangliosides, active gliosis, as well as the infiltration of inflammatory cells from the periphery leading to neuronal cell death and lethal neurodegeneration.

The cytokine environment plays an important role in the perpetuation or suppression of immune cell infiltration and expansion within the CNS (Wu and Proia, 2004; Kyrkanides et al., 2008). Interleukin

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(IL)-15 is a 14 kDa 4-alpha-helix bundle cytokine that is expressed in dendritic cells, macrophages and even glia and neurons within the CNS (Hanisch et al., 1997). The pleiotropic function of IL-15 is evident by its role in the maintenance of lymphoid cells (including NK, NK T, $CD8^+$ and γδ intra-epithelial lymphocytes), muscle growth (Quinn et al., 1995), and neurogenesis (Gomez-Nicola et al., 2011). Although IL-15 mRNA is constitutively expressed, IL-15 is poorly translated and targeted due to multiple 5′UTR AUG sites, a short signal peptide and its carboxyl terminus (Bamford et al., 1998). IL-15 forms a high affinity complex with IL-15Rα and interacts in *trans* with IL2Rβ and IL2R-γ-c, which are expressed on resting NK, NK T and $CD8^+$ memory T cells (Burkett et al., 2004). IL-15 signals through JAK1/3 and STAT3/5 (Burkett et al., 2004), PI3K, MAPKs and NF-kB to induce survival, proliferation and activation of its target cells (Kennedy et al., 2000). As well, through IL-15Rα signaling, IL-15 modulates the cytokine secretion from macrophages and dendritic cells (Alleva et al., 1997). *In vitro* experiments have demonstrated that IL-15 attenuates nitric oxide production and supports microglia proliferation (Hanisch et al., 1997). Peripheral derived IL-15 may also stimulate cerebral endothelial signaling events (Stone et al., 2011). Tumor necrosis factor alpha (TNFα) stimulates IL-15 secretion and up regulates IL-15 receptors in cerebral endothelia (Pan et al., 2009). The contribution of IL-15-dependent immune cell populations (*i.e.* $CD8^+$ cytotoxic T lymphocytes and NK cells) to lysosomal storage diseases in general and Sandhoff disease in particular has largely been unexplored. Previous work in our lab has shown that deletion of TNFα in *Hexb* −/− mice extends their lifespan, and reduces apoptosis and astrogliosis in the CNS (Abo-Ouf et al., 2013). Since TNFα is known to induce IL-15, one possibility is that the removal of IL-15 and as a result IL-15-dependent leukocytes would abolish some of the pleiotropic effects of TNFα.

In this study, we show that removal of IL-15 shortened *Hexb* −/− mouse lifespan and accelerated the loss of balance and coordination of motor behaviour. Removal of IL-15 lowered the prevalence of both $CD4^+$ and $CD8^+$ T cells in the brains of *Hexb* −/− *Il-15* −/− mice, and suppressed the relative prevalence of $CD8^+$ cells in the blood, and potentially increased a Th17 response. In the cerebellum, normally a region of relatively high IL-15 expression, we observe decreased astrogliosis in the *Hexb* −/− *Il-15* −/− mouse compared to *Hexb* −/−. These findings suggest that IL-15-dependent NK and CD8 + T cells plays a role in astrogliosis which in turn may play a protective role during neurodegeneration in a Sandhoff mouse model.

2. Materials and methods

2.1. Generation of mice

The *Hexb* −/− and *Il-15* −/− mice were generous gifts from Dr. Roy Gravel (University of Calgary, Canada) and Dr. Ali Ashkar (McMaster University, Canada) respectively. Both transgenic mice were on a C57Bl/6 background. *Hexb* −/− mice were crossed to *Il-15* −/− mice to generate double heterozygous *Hexb* +/− *Il-15* +/− mice, which were bred to generate the double knockout mice, as well as *Hexb* −/− *Il-15* +/− mice. Mice were genotyped using isolated tail genomic DNA as a template for polymerase chain reaction (PCR). PCR primers were synthesized by Integrated DNA technologies (Coralville, Iowa, USA). For *Hexb* genotyping, 2 forward primers were used for wild type 5′-GGTTTCTACAAGAGACATCATGGC-3′ and knock out 5′-GATATTGCTGAAGAGCTTGGCGGC-3′, with a common reverse primer 5′-CAATCGGTGCTTACAGGTTTCATC-3′ to generate a 141 bp product for the wild-type allele and a 700 bp product for the knockout allele. The thermocycler program consisted of 35 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 45 s. For *Il-15* genotyping, the following primers were used to detect the wild type allele 5′-GAGGGCTAAATCTGATGCGTGTG-3′ and 5′-GAGCTGGCTATGGCGATGGGC-3′ of 240 bp, and the knock out allele 5′-GAATGGGCTGACCGCTTCCTCG-3′ and 5′-TCATATCCTCTGCACCTTGACTG-3′ of 520 bp, using a thermocycler program of 35 cycles of 94 °C for 60 s, 64 °C for 120 s, and 72 °C for 40 s. All procedures involving animals were approved by the McMaster Animal Research Ethics Board and were in accordance with the Canadian Council for Animal Care.

2.2. Behaviour tests

Female and male mice between the ages of 6 and 20 weeks were used in behaviour tests. A rotarod test was used as a measure of motor function, including coordination and physical condition (n = 9–25 mice per genotype). Briefly, an EZ-Rod apparatus (AccuScan Instruments, Columbus, USA) with EZ-Rod software v.1.20 was used, along with a program that consisted of acceleration to a maximum of 40 rpm, with the no fall option enabled. Each mouse was tested once per week, and given 3 trials each time. The best time from each session was included in the data set. A wirehang test was used to assess grip strength once per week (n = 6–20 mice per genotype). Mice were placed on a wire mesh grate, and suspended upside down, at a height of approximately 40 cm, and for a maximum of 3 min. Mice that jumped were given an additional trial per session. The righting reflex test was carried out by placing each mouse on their back and measuring the time each mouse needed to right itself (*Hexb* −/− n = 21, *Hexb* −/− *Il-15* −/− n = 18). A test of 45 s was indicative of welfare endpoint for the Sandhoff disease mice.

2.3. Immunophenotyping

Peripheral blood was collected from mice by terminal cardiac puncture with a heparinized needle (BD, Mississauga, Ontario, Canada) or by facial blood sampling using a 5 mm lancet (Goldenrod Animal Lancet, Braintree Scientific, Inc. Braintree, MA, USA). Erythrocytes were lysed using BD Pharm Lyse (BD Biosciences, Mississauga, Ontario, Canada) and leukocytes were counted. Cells were pre-incubated with rat anti-mouse CD16/CD32 (Fc Block, 10 μg/ml, BD Pharmingen, Mississauga, Ontario, Canada) and immunostained for cell surface markers using 1 μg of each antibody for 10^6 cells in PBS, 3% BSA (all sourced from BD Biosciences, Mississauga, Ontario, Canada): hamster anti-mouse CD3ε-APC-Cy7 (clone 145-2C11), rat anti-mouse CD4-PE (clone GK1.5), rat anti-mouse CD8a-Pacific Blue™ (clone 53–6.7), and rat anti-mouse TER-119-Alexa Fluor® 700 (clone TER-119). Monocytes and granulocytes were immunostained using rat anti-mouse Ly6C-v450 (clone AL-21), rat anti-mouse CD115-PE (clone AF598) antibodies. Samples were washed with PBS 3% FBS buffer, fixed with BD Cytofix™ Fixation Buffer (BD Biosciences, Mississauga, Ontario, Canada) and washed before they were run on a LSR II flow cytometer (Beckman Coulter, Mississauga, Ontario, Canada).

For analysis of brain leukocytes, mice were anaesthetized, perfused with ice cold Hank's Balanced Salt Solution (HBSS, Life Technologies, Mississauga, Ontario, Canada), followed by collagenase A and DNAse I solution. The brains were then dissected and homogenized in a Dounce homogenizer in RPMI (Life Technologies, Mississauga, Ontario, Canada). Percoll (GE Healthcare Life Sciences, Mississauga, Ontario, Canada) and HBSS were added to the homogenate to make a final concentration of 30% Percoll, and the mixture was layered onto a 70% Percoll solution. The samples were centrifuged at 500 ×g in a swinging bucket rotor for 30 min at 18 °C, without the brake set. The cells at the interface between the 70% and 30% Percoll layers was collected, HBSS was added to dilute the Percoll, and samples were centrifuged for 7 min. at 500 ×g at 18 °C with the brake set. The cell pellet was resuspended in PBS, 3% BSA and the staining procedure was carried out as above.

2.4. Enzyme-linked immunosorbant assay

Sera from approximately 120 day old wild type, *Il-15* −/−, *Hexb* −/−, and *Hexb* −/− *Il-15* −/− mice were analyzed for IL-17A and IL-10 (BD paired antibodies and BD OptEIA kits, BD Biosciences, Burlington, Ontario, Canada).